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Abstracts

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Abstract Book

Taphonomic bias in the taxonomic identification of *Lystrosaurus* in the Karoo Basin, South Africa

Caroline Abbott

The Permo-Triassic Mass Extinction (PTME) occurred 251.9 Ma and was the worst biodiversity crisis in all Earth's history, and is well-preserved in the Karoo Basin, South Africa. Taxa like the genus *Lystrosaurus*, are useful for understanding the PTME for their large sample size and broad geographic distribution. Changes in *Lystrosaurus*' body size, histology, and ontogeny during the PTME are a compelling system for understanding life history shifts with climate change.

Historically, *Lystrosaurus* species were over-split, many of which were erected based on deformation style. Today, four species are recognized in the Karoo Basin: *L. maccaigi*, *L. curvatus*, *L. declivis*, and *L. murrayi*. While these species boundaries are more reliable, identifications still suffer from taphonomic biases. Given that *Lystrosaurus* is a crucial taxon for understanding the PTME, recognition of how taphonomy affects their identification is needed.

I surveyed cranial characters of 150 *Lystrosaurus* specimens in South African collections. *Lystrosaurus* exhibits varied brittle and plastic deformation modes. The two Permian species are distinct regardless of ontogeny, size, and deformation. The two Triassic species appear to be problematic, as their classic diagnostic features are subject to alteration based on taphonomic processes. These results highlight the necessity of ongoing revisions to taxonomy, ontogeny, and phylogeny of this taxon. Additional ordination analyses, cluster analyses, and phylogenetic analyses, are planned to provide additional insight into *Lystrosaurus* taxonomy and ontogeny.

Oceanography's Diversity Deficit: Identifying and Addressing the Challenges for Marginalized Groups

Salma Abdel-Raheem, Allison Payne, Milagros Rivera, S. Sturdivant, Nia Walker, Melissa Márquez, Armando Ornelas, Mo Turner, Kelsey Byers, Roxanne Beltran

Centuries of exclusion have resulted in a tangible diversity deficit, where the diversity of oceanographers does not represent the global diversity of people impacted by ocean processes. We present six challenges faced by oceanographers with one or more marginalized identities: 1) Historical practices of conquest, discrimination, and exclusion thwart attempts to address oceanography's modern diversity deficit; 2) Undervalued and uncompensated labor by minority oceanographers can perpetuate a lack of representation by leading to burnout and attrition; 3) Marginalized individuals are often forced to hide parts of their identities (languages, appearances, partners, behaviors) that deviate from outdated expectations of professionalism; 4) Oceanography requires trainees to navigate extensive logistical and financial hurdles; 5) Individuals from non-Western cultural and religious traditions often conceal their spiritual obligations in attempts to assimilate or avoid forgoing valuable research experiences; 6) Limited planning and transparency in oceanographic fieldwork can threaten the physical and mental safety of marginalized individuals. We highlight how holding multiple, intersecting identities can compound negative impacts on the well-being of marginalized oceanographers. Finally, we recommend solutions that individuals, mentors, professional societies, funding agencies, and institutions should undertake to move toward a more diverse oceanographic community.

Tracking symbiont uptake rates and immunity in a model anemone *Exaiptasia diaphana*

Peyton Abdelbaki, Lauren Fuess, Erin Borbee

Coral bleaching events are threatening coral reefs across the globe. In the absence of stress corals have the ability to recover from these events by reacquiring their algal symbionts. Recent data has shown that the onset of this symbiosis however comes at the cost of suppressed host immunity. In order to better understand this process we used an anemone model system, *Exaiptasia diaphana*, to investigate uptake rates of algal symbionts. To do this

we started with four clonal lines of bleached anemones and inoculated each with their homologous symbiont (i.e. the species of symbiont they are naturally found with) and a heat tolerant symbiont species. On a weekly basis, we excised tentacles from the anemones and tracked chlorophyll a fluorescence to monitor the rate of symbiont uptake in each clonal line. At the end of the experiment the anemones were preserved and processed for a standard suite of immune assays. These immune assays allow us to characterize important pieces of the cnidarian immune systems including catalase, phenoloxidase, and antimicrobial activity, as well as melanin concentration. The results from this study will be used to further develop our overall understanding of coral-algal symbiosis and will inform restoration efforts for coral reefs.

The Developmental Genetic Basis of Appendage Patterning in the Brown Garden Snail, *Cornu aspersum*

Kaitlyn Abshire, Ethan Laumer, Sophie Neu, Charlotte Wood, Prashant Sharma

Mollusks are one of the most morphologically diverse clades of metazoans, exhibiting an immense diversification of evolutionary novelties. One innovation explored recently in mollusks has been the derivation of limb axes from the foot (arms and tentacles) of cephalopods, exemplifying cooption of limb axis patterning genes. However, the developmental genetic basis underlying appendages in other mollusk classes has not been explored. To begin investigating the development of the ommatophore (eye stalk) and rhinophore (olfactory tentacle) of air-breathing terrestrial gastropods (Superorder Eupulmonata) we established modern genomic resources for the brown garden snail, *Cornu aspersum* (Müller, 1774), spanning a developmental transcriptome, a draft genome, complete embryonic staging system, and protocols for gene expression assays. We employed these tools to survey a suite of limb axis patterning genes that have been well-characterized in cephalopod, arthropod, and vertebrate literature. Here, we show that a subset of limb patterning genes is comparably expressed in the appendage primordia of gastropods and outgroup taxa. By contrast, other members of this suite of genes exhibit evolutionary lability. Taken together, these data suggest another case of cooption of limb patterning genes in the appendage primordia of Eupulmonata. Future investigations of appendage development in gastropods must emphasize the establishment of functional toolkits in *C. aspersum* with the goal of linking changes in gene expression domains with phenotypic divergence and specialization of appendages.

A molecular evolution facilitates nematode parasitism of prey carrying toxic cardiac glycosides

Perla Achi, Simon Groen, Adler Dillman

Target-site insensitivity (TSI) is an important mechanism of animal resistance to natural and man-made toxins. TSI evolved in parallel in the monarch butterfly and other insects that specialize on milkweeds and is thought to have facilitated sequestration of cardiac glycosides (CGs) that may protect these insects from predation and parasitism. Substitution N122H in the CG-binding pocket of the molecular target, the Na⁺/K⁺-ATPase alpha subunit (ATPα), strongly enhances TSI and evolved in parallel in CG-sequestering insects across six orders. Upon performing a genetic screen of the Na⁺/K⁺-ATPase, we recently identified N122H in the entomopathogenic nematode (EPN) *Steinernema carpocapsae*, which parasitizes insects around milkweeds. This sets up the possibility that parallel evolution of N122H may not only have facilitated CG sequestration by insects, but also at the third trophic level for nematode parasitism of CG-carrying insects. Here, we show that N122H is rare among nematodes and that, among species tested for CG tolerance, *S. carpocapsae* showed significantly stronger insensitivity to diverse CGs than nematodes without N122H, including free-living *Caenorhabditis elegans* and parasites of milkweed roots. CRISPR gene editing in *C. elegans* showed N122H is sufficient for overcoming toxicity of CG levels found in sequestering insects. However, N122H was accompanied by costs related to nervous system robustness, potentially explaining its rarity among nematodes. Finally, *S. carpocapsae* was the only EPN tested that was highly successful at infecting CG-carrying insects

and that displayed attraction to CGs. Taken together, our results suggest that a molecular evolutionary cascade of parallel substitutions across hosts and parasites, last sharing common ancestry 600 million years ago, may shape multitrophic interactions across plant communities.

Evaluating the Impacts of Urbanization: Are Deer Mice Stressed?

Lauren Acuff, Elizabeth Addis, Louis Nipp

Urbanization is a global phenomenon that can cause dramatic habitat changes. Because rates of urbanization are increasing, the need to understand how organisms respond to urbanization induced habitat is paramount. We investigated if urbanization can create stressful environments for animals. We selected *Peromyscus maniculatus* (the Western deer mouse) as our model organism because they are widely distributed in urban and rural areas. For our study, deer mice were trapped along a gradient ranging from urban to rural. For each mouse, we collected fecal samples prior to the release of glucocorticoid metabolites (GCMs) levels induced by trapping, and again succeeding the release of GCMs (less than four hours and more than six hours, respectively). We used the amount of GCMs in the feces as an indicator of stress. To assess if habitat structure varies among urban and rural sites, and if it impacts deer mouse stress, we calculated vegetation cover and the percent of organic litter at each trap site. We hypothesized that urban populations will have higher baseline GCMs levels than rural populations, but trapped induced GCMs levels will be higher than baseline GCMs levels for both mouse populations. We also hypothesized that habitat structure will differ between urban and rural locations and correlate with GCMs levels in mouse populations. Our results from this study will be reviewed in this poster.

Does isotocin mediate fear learning in mangrove rivulus fish?

Riley Adam, Elayna Griffaw, Gemma Hodgkiss, Zara Jadol, Mary Saylor, Maddie Wieringa, Ryan Earley

Oxytocin and the fish homologue, isotocin, are nonapeptide neurochemicals that have evolved to serve multiple functions within the brain and periphery, including responses to social stress and mediation of social behavior, particularly affiliation. Interfering with nonapeptide signaling alters animals' emotional recognition as well as their abilities to make sense of context clues and process cues in cases of multi-stimulus learning. We designed an experiment to test the hypothesis that, in mangrove rivulus fish, isotocin signaling would affect performance in multi-stimulus fear learning tasks. Isotocin signaling was elevated (isotocin administration), attenuated (isotocin receptor antagonism) or left unmanipulated (control). Subjects in each treatment were first challenged to learn the association between light and shock. After 5-10 associative learning trials, we recorded each subjects' behavior in 5-10 additional tests where only light was presented (no shock). Rates of fear learning and extinction were measured across the respective trials, and indicated by both the latency to respond after presentation of light stimulus and type of response (e.g., time to freeze or flee). We predicted that rivulus with less circulating isotocin would take longer to learn and perhaps longer to extinguish what was learned. We are still in the process of collecting data but will present initial findings on the role of isotocin in governing the extent to which animals learn about potentially dangerous stimuli.

Stiffness and density of the skull and mandible across cetaceans and artiodactyls

Danielle Adams, Brad Boyce, Daniel Hooks, Benjamin Klitsner, Kevin Garber, Sam Price, Richard Blob

Reentering aquatic habitats involved drastic evolutionary changes in the physiology and morphology of whales and dolphins (Cetacea) compared to their terrestrial relatives (Artiodactyla). Properties of the skull and mandible, including density and material stiffness, are critically important for understanding mechanical loads associated with the evolution of feeding and sound reception in cetaceans. However, the extent of variation in the material properties of bone within and between vertebrate taxa is unclear. To evaluate bone material properties across cetaceans and their close terrestrial relatives, we sampled density and stiffness at five locations across the skull and mandible.

Bone mineral density was tested using microCT and bone sample stiffness was measured using nanoindentation. Nanoindentation was performed in both air and liquid (reflecting the naturally hydrated state of bone in vivo) to test for possible stiffness differences between different methodologies. The stiffness values we obtained were consistent with previously measured values from mammalian bone. Within the skull of both cetaceans and artiodactyls, stiffness differed along the anterior-posterior axis, with the anterior rostrum and mandible showing lower stiffness values than the posterior bones. This result likely reflects functional differences between these elements. These data contribute to a wider understanding of the evolution of bone material properties within and between vertebrate taxa and, specifically, how this variation might contribute to functional specialization for different habitats and life habits within Cetartiodactyla.

How terrain impacts activity and energetics of salamanders and lizards in mechanistic niche models

David Adams, Eric Riddell

Predicting organismal responses to changing conditions is crucial for understanding how species may be affected by climate change. By linking microclimates to an organism's physiology and behavior, mechanistic niche models serve as powerful tools for predicting the ecological impact of climate change. Mechanistic niche models are dependent on calculations of microclimates, which can be complex in forested, mountainous, and heterogeneous environments. We developed an algorithm to adjust solar radiation calculations for various effects of topography including self-shading, topographic shading, and the angle of incidence based on slope and aspect of the terrain. Then, the terrain algorithm was incorporated into mechanistic models that predicted operative temperatures, metabolic costs, and activity for lizards and salamanders under current and future climate conditions. The overall effects on model predictions were more pronounced for lizards in Death Valley compared to salamanders in the Appalachians. However, for both lizards and salamanders, including terrain corrections generally decreased predicted metabolic costs on northern slopes (-4.5% and -2.7%, respectively) and increased predicted costs on southern slopes (3.8% and 1.5%, respectively). Changes in activity were not strongly tied to aspect, with varying effects for lizards (-18.6% to 28.6%), while having no impact on salamanders. Our work emphasizes the context-dependence of topography in mechanistic niche models, offering critical insight into the role of topographic shading in driving ecological responses to climate change.

If the Shoe Fits: Strike Responses of the Round Stingray (*U. halleri*) to Different Human Foot Sizes

Hanna Adamson, Benjamin Perlman

Injuries from stingray strikes are commonly experienced by beachgoers as stingray habitat often overlaps with human recreation zones. Southern California is known for its stingray populations and associated human injuries, many of which are caused by Haller's round ray (*Urobatis halleri*), one of the most abundant species of stingrays in Southern California. The severity of injury resulting from a stingray strike can vary greatly and may be influenced by many factors. We previously studied the tail kinematics of *U. halleri* striking in response to stepping on them with a pseudo-cadaveric force application device (FAD), representing a human foot stepping on a stingray in situ. Our goal here was to assess how the strike response might change with scaled versions of our FAD, representing different sized human feet when stepping on rays. Our data using the adult-sized FAD indicated that rays escaped most of the time when stepped on a body region other than its midbody, and were only likely to strike when pinned down at the center of their dorsal surface. With smaller and medium-sized FADs, there may be a lower chance of pinning down the ray, so we predicted to see an increase in the frequency of escape attempts. This would suggest that people with larger feet might be at risk for more severe injury than those with smaller feet.

Population Genomics of the Endangered Freshwater Mussel, *Arcidens wheeleri*, in the Little River, AR

Mia Adcock, Nathan Whelan, Samantha Donohoo, Kendall Moles, Nicole Garrison

Over the last century, many anthropogenic stressors have led to substantial declines of freshwater mussels globally. The Little River in Arkansas is home to one of the last standing populations of the federally endangered freshwater mussel, *Arcidens wheeleri* (Ouachita Rock Pocketbook). Population demographic information is lacking for *A. wheeleri*, and no population genetic study has been conducted on this species. A greater understanding of the genetic diversity within a population can serve as a benchmark for developing an effective management plan. We sampled *A. wheeleri* from three locations in the Little River: upstream, middle, and downstream. Genomic data were generated with a single-enzyme restriction-site associated DNA sequencing (RADseq) approach to assess genetic diversity and structure of *A. wheeleri* in the Little River. ADMIXTURE analyses indicated one genetic cluster among the three locations, with no genetic structure or sub-populations. Observed heterozygosity values were lower than expected heterozygosity values, with $H_o = 0.14$ and $H_e = 0.22$, indicating a genetic bottleneck. Coalescent-based demographic analyses also suggest a historical bottleneck but fails to recover any signal of recent population decline. The survival of *A. wheeleri* may be at high risk of extinction because of high inbreeding rates and a loss of genetic diversity. With little knowledge of this species, our study provides valuable information about the basic biology of *A. wheeleri* and for informing management plans.

Tolerance of infection could support resilience at multiple levels

James Adelman

Tolerance of infection, or the ability to minimize the fitness losses associated with a given number of parasites, is increasingly recognized as a critical host strategy for combatting infectious diseases. In animals, tolerance is typically considered and measured during active infection, but it likely has important consequences for post-infection performance and fitness, or individual resilience. Moreover, the evolution of tolerance in the face of emerging infectious diseases provides one potential mechanism of population-, species-, or community-level resilience following novel epizootics. In this talk, I will briefly review recent progress in our understanding of tolerance, with particular focus on house finches and their bacterial pathogen *Mycoplasma gallisepticum*, which emerged from domestic poultry in the early 1990s. I will explore the evolution of tolerance, its potential mechanisms, and behavioral and physiological links to resilience, both at individual- and population-levels, highlighting productive avenues for future research.

Optimal foraging height of arboreal lizards: the role of visual acuity

Stephen Adolph, Deniz Korman, Brennan Plassmeyer

Many lizards are arboreal sit-and-wait predators that forage from elevated perches on trees and other habitat features. We modeled how foraging success (expected daily energy intake) would vary with perch height, and therefore predicted optimal foraging heights for lizards of different body sizes. Our model focuses on lizards that eat small arthropod prey, using typical prey size distributions from field studies. We assume that visual acuity scales with lizard body size based on the allometry of eye size. We also assume that all prey are taken from the ground. The model finds that optimal foraging height increases with lizard body size, due to the allometry of visual acuity. This result is consistent with some commonly observed patterns of perch heights in *Anolis* and *Sceloporus* lizards, in which larger species (or individuals within species) choose higher perches. Our results suggest that limitations of visual acuity, combined with the small sizes of potential prey items, could contribute to interspecific and intraspecific partitioning of perch heights via optimal foraging mechanisms. Our model also finds that visual acuity may be more important than energetic considerations in determining threshold distances at which lizards will no longer pursue prey.

Architectures and key attributes of wing neuromechanical system of natural fliers

Suyash Agrawal, Christopher Rahn, Md Zafar Anwar, Bo Cheng

Flapping flight in natural fliers emerges from complex interactions among a collection of coupled neural and mechanical components in their flight apparatus that generates and controls the motion of wings. Here we survey and synthesize the literature on the wing neuromechanical systems in locusts, flies, hawkmoths, and hummingbirds, with the aim of generating insights regarding wing motor control in natural fliers and their implications on bioinspired robotic flight. We propose that there are two fundamental architectures of wing motor system, i.e., Coupled Neural Oscillator and Mechanical Oscillator (Coupled NO-MO), and Single NeuroMechanical Oscillator (Single NMO), that correspond to natural fliers with synchronous and asynchronous power muscles, respectively. These architectures help us to further understand the separation of power and control, separation of central and peripheral control, emergence of flapping frequency or rhythm, the synchronization of motion, etc. Further, we propose a framework that centers on the effects of body size and the contributors to maneuverability, which can be applied to integrate and draw insights on the key attributes such as tonic vs phasic muscle force production, precise timing of muscle activation, the role of proprioceptive feedback, force vectoring capacity, single vs multiple power and control actuation. Lastly, we discuss the attributes of the wing neuromechanical systems in natural fliers that are and are not conducive to emulation in robotic flight.

How do circulating energy metabolites relate to variation in aggression?

Elizabeth Aguilar, Emily Levy, Kimberly Rosvall

Energy is the currency that fuels all biological functions, including behavior. Aggression, for example, often involves physical exertion that can trigger metabolic changes in the brain. For tree swallows (*Tachycineta bicolor*), aggressive interactions usually occur in flight, and more aggressive females are better able to secure a nesting site. We hypothesized that energetic metabolites, such as triglycerides and glucose, function to facilitate aggression during competition. First, using a correlative approach, we asked whether consistent individual differences in aggression relate to metabolic energy sources, predicting that highly aggressive birds would have higher metabolite levels in circulation. Second, we used an experimental approach, asking whether induced competition promotes higher metabolite levels to meet the energetic needs of a social challenge. For both approaches, we captured females, collected blood, and measured triglyceride and glucose levels in plasma using point-of-care handheld meters. Contrary to our predictions, these metabolites did not relate to individual variation in aggression, nor did they differ among control and socially challenged birds. However, triglycerides were remarkably variable among individuals. Future work should investigate causes or consequences of this variation, including the potential role of tissue-specific storage, production, and use of energetic metabolites. Together, these efforts will integrate metabolic perspectives into an ecological and evolutionary framework.

On the demographic history of the Western European house mouse, *Mus musculus domesticus*

Kennedy Agwamba

Human commensal, *Mus mus domesticus*, is native to the European continent, with the subspecies' range extending from the Middle East to western Europe by the end of the Iron Age. Wild populations of *M. m. domesticus* are now distributed across Africa, the Americas, and Oceania, a range notably consistent with the global migration patterns of western Europeans that began in the early 16th century. Despite its standing as the premier mammalian model organism for biomedical, ecological, and evolutionary research, important details surrounding the population history of wild house mice remain a mystery. To investigate patterns of genetic structure and infer the demographic history of the Western European house mice, *M. m. domesticus*, we analyze a collection of 183 house mice sampled from western Europe and the Americas, including 59 new whole genome sequences from historically relevant regions of western Europe. Principal component analysis clusters samples by geographic location, uniquely identifying a northern European,

Mediterranean, and Iberian population clade amongst our western European samples. Admixture graphs reveal the Iberian clade to be sister to all populations of house mice in the Americas, and a migration edge from the UK to the base of the North America clade indicates a distinct secondary introduction of house mice to the Americas. Demographic models reveal American populations diverged largely within the last 500 years, consistent with the timing of European colonization history in the America. Altogether, these results provide clarity around the recent introduction of Western European house mice to the America, highlighting the effects of human migration and global colonization on the concurrent spread of an invasive human commensal.

Effects of short-term exposure to pesticides mixture on tissue architecture in the American oyster

Asif Ahmed, MD Rahman

Pesticides are used widely to control weeds and pests in agricultural settings but harm non-target aquatic organisms. Marine bivalves are a prominent seafood and animal protein source for people worldwide, including the United States. In this study, we examined the effect of short-term exposure (one week) to pesticide mixtures (low dose: 0.5 mg/L Roundup®, 0.5 mg/L 2,4-D, and 0.4 mg/L atrazine; high dose: 1 mg/L Roundup, 1 mg/L 2,4-D, and 0.8 mg/L atrazine) of American oyster (*Crassostrea virginica*, an important shellfish and seafood species) under controlled laboratory conditions. Histological analyses demonstrated the atrophy in the gills and digestive glands of oysters. The pH levels of extrapallial fluid remained unchanged, although the protein concentrations dropped significantly in the high-dose treatment group. The number of hemocytes in connective tissue increased in low- and high-dose treatment groups. However, pesticide treatments decreased the amount of mucous in the digestive glands. Our results suggest that exposure to pesticide mixtures causes morphological changes in oysters' tissues and alters body fluid conditions, which may lead to impaired physiological functions in oysters.

Keywords: environmental chemicals, oyster

Cryptic coral diversity shapes bleaching patterns in Bocas del Toro, Panama

Hannah Aichelman, Carsten Grupstra, Annabel Hughes, Viviana Guerra, Sarah Davies

There is a growing appreciation for cryptic coral lineages—genetically distinct yet visually similar groups that have been identified in at least 23 coral genera, including many abundant Caribbean reef builders. These lineages often exhibit functional diversity, including variation in their ability to cope with warmer oceans. We have previously identified at least three cryptic lineages of *Siderastrea siderea* in Bocas del Toro, Panamá that differ in their distributions (across an inshore-offshore and depth gradient), algal symbiont associations, skeletal light scattering abilities, and thermal tolerance under experimental heat challenge. Together with differences in historical growth patterns, these findings suggest that these lineages are ecologically specialized. Analysis of bleaching severity and frequency in tagged colonies during the 2023 thermal anomaly suggests that in situ bleaching tolerance is shaped by a combination of cryptic lineages and environmental history. This work illustrates that failure to recognize the different susceptibilities of cryptic lineages to climate change stressors can underestimate threats to local populations, and lead to unknown biodiversity losses during coral bleaching events.

The evolution of distinct flight strategies in bombycoid moths and the development of a model clade

Brett Aiello, Usama Sikandar, Sarah Maccarelli, Katie Pfuhl, Nicole Mason, Jared Johnson, Joanna Baker, Ethan Wold, Leo Wood, Chris Hamilton, Milton Tan, Akito Kawahara, Simon Sponberg

Physiological and mechanical systems interact together with the environment to generate locomotion. A robust understanding of how these processes interact to generate locomotion across species can be bolstered through the use of a model clade, leveraging natural diversity. However, a model clade needs closely related species sharing general morphology and sensorimotor systems with demonstrated diversification of the locomotor system. Here

we identify Bombycoidea, a diverse moth superfamily, as a model clade. Bombycoidea includes hawkmoths and silkmoths, sister clades divergent in life history. The integration of morphometrics, kinematics, and aerodynamic modeling reveals two distinct flight strategies also evolved between these clades. Hawkmoths evolved smaller wings shaped favorably for power reduction and use high frequency wing beats to complete rapid maneuvers. Silkmoths evolved larger wings shaped favorably for maneuverability and reduce power using slow high-amplitude wing strokes. The rapid divergence in flight strategy between these clades is best explained by an adaptive shift and the subsequent diversification of traits within each clade along distinct trajectories. Bombycoidea thus facilitates the examination of how the multitude of variables contributing to flight performance evolve relative to interspecific differences in flight strategy and life history. We end by integrating results on bombycoid motor control, thoracic mechanics, flight myology, body kinematics, and wing stiffness to demonstrate how these different determinants of flight performance diverge or remain conserved across these diverse, agile organisms.

100-fold increase in brain size during through development in the white-lined sphinx moth (Hyles lin)

Isabel Aksamit, Felipe Dorigão-Guimarães, Wulfila Gronenberg, R Keating Godfrey

The larval brain of holometabolous insects contains cells differentiated for larval function and those arrested in embryonic form for the development of the adult brain during metamorphosis. While factors regulating larval growth and determinants of adult body size are described for several insects, less is known about brain size scaling through development. Here we use the isotropic fractionation ("brain soup") method to estimate the number of brain cells and cell density for the white-lined sphinx moth (Lepidoptera: Hyles lineata) from the first instar through the adult stage. We measure mass and brain cell number through development and find that, while body mass shows an exponential relationship with head width, the total number of brain cells increases logarithmically during the larval stages. Larval brain cell number increases by a factor of ten from 8,002 (sd = 543) in the first instar to 81,806 (sd = 6,563) in the fifth instar. The brain increases by another factor of 10 during metamorphosis, with the adult brain containing 914,816 (sd = 56,334) cells. This is generally consistent with developmental studies of the vinegar fly (*Drosophila melanogaster*) and the black soldier fly (*Hermetia illucens*). Furthermore, adults show slightly smaller brain-to-body mass ratios than wasps or bees of a similar body size, but brain cell density is comparable, indicating a high level of conservation in cell density across these divergent lineages.

Luxury effects in NYC: Socioeconomic effects on biodiversity across taxa

Valentina Alaasam, Rafael Baez-Segui, Emerald Lin, Gaia Rueda-Moreno, Kristin Winchell

Urban wildlife worldwide are facing similar environmental challenges. Increases in noise, light at night, heavy metals, non-native species, heat island effects, and impervious surfaces, result in spatially distant cities often resembling each other more than they do the surrounding natural habitat. However, within urban landscapes there is also relatively unexplored heterogeneity. The size, connectivity, and general maintenance of green spaces are unevenly distributed and known to covary with socioeconomic variables such as household income and historical redlining. The "Luxury Effect" describes a phenomenon where biodiversity is positively correlated with household income. To date, the strongest support for the Luxury Effect comes from arid regions, where wealthier neighborhoods that use more water benefit from a subsequent richness of plant communities. Here, we investigate whether there is support for the Luxury Effect across taxa in New York City. We conducted surveys of birds, insects, and amphibians across green spaces managed by New York City Parks in Manhattan and the Bronx. We predicted that surrounding household income levels and historical redlining would predict species biodiversity, but that the direction of the effect would vary across taxonomic group. We compare our results with biodiversity predictions based on iNaturalist and

eBird data to investigate potential biases in user demographics. Results shed light on how socioeconomic heterogeneity in cities can lead to the reorganization of animal communities.

Phylogeny and Diversification of Ancient New Zealand Mite Harvesters

Zade Alafanji, Peter Aspholm, Haley Heine, Rina Morisawa, Phoebe Fu, Nathaniel Moyes, Shanta Hejmadi, Shahan Derkarabetian, Sarah Boyer

New Zealand is home to a remarkable number of endemic taxa. Some of these taxa existed on the archipelago before the breakup of Gondwana. The mite harvesters (suborder Cyphophthalmi), tiny arachnids which dwell in forest leaf litter and caves, are one such group. The mite harvester family Pettalidae exhibits a classic Gondwanan distribution, and has proven informative for describing ancient patterns of diversification throughout the last hundreds of millions of years. Within New Zealand, there are three genera of pettalids; our research focuses on the phylogeny of the most widespread and diverse of these: Rakaia. We generated a dataset of hundreds of loci through hybrid target enrichment of ultraconserved elements. We conducted a phylogenetic analysis of this dataset for specimens collected across New Zealand's North Island, South Island, and Stewart Island. Furthermore, a fossil-calibrated molecular clock approach was used to calculate approximate divergence dates across Rakaia. This study provides a window into patterns of ancient diversification, and is informative for describing historical biogeographic trends within New Zealand. Future goals of the study include elucidating undescribed species of Rakaia, and confirming the suspected ancient origins of the genus.

The effect of female mate choice on offspring sex ratios in a freshwater amphipod species

Joseph Alcuitas, Rachel Uhlig, Rickey Cothran

Theory predicts even sex ratios because parents always benefit from producing the rarer sex. However, deviations in nature may occur to maximize reproductive success. On an individual brood basis, females may allocate resources to the sex that returns the most fitness. We hypothesized that choosy females bias sex ratio of broods in favor of sons. Amphipods (*Hyalella* sp.) were used as it is possible to identify chosen mates because they physically pair for a short period before copulation. We collected mating pairs and a background sample of amphipods from a freshwater spring. Half of the females were separated and re-paired with their original mate—i.e. they chose their mate. The other half of the females were separated and assigned a new male randomly from the background sample. Each pair was placed in a jar inside of an environmental chamber. We checked the pairs three times weekly and recorded offspring sex ratios. As predicted, choosy females produced a higher proportion of sons in their broods than females mated at random. This strategy allows females to maximize fitness by investing more heavily in sons when mating with a high quality male.

Do social interactions influence testosterone levels in the red-capped manakin?

Camilo Alfonso, Amalia Moore

The original Challenge Hypothesis proposed that variability in circulating testosterone levels can be attributed to social challenges. These challenges, such as male-male aggressive encounters, can increase testosterone and help males gain access to mating opportunities. In addition, the Challenge Hypothesis also proposes that male-female interactions related to mating opportunities can influence testosterone levels because the hormone can enhance mating performance in males. Most tests of the Challenge Hypothesis have been conducted on male-male aggressive interactions of species that are socially monogamous and where males provide parental care. In this study, we aimed to test the Challenge Hypothesis in a socially polygynous bird lacking male parental care. We study the red-capped manakin (*Ceratopipra mentalis*) in the tropical rainforest of Gamboa, Panama. Males of this species perform elaborate display behaviors within leks, competing for mating opportunities with other males. Our primary focus was to investigate how males respond hormonally and behaviorally to both male-male and male-female social interactions. We

employed taxidermic male and female decoys to simulate territorial intrusions and mating opportunities, respectively. We recorded the behaviors exhibited and measured plasma testosterone levels immediately after capture. Our aims were to enhance our understanding of the Challenge Hypothesis, particularly in the context of understudied mating systems and to motivate fellow biologists to investigate tropical species, exploring established ideas with new perspectives gained from studying understudied species.

Morphology and transcriptomics illuminate silk adhesion to water by the spider *Wendilgarda clara*

Angela Alicea-Serrano, Todd Blackledge, Ali Dhinojwala, Jessica Garb

Life at the air-water interface is a fundamental challenge for small animals. Remarkably, the spider *Wendilgarda clara* uses a reduced orb web anchored to the water surface of streams to catch floating prey. Neither the mechanisms of water attachment and prey capture, nor the material used to make these webs is known. This web potentially requires unique attachment disc architecture and chemistry for water anchoring and tuning of silk properties through the evolution of novel proteins. We investigated how *Wendilgarda* webs attach to water through silk chemistry and spigot morphology. We imaged spinneret morphology using SEM to test whether *Wendilgarda* trades off allocation of piriform attachment silk from many thin fibers to fewer longer fibers, increasing drag on water surfaces. We also sequenced mRNA from *W. clara* silk glands to determine the abundance of silk protein transcripts in different silk glands. We did this to understand how *Wendilgarda* silk proteins evolved to meet new mechanical demands of their semi-aquatic environment. "Reduced webs" in challenging habitats illuminate how natural selection can functionalize silks for different applications. The work will advance knowledge of the functional links between silk structure, mechanics, and evolutionary ecology, and will expand the range of possible silk applications from single threads to thread networks, as well as universal glues that could stick even to the surface of water.

Function of FGF signaling in neural and mesodermal specification in the annelid *Capitella teleta*

Tessa Allan, Neva Meyer

During early development of bilaterians, tissue is specified into mesoderm, endoderm, and ectoderm, which is critical for later development and body plan formation. Based on animal phylogenies, the last common ancestor of Bilateria likely had mesoderm. However, the molecular mechanisms controlling mesoderm formation differ across bilaterian clades. One recurring theme is the involvement of fibroblast growth factor (FGF) signaling, particularly via activation of the intracellular RAS/MAP kinase pathway. In *Spiralia*, FGF/MAPK signaling has been found to be essential for mesoderm development as well as gastrulation and axial elongation. Previous preliminary experiments in the spiralian annelid *C. teleta* demonstrated a loss of posterior trunk tissue with the pharmacological inhibition of FGF/MAPK signaling, indicating that FGF signaling may take on a similar function in the specification of mesodermal tissue in *C. teleta*. Thus, we hypothesize that the specification of mesodermal tissue is induced by FGF signaling via the MAPK pathway in *C. teleta* and that this mechanism is conserved across *Spiralia*. To investigate this, pharmacological inhibition of FGF using SU5402 was performed on embryos at varying developmental stages. We found that there was an observable loss of posterior tissue and a decrease in the expression of mesodermal markers. Taken together, this indicates that FGF/MAPK signaling is necessary for mesoderm development in *C. teleta* and that this mechanism may be conserved within *Spiralia*.

How octopuses process black, white, and light we can't see

Angelique Allen, Judit Pungor, Cristopher Niell

Cephalopods have a complex visual system, with camera-type eyes that resemble our own and a large brain. This system supports a wide array of visually guided behaviors such as prey capture, predator avoidance, and camouflage. Most of our understanding of neural function in the cephalopod visual system comes from studies investigating retinal responses, leaving many unknowns in how light information is processed, where throughout the brain this information is distributed, and ultimately how this gives rise to behavioral

responses. To investigate how the octopus brain encodes different aspects of the visual scene we are using two-photon calcium imaging to record large-scale organization of neural activity in the optic lobe of *Octopus bimaculoides*. By presenting a variety of luminance-based stimuli we discovered localized receptive fields, retinotopic organization, and ON and OFF processing pathways. To further understand how octopuses process an aspect of the visual scene that is not visible to most vertebrates we also recorded while presenting stimuli that are defined by the polarization of light. These results suggest that there are distinct yet overlapping spatial patterns of activation for each light characteristic. In order to further investigate the neural encoding of polarization processing, we aim to image single-cell response properties to polarization or luminance stimuli in the optic lobe.

Biologgers reveal physiology and behavior trade-offs under immune challenge in a free-ranging mammal

Austin Allison, Helen Chmura, Cory Williams

Immune responses against pathogens undoubtedly benefit animals via increased short-term survival. However, mounting an immune response is energetically costly. Hence, life-history theory predicts sickness behavior alongside physiological immune responses to compensate for the increased energetic output required to fend off an immune challenge. Collecting detailed individual-level data to test this prediction in free-ranging animals was difficult until recent advances in biologging made such field studies possible. We surgically implanted heart rate and temperature loggers in free-ranging adult male Arctic ground squirrels and fitted the squirrels with collars equipped with accelerometers and light loggers to simultaneously measure physiological and behavioral responses to immune challenge. We experimentally induced an immune response in the squirrels via intraperitoneal injections of lipopolysaccharide (LPS). Squirrels injected with LPS exhibited significantly elevated heart rates (20.2 bpm greater) and body temperatures (0.7 °C greater) compared to squirrels injected with saline during the night following injection, physiological evidence of an activated immune response. LPS-injected squirrels also spent 19.0% less time above ground and had a 42.0% lower overall dynamic body acceleration (a measure of activity-specific energetic output) than saline-injected squirrels during the day following injection, as predicted by life-history theory. Thus, the novel combination of biologgers we deployed on free-ranging squirrels allowed us to detect a trade-off between energetic allocation to physiological responses and activity under immune challenge.

Altitudinal Adaptations: Exploring Ecophysiology Diversity in *Plethodon* Salamanders

Nathalie Alomar, Eric Riddell, Martha Munoz

Woodland salamanders (genus: *Plethodon*) reside in the North American Appalachian Mountains, the global hotspot for salamander biodiversity. The complex is broken up into three sublineages: the slimy salamander (*P. glutinosus* complex), the red-backed salamander (*P. cinereus* complex), and the *P. welleri/wehrlei* complex. Within these sublineages their elevation ranges vary; some species are restricted to narrow elevational ranges while others span a wide elevational gradient. As lungless, terrestrial, amphibians considering how their physiology reflects these elevational conditions could provide insight into the diversification of this clade. For example, those found in high elevations may have lower resistance to water loss rates, lower metabolism, and be more cool-adapted compared to those in low elevations. We tested this hypothesis by collecting species across the three different sublineages from elevations ranging from 200m to 1600m. In the lab, we collected each species' resistance to water loss rates, metabolic rates, critical thermal maximum, minimum, and thermal preference. Preliminary results show how some, but not all, physiological traits are shown to adapt to species' elevation. This shows how *Plethodon* salamanders may adapt to mountainous habitats through physiological responses, enhancing our understanding of their ecophysiological diversity.

Restraint stress rapidly impacts reproductive neuroendocrinology and gonads of big brown bats

Mattina Alonge, Lucas Greville, Xuehao Ma, Paul Faure, George Bentley

Animals face unpredictable challenges that require rapid, facultative physiological reactions that support survival but may compromise reproduction. Bats have a reputation for being highly sensitive to stressors, yet little is known about how stress affects the signaling regulating reproductive physiology. Here we provide the first description of the neuroendocrine and gonadal response within the hypothalamic-pituitary-gonadal (HPG) axis of male big brown bats (*Eptesicus fuscus*) in response to acute stress using 1hr restraint. This stressor was sufficient to upregulate plasma corticosterone and resulted in a decrease in circulating testosterone after 1hr. While we did not find differences in the mRNA expression of key steroidogenic enzymes (StAR, aromatase, 5-alpha reductase), seminiferous tubule diameter was reduced in stressed bats coupled with a 5-fold increase in glucocorticoid receptor (GR) mRNA expression in the testes. These changes, in part, may be mediated by RFamide-related peptide (RFRP) because fewer immunoreactive cell bodies were detected in the brains of stressed bats compared to controls—suggesting possible secretion—and increased RFRP expression locally in the gonads. The rapid sensitivity of the testes to stress may indicate deleterious impacts on tissue function, supported by significant transcriptional upregulation of key pro-apoptotic signaling molecules (Bax, cytochrome-c). These data contribute to our understanding of the rapid impacts of stress in bats which may impact decisions surrounding animal handling and conservation approaches.

Proliferative abilities of saccular hair cells in Atlantic Croaker after exposure to noise

Ariel Alonso, Kelly Boyle

Anthropogenic noise is a growing threat to marine animals such as fish which use hearing to detect predators, hunt for food, and detect spawning calls. Sensory hair cell proliferation (addition of hair cells by growth) and regeneration (addition following noise damage) in the ear are documented from relatively few teleost species. In this study, we aim to determine if Atlantic Croaker (*Micropogonias undulatus*) saccular hair cells proliferate and regenerate after noise exposure. We conducted laboratory experiments to test for hair cell proliferation and regeneration following exposure to 36 hours of white noise (150 dB re: 1μPa). We used *in vivo* injections of BrdU (Bromodeoxyuridine) to assess cell proliferation with immunohistochemistry. We will test for differences between cell proliferation rates among a control group and four recovery period treatments (0, 2, 4, and 6 days) following noise exposure. Preliminary results indicate that hair cell proliferation occurs in both control group and noise-exposed group fish: 144 + 162 (mean + SD) proliferated cells per mm² for noise-exposed fish and 93 + 92 (mean + SD) under control conditions. The analysis (in progress) will determine if regeneration occurs after noise exposure and if so, the timing of peak regeneration. Regeneration of damaged saccular hair cells may be important to maintain hearing and vestibular senses and thus has important fitness consequences for soniferous species like Atlantic Croaker.

Age and growth characteristics of smallmouth basses exhibit multiple responses to a thermal gradient

Mitdalia Alonso, Michael Newbrey, Ashley Desensi, Jennifer Newbrey

The effects of a thermal gradient on the age and growth characteristics of the Smallmouth Bass (*Micropterus dolomieu*) species complex are poorly understood. Therefore, we described the relationships between age and growth characteristics and cold, average, and hot thermal gradients to identify variability in the data, which would suggest a multispecies response. Specifically, we examined relationships among mean annual temperature (MAT) at 24 hr, maximum MAT, and minimum MAT at ages 3, 5, and 8 years old and longevity for 53 populations of Smallmouth Bass from published literature. The Smallmouth Bass species complex ranges from southern Ontario/Quebec to Oklahoma/Georgia, USA, so the thermal gradient data examined ranged from 5.5 MAT_{24hr} °C-15.9 MAT_{24hr} °C. We also examined total length for 39 state records of Smallmouth Bass from 3.7 MAT_{24hr} °C-21.2 MAT_{24hr} °C. We

found significant positive relationships among MAT24hr and TL3,5,8, among MATMax and TL3,5,8, and among MATMin and TL5,8. An analysis of state records showed significant positive relationships among MAT (minimum and 24 hr) and ultimate TL. Most analyses exhibited a similar response pattern, suggesting an overlap in age and growth characteristics of all species in the complex. However, the spread of the data for MTL for each population was in the shape of a triangle, and an overlay of locations showed geographic patterns, suggestive of a multispecies response (northern and southeast/south central).

Serotonin and Its Effects on the Chronic Stress Response in Crayfish Following Nonylphenol Exposure

Nina Alpers, Daniel Bergman, Tara Olen, Madelyn Mauer, Abby Niessink

Crayfish are a well-studied neurophysiological model and consequently are used to examine the mechanistic causes of neurophysiological stress. One potential stressor for aquatic species is nonylphenol, a hydrophobic chemical widely used in agricultural products and detergents that can pollute rivers, streams, and lakes. Nonylphenol is a known endocrine disruptor and can lead to physiological and behavioral impairments in crayfish. Long-term non-lethal exposure to nonylphenol likely induces chronic anxiety-like stress in crayfish. Stress is analyzed by observing behaviors like avoidance and shelter occupancy, and by measuring crayfish hyperglycemic hormone (CHH) levels in the hemolymph. Previous research demonstrates that serotonin can modulate aggression and acute stress in crayfish. We, therefore, administered serotonin agonist and antagonist treatments to influence chronic stress levels of crayfish exposed to nonylphenol, with the intent to modulate their stress levels.

Sei Whale, Say What? Acoustic Occurrence of a Rarely Observed Rorqual in Oregon Waters

Mariam Alsaïd, Dawn Barlow, Kate Stafford, Holger Klinck, Leigh Torres

Despite being the third largest rorqual inhabiting our oceans, the behavior, distribution, and life history of sei whales (*Balaenoptera borealis*) is largely unknown, particularly in the North Pacific. To investigate sei whale acoustic presence in this region, a hydrophone deployed 45 miles off Newport, OR recorded continuously from October 2021 to December 2022. Acoustic data was then visually and aurally reviewed in 5-minute intervals in the 0-150 Hz bandwidth. Sei whale calls were categorized by confidence levels established based on the published literature. High confidence sei whale calls recorded in this study are characterized by broadband downsweeps with an average frequency range of 105 - 35 Hz, which aligns with previous sei whale vocalizations recorded in the Pacific. High confidence sei whale calls were detected on 30 days of our study period. Peak sei whale acoustic presence occurred in June, October, and November. A strong peak in sei whale calls occurred in October 2022, when 596 individual high confidence sei whale calls were recorded over 4 days and a prevalence of a unique multiplet call series pattern was detected. No significant diel pattern was observed in sei whale calls. Learning more about sei whale occurrence and habitat use in the North Pacific can guide management efforts for the conservation of this endangered species.

Convergent evolution in adaptations to low-light environments across diverse fish clades

Liz Alter

Adaptation to low-light or lightless environments has evolved frequently across diverse clades of marine and freshwater fishes, making traits associated with these habitats particularly useful for studying convergent evolution across many levels of biological organization, from molecular to morphological. In this study, we investigated genetic mechanisms that may drive recurrent features in such taxa. We investigated positive selection in candidate genes, including the opsin gene family and *Grk1*, in rockfishes (genus *Sebastes*), a diverse clade of marine fishes in which closely related species occupy different depths and light environments. We compared substitution patterns, signatures of positive selection, and loss-of-function mutations in these candidate genes across 40 rockfish species, as well as representatives

of 18 families of teleost fishes spanning a range of habitats including freshwater, marine, cave, deep river, and deep sea. While specific sites under selection within genes largely differ across species that are separated by large evolutionary distances, common patterns at the gene and gene family level provide compelling evidence for the prevalence of convergent evolution toward low-light environments across a wide array of fish clades.

Shifts in the thermal sensitivity of resting metabolic rate in transplanted *Anolis* lizards

Karla Alujevic, Carrie Alfonso, Leah Bakewell, John David Curlis, Samantha Fontaine, Jaden Keller, Yanileth Lopez, Daniel Nicholson, Renata Pirani, Nathaly Ponce, Noa Ratia, Adam Rosso, Alejandro Vivas, Claire Williams, Kelly Wuthrich, W. Owen McMillan, Christian Cox, Michael Logan

The rate at which the resting metabolic rate (RMR) of ectotherms increases with temperature plays a critical role in their ability to respond and adapt to the challenges posed by climate change. The thermal sensitivity of RMR influences energy expenditure and activity levels, and thus overall survival and reproductive success. While RMR typically increases exponentially with increasing temperature, the rate of increase is predicted to be lower in animals that occupy thermally variable conditions as an adaptation to the fluctuating energy demands associated with variable temperatures. To test this hypothesis experimentally, we transplanted slender anoles (*Anolis apletophthalmus*) from a thermally stable mainland population to warmer, thermally variable islands in the Panama Canal. In addition to measuring RMRs at two ecologically relevant temperatures, we assessed operative temperatures, lizard activity, and field-active body temperatures in hundreds of individual lizards occupying six islands and across seven generations. We hypothesized that, compared to mainland lizards, island populations will display higher baseline metabolic rates but reduced thermal sensitivity of RMR as an evolutionary and/or plastic response to the warmer and more variable thermal conditions on the islands. Our results have implications for the energetics of ectotherm populations in our rapidly changing world.

Toxin tales: alkaloid load correlates with gene expression differences in poison frogs

Aurora Alvarez-Buylla, Elicio Tapia, Dania Nanes-Sarfati, Nora Martin, Mabel Gonzalez, Luis Coloma, Lauren O'Connell

Chemical defense is an adaptive strategy that involves the use of toxic or unpalatable compounds to avoid predation. South American poison frogs (family *Dendrobatidae*) are a diverse group best known for their bright warning coloration (aposematic frogs) and ability to sequester dietary alkaloids onto their skin as chemical defense. Within the *dendrobatidae* family, however, there also exist species without warning coloration (cryptic frogs), most of which are undefended with a few exceptions. In this study, we collected eight species of *dendrobatids* at six different locations in Ecuador, focusing specifically on defended and undefended species that are found sympatrically. For all of these samples we quantified the alkaloid profiles and the levels of gene expression in the liver, skin and intestines. We found that the cryptic *E. boulengeri* and *E. darwinwallacei* have intermediate levels of alkaloids on their skin as compared to their sympatric aposematic counterparts. We also found that there are orthologs across species whose expression correlates positively and negatively with alkaloid load. Our findings provide quantitative evidence for alkaloid load in field collected poison frogs existing on a spectrum, and find that the expression of certain genes correlates with this total alkaloid load, suggesting an important role of expression regulation in the evolution of toxicity in poison frogs.

Early-life cortisol responses to common lab stressors and associations with behavior in a cichlid

Alyssa Alvey, June Lee, Abigail Parrish, Tessa Solomon-Lane

Stress has highly conserved effects across species, including on growth, metabolism, immune function, and fitness. Cortisol, a steroid hormone, increases in response to a stressor, with effects on physiology and behavior.

We investigated cortisol and behavior in juvenile (< 1 week old) *Astatotilapia burtoni* (Burton's Mouthbrooder), a highly social cichlid fish. The stress axis has been studied in adult *A. burtoni* related to social behavior and reproduction, but juveniles are understudied. We quantified water-borne cortisol at baseline and in response to common lab stressors. Fish were first placed in an open field exploration and a social cue investigation. Next, fish were exposed to either a collection beaker (90 min, control), a standard lab protocol; three sequential beakers (30 min each) to measure change over time; net confinement; and movement via an orbital shaker. The net and orbital treatments also had three sequential collections. The net, orbital, and beaker transfer treatments all had significantly higher cortisol than the control. The net confinement was highest. This suggests common lab stressors successfully elicited a stress response in young juveniles. Cortisol also varied among individuals, and we then analyzed whether behavior explained this variation. This work provides insight into the early development of the stress axis and is a foundation for future research on stress and development in early-life.

The Influence of Turgor Pressure Dynamics of Poricidal Anthers: Implications for Buzz Pollination

Mitchell Alvord, Jenna McNally, Mark Jankauski

Buzz pollination is a critical process for the reproduction of tomato, potato, eggplant and other economically valuable crops. During buzz pollination, bees use their flight muscles to vibrate tube-like flower anthers to remove pollen. The success of pollen release relies on the dynamic properties of the anther. Studies show that environmental factors such as drought may influence the turgor pressure in anthers – yet the extent to which turgor pressure affects anther dynamics is unknown. In this study, we experimentally measured the first and second natural frequencies of *Solanum Sisymbriifolium* anthers using experimental modal analysis. Turgor pressure loss was induced by removing the anther from the flower. The anther was vibrated with an electrodynamic shaker and a laser vibrometer was used to measure the anther's deformation velocity. The anther's first and second natural frequencies were then estimated from the fast Fourier transform of deformation velocity. On average, the first natural frequency decreased by 19.8% and the second natural frequency decreased by 20.0% over the duration of the experiment. Using a dynamic model, we estimate that a 58.3% decrease in turgor pressure influences anther dynamics nontrivially, and significant reductions in turgor pressure brought on by changes in the environment may adversely affect a bee's ability to remove pollen.

Evolutionary Influence on Marine Actinopterygian Hearts: Chamber Landmark Morphometrics

Kyra Amacker, Stacy Farina

Fish heart anatomy differs considerably from other vertebrate hearts, due to their more simplistic, single-circuit circulatory system with a single atrium and ventricle. Despite this simplicity, the teleost cardiovascular system addresses the specific demands of the varying conditions of oceanic environments for 34,000+ species. Our study seeks to establish the ecological influence on heart form and function via morphological analysis and phylogenetic comparisons. We studied ten ecologically distinct species from across the Actinopterygii clade (pelagic, benthic and intermediate) via size and volume landmarking analysis. We used 3D Slicer to landmark the chambers of iodine-stained CT scanned hearts. We converted these chamber landmarks to meshes using a custom MATLAB script. We visualized and analyzed the meshes in MeshLab for size, shape, and volume measurements for interspecies comparison. We used phylogenetic ANOVA analysis to investigate interspecies variations and confirm heart function specificity in similar zone species for preliminary results indicate that ecological factors influence the anatomical and physiological function of these fish species. The benthic species' heart exhibited more rounded and thin-walled ventricles and large atria, while the pelagic species' heart has a more triangular shape to the ventricles with comparably smaller atria.

Scaling of stick insect stickiness

Guillermo Amador, Brett Klaassen-van-Oorschot, Benjamin Karman, Rutger Leenders

The climbing abilities of animals, like insects and tree frogs, have stimulated the curiosity of scientists for centuries. How are these animals capable of this spectacular feat with seemingly effortless execution? Scientific progress within the last few decades has revealed the functional morphology of an insect's sticky footpads – a soft, sponge-like pad that secretes a thin fluid film. However, the mechanisms underlying their adhesion remain elusive. Are fluid forces (i.e., capillary, viscous) dominant over molecular forces (i.e., van der Waals, electrostatic)? Do the underlying mechanisms vary with size? In this presentation, we explore these underlying mechanisms by directly quantifying the adhesive stress (or tenacity) of the footpads of live, freely walking Indian stick insects *Carausius morosus*. Throughout their lives, these insects span more than two orders of magnitude in size, from a few milligrams up to a gram. By understanding how adhesive performance scales with footpad size, we will shed light on the underlying physics of biological adhesion, as well as inform the biomimetic design of scale-independent reversible adhesives.

Genetic Accommodation of Temperature Dependent Color Changes in *Manduca sexta*

stephanie Amaya, Yuichiro Suzuki, Paula Gonzalez, Daniela Becerril

Genetic accommodation is an adaptive process by which natural selection acts on developmental plasticity to generate novel phenotypes. In this study, we explored the molecular basis of genetic accommodation using two genetically accommodated strains of the tobacco hornworm, *Manduca sexta*, which change color to different degrees in response to temperature. RNA-seq on the brain/corpora allata complex revealed changes in genes associated with juvenile hormone (JH) and ecdysteroid signaling. Our findings demonstrate that although both JH and ecdysteroid levels fluctuate in response to temperature, JH is responsible for the strain-specific response to temperature. The expression analysis of regulators of JH titers suggest selection acting on polygenic targets.

Digital Evolution: Genetic algorithms show emergence of complex color patterns and genes

Birch Ambrose, Jason Davis

A genetic algorithm is a simple type of machine learning program that uses principles of biological evolution to solve a problem. It is fairly simple to use these algorithms to create digital environments that approximate patterns observed in real ecosystems, particularly when algorithms are shaped by the actions of natural selectors. We created one such environment that can reproduce the emergence of complex defensive coloration using human agents to apply selective pressure. Under varying conditions this has led to the evolution of camouflage, aposematism, and mimicry in the digital organisms. It should be possible to use a similar approach to study the emergence of a range of evolutionary patterns using this technique, including the interface of factors such as mutation rate, population size, species interactions and developmental/modulatory genes. In this poster we discuss and demonstrate the overall format of our software as well as the varying patterns and processes that it has produced.

Exploring Variations in Strength and Mechanical Properties of Lumbar Vertebrae in *Eulipotyphla*

Myleen Amendano, C. Tristan Stayton

This study examines potential strength variations among lumbar vertebral units, driven by functional trade-offs. Microindentation testing is employed to elucidate mechanical property discrepancies across distinct vertebral units. Hypothetically, shrew lumbar vertebrae features will be correlated with mechanical attributes, including stiffness and load-bearing capacity. We are predicting that the vertebrae may show adaptations in strength with a trend of increasing strength as one descends the vertebral column. This could be driven by the need to balance the mechanical demands of supporting weight and providing stability with the requirement for flexibility and mobility in the

lower lumbar region. In other words, the transition from weight-bearing to facilitating movement prompts a distribution of strength to address these functional trade-offs, consequently yielding the observed pattern.

Effects of season on the regulation of CIRBP, HUNK, and TPH2 in green anole lizards

Brooke Andel, Spencer Harstad, Rachel Cohen

The green anole lizard (*Anolis carolinensis*) is seasonally breeding, with dramatic behavioral, physiological, and morphological changes between the breeding and non-breeding season. Understanding how seasonal environmental changes (photoperiod and temperature) alter gene expression can provide valuable insight into which pathways may be involved in regulating seasonal reproduction. Preliminary RNA-seq studies in male anole lizard brains ($n=3$) revealed approximately 150 genes that may be different across seasons. Our study sought to further analyze several genes that were identified. We selected three gene candidates to examine which are regulated seasonally in the anole: cold inducible RNA binding protein (CIRBP), hormonally upregulated neu-associated kinase (HUNK), and tryptophan hydroxylase 2 (TPH2). CIRBP is a cold-shock and stress response protein that can be induced after exposure to low temperature, HUNK likely plays a role in synaptic plasticity, and TPH2 regulates serotonin production. We utilized real-time quantitative PCR on mRNA isolated from the hypothalamus of breeding and non-breeding male and female anoles ($n=6$) to examine expression levels of the three selected genes. CIRBP mRNA expression did not differ across groups ($H(3)=4.75$, $p=0.193$). HUNK expression was highest in females ($F(1,19)=4.27$, $p=0.053$) but did not differ seasonally. TPH2 expression was higher in breeding females compared to non-breeding males ($H(3)=8.32$, $p=0.04$). TPH2 and HUNK expression patterns suggest that more work is needed to understand what role these genes might have in regulating seasonal changes.

Who stays home? Parental role, plasticity, cross-sexual transfer, and hormones in a cichlid genus

Andrew Anderson, Suzy Renn

Cichlid species in the genus *Julidochromis* are known to have diverse typified sex-biased parenting and pairing behaviors that can be plastically shifted depending on the social environment. Instances where one sex takes on phenotypes common in the other are termed cross-sexual transfer. What is unclear is the mechanisms that govern cross-sexual transfer and whether they are convergently evolved or have shared ancestry. Using the Ancestral Modulation Hypothesis we predict the behaviors should have a shared mechanism across sexes and species. Here we investigate the hormonal and behavioral changes with partner size in two species *Julidochromis* that typically form male-larger (*J. transcriptus*) and female-larger pairs (*J. marlieri*). For both species, individuals are exposed to both a larger and smaller partner and have behavioral and hormonal data collected following successful pairing. We then correlate circulating hormones and behaviors with the role, size, sex, and species to confirm if individuals use similar mechanisms to govern the associated behaviors.

Contrasting Topology and Interaction Strengths Drive Stability in Mutualistic Networks

Chris Anderson, Alva Curtsdotter, Berry Brosi, Fernanda Valdovinos, Phillip Staniczenko

Ecological networks continually face multiple demands—they must remain high functioning, yet resistant to the constant threat of various forms of perturbations. Ecologists have long been interested in how the structure of an ecological network impacts its stability and resilience. Mutualistic networks, such as plant-pollinator communities, almost always exhibit a nested interaction pattern in nature. This near-ubiquity is puzzling given that nestedness seems to both reduce local stability and increase resource sharing (i.e. reduce resource complementarity). By contrast, nestedness tends to improve another stability-related outcome, robustness to coextinction. These competing tensions could potentially be ameliorated in mutualistic networks by considering interaction strengths in addition to the simple presence or absence of

interactions. In particular, complementarity in interaction strengths is predicted from adaptive foraging theory (and has been demonstrated in natural ecosystems) and also has analytical justification for potentially enhancing stability in networks with nested interaction patterns. To investigate if the interplay of interaction strengths and presence-absence topology influences stability, we adopted a community-matrix approach, simulating networks of varying nestedness and interaction strengths and assessing local stability. We predicted that complementarity in interaction strengths will confer local stability to nested networks, ultimately resulting in networks that are both more locally stable and robust to coextinction perturbations.

Lizard Skin Detects and Blocks Light: A Study of Non-visual Opsin Expression

Grace Anderson, Violeta Trejo-Reveles, Troy Murphy, Jim Shinkle, Zhou Wu, Alex Johnston, Simone Meddle, Michele Johnson

Vertebrate skin is the organ with the most exposure to sunlight, and emerging literature has shown that nonvisual opsins (a group of light-sensitive proteins) are expressed in dermal tissues in many taxa. Light detection is particularly important in terrestrial reptiles for physiological and behavioral regulation, yet opsin expression is understudied in this group. In this study, we examine the green anole lizard, *Anolis carolinensis*, to map opsin expression in the skin and internal organs, and to quantify the exposure to sunlight experienced by the exterior and interior of the lizard. Using qPCR, we quantified the expression of the four major non-visual opsins and found that a series of them are more highly expressed in dorsal skin, which is directly exposed to sunlight, than in ventral skin, which is generally sheltered from direct sun. We also found that nonvisual opsins are abundantly expressed in internal organs. Yet, our measures of light transmittance through tissues reveal that very little light penetrates the body cavity wall to reach the internal organs. The light that does get through is noticeably shifted to wavelengths longer than 700 nm, outside the conventional opsin sensitivity range. These preliminary findings suggest that tissue-specific expression of dermal opsins may be critical for light detection, yet opsins in internal tissues may perform a different primary function.

How does height influence perch-related locomotor behaviors in arboreal snakes?

Jeffery Anderson, Jake Socha, Amalia Moore, Joshua Pulliam, Jerry Wong, Collin Barnett, Ulmar Grafe, Salwa Khalid

When traversing gaps within tree canopies, snakes have been observed using a range of locomotor behaviors, including cantilevering, lunging, jumping, and gliding. As these behaviors become more dynamic, the risk of slipping or falling likely increases, with injury potential increasing with height. Do arboreal snakes modulate their locomotor behavior based on perch height? We hypothesized that snakes would be less likely to leave the perch and perform dynamic behaviors at high heights due to the threat of falling. We examined the behavior of two closely related species of arboreal snakes (*Dendrelaphis pictus* and *Ahaetulla prasina*) at low ($h=1.5$ m) and high ($h=5.0$ m) heights. At each height, we examined behavior in the presence or absence of a nearby target placed at a distance (85% SVL, snout-vent length) chosen to elicit dynamic, non-cantilever movements. In each trial, snakes were observed for a set duration while given periodic stimuli to induce movement. Analysis of video records suggests that height does not influence behavioral choice in *Dendrelaphis* but does so in *Ahaetulla*. Specifically, *Dendrelaphis* were more prone to perform dynamic behaviors and were more likely to leave the perch. However, both species were less willing to perform when the target was absent, suggesting target presence is a significant variable for arboreal locomotion. Supported by NSF 1922516 and 2027523.

Response of larval Pacific krill (*Euphausia pacifica*) to ocean acidification and warming

Meredith Anderson, Shalin Busch, Paul McElhany, Michael Maher, Danielle Perez, Kate Rovinski

As climate change alters the ocean's temperature and pH, many ocean ecosystems will diverge beyond their historical state due to environmental conditions crossing the biological tolerance thresholds of its organisms. We conducted experiments on larval Pacific krill, *Euphausia pacifica*, from the Puget Sound to understand their sensitivity to current and future ocean conditions to identify the impacts of a changing ocean on a critical trophic link in the California Current Ecosystem (CCE). Pacific krill support a variety of commercially and ecologically important species in the CCE, such as Pacific hake, Chinook salmon, and baleen whales.

We utilized ten CO₂-by-temperature treatments (400 ppm, 2000 ppm CO₂ by 8°, 10°, 12°, 14°, 16°) to address the sensitivity of early life stages of Pacific krill to ocean acidification and warming. Measured response metrics include egg extrusion and hatch and larval development, survival, and size. We will present the preliminary results on some of these metrics. Understanding how future conditions impact larval krill can inform population dynamics and ecosystem modeling and the utility of krill as an ecological indicator of ecosystem health.

Perceptual bias and behavioral mechanisms set scene for convergent evolution in foot-flagging frogs

Nigel Anderson, Doris Preininger, Matthew Fuxjager

Convergent evolution occurs when species independently evolve similar phenotypic traits to solve the same environmental problem. Still, an outstanding question regarding convergent evolution, particularly with communication signals, is what mechanisms allow similar traits to evolve independently in different species. One possible explanation is that selection favors traits that excite receiver perceptual biases, while additionally selecting for the evolution of underlying physiological mechanisms that enhance the favored traits to a receiver. We suggest that foot-flagging displays in frogs provide an excellent example of the co-option of perceptual bias and physiological systems for the convergent evolution of novel communication signals. The foot-flag display is an agonistic gestural display produced when a frog fully extends its hindlimb out from its body and moves it downwards in an arc like motion. Past studies show that the emergence of this display in unrelated frog taxa is marked by an increase in the expression of androgen receptor (AR) in the muscles that move the leg. Additionally, we find that the foot-flagging frog *Staurois parvus* reacts to a conserved perceptual bias similarly to how it reacts to the foot-flag display of rivals, and these targeted display traits are enhanced by an increase in circulating testosterone. Thus, our findings provide a possibility in which perceptual biases work with underlying physiological systems to facilitate the evolution of pre-existing behavior into a communication signal.

Adventures in experimental impact dynamics and puncture mechanics

Philip Anderson, Bingyang Zhang

Puncture, the act of creating fracture in a tissue and inserting a tool, is widespread in biology. Some puncture systems are slow and methodical, like a parasitoid wasp burrowing its ovipositor through rotten bark to deposit an egg. Others are dynamic, such as the predatory strike of a snake, or sting of a jellyfish's nematocyst. Still others are fully passive, like the spines some cacti use to hitch a ride on passing mammals to spread clones. While these diverse systems must all achieve the same end-goal of puncture, they deal with a variety of tissue resistances and dynamic situations. In order to create the framework for puncture energetics that our lab has been building for the last couple years, we have performed a variety of experimental tests to understand how variables such as morphology, material properties and dynamics influence puncture. Here, I summarize some of the findings from these experimental analyses including: 1) The influence of morphology on puncture performance appears to decrease at higher speeds, especially in the ultra-high-speed range, 2) Certain material responses to puncture, such as

the stretch-based damage pattern in soft materials, are invariant with regards to tool morphology for a given dynamic range and 3) Multi-layer materials can increase puncture resistance, even when the outer layer is thin and compliant.

A new model for lizard thermoregulation derived from field data on an intensive foraging lizard

Roger Anderson

The Western Whiptail Lizard, *Aspidoscelis tigris*, is a physiology-assisted, etho-thermoregulating ectotherm, and inhabits the Sonoran, Mojave and Great Basin Deserts. At the northern and southern extremes, a combined total of about 750 field-active lizards were captured when they were performing obvious behaviors and for which body temperatures were measured. Body temperatures of lizards when foraging and also at the beginning, middle and end of basking behavioral bouts and the beginning, middle and end of cooling behavioral bouts revealed data that can be used to infer a new model for lizard thermoregulation: dual limit setpoints for both warming (begin v end) and cooling (begin v end), with stochastic foraging temperatures between the begin-bask and begin-cool body temperatures. The basking body temperatures were similar in both Sonoran Desert and Great Basin Desert lizards. Begin-bask body temperatures, however, and also foraging body temperatures averaged about 0.5°C higher in the southern desert than in the northern desert. Whereas higher foraging temperatures in the southern desert largely can be attributed to the warmer environmental temperatures there, the begin-bask temperature differences between sites may be features of adaptedness to differing thermal conditions.

The effect of developmental environments on methylation of DNMT3 in a cooperatively breeding species

Susan Anderson, Andrea Liebl, Andrew Russell

Epigenetic regulation, such as DNA methylation, alters gene expression without changing the underlying genomic code. Variable environments, such as temperature, diet, sociality, and other ecological pressures can induce epigenetic responses, which, in turn, influence phenotypes. Developmental environments, such as parental care or abiotic factors, have profound, and sometimes permanent, effects on DNA methylation and their resulting phenotypes. Although there are two main enzymes that control methylation, DNA methyltransferase 3 (DNMT3) is primarily responsible for interacting with the environment to induce de novo methylation signals, making it a particularly important conduit between the environment and genome. Here, using the cooperatively breeding chestnut-crowned babbler (*Pomatostomus ruficeps*), we analyze how variable developmental environments (e.g. brood and helper number, climate) influence epigenetic regulation of DNMT3, with the assumption that increased methylation of the promoter of this gene would decrease an individual's ability to respond to the environment. We assessed methylation in the promoter of DNMT3 in chicks at hatching and just before fledging to analyze how epigenetic signals in this gene changed across different conditions. We predict that environmental conditions during development will be related to methylation of the DNMT3 promoter, thus affecting its ability to regulate other genes. This research will add to the growing body of ecological epigenetic work in understanding how wild organisms respond to abiotic and biotic environmental factors.

Nervous system evolution in deuterostomes

José Andrade-Lopez, Laurent Formery, Lauren Lubeck, Chris Lowe

The origins and evolution of the chordate nervous system have a long and contentious history in Zoology. Although reconstructing ancestral deuterostome anatomical characters has been challenging due to the large morphological disparities in body plan organization between the major groups, new developmental and genomic data are providing critical insights by facilitating the reconstruction of ancestral molecular characters. Enteropneust hemichordates, as an outgroup to the chordates, are crucial for testing hypotheses of chordate origins. We present a combination of molecular genetic and neuroanatomical data during the development of the direct-developing enteropneust, *Saccoglossus kowalevskii*, to highlight how highly conserved molecular genetic regulatory networks can support strongly

divergent neural architectures. We will also present data for a novel neuro-secretory center that shows strong molecular and neural similarities with the vertebrate hypothalamus and pituitary.

Diversity in thermal tolerance, performance, stress and buffering in Northeastern GOM Fishes

Alyssa Andres, Dean Grubbs

Average temperatures in the waters surrounding St Teresa, FL, have warmed ~3°C in the last 15 years, far exceeding the rate of thermal rise in Gulf of Mexico coastal waters. Ecological data from the region suggests thermal shifts of such magnitude are causing longer perturbations in ecosystem dynamics, and on species movement and residency within the studied systems. However, a mechanistic understanding of species-specific thermal tolerance is lacking for most coastal fish species, as is the degree to which species within an ecosystem may vary in their responses to such environmental change. Using whole organism performance metrics such as metabolic scope, oxygen supply capacity, and critical thermal maximum (CT_{max}), we identify differences in species-specific trends in thermal performance, test the causes of declining thermal tolerance at temperature extremes, and calibrate indices of viable habitat for a diversity of fish species of the Northeastern Gulf of Mexico, inclusive of the Atlantic stingray (*Dasyatis sabina*), spotted sea trout (*Cynoscion nebulosus*) and Gulf toadfish (*Opsanus tau*). In addition, hematological indicators of metabolic change and thermal buffering, such as the abundance of heat shock chaperone proteins (HSP70), and shifts in metabolites and electrolytes with temperature, further indicate the onset and degree of systemic disturbance in these species under elevated temperatures. Comparing physiological responses across diverse fish taxa may reveal differential vulnerability to climate change within this ecosystem.

Defining bull shark movement, habitat use, and nursery habitat in a unique spring-fed ecosystem

Alyssa Andres, Harrison Clark, Jasmin Graham

As top predators, bull sharks are key direct and indirect ecosystem modifiers, economic resources, and facilitators of ecosystem health. Their population status, habitat use, and responses to anthropogenic/environmental factors have been made a research priority for management, with loss of nursery habitat recognized as one of the largest threats to this species success and survival. Kings Bay/Crystal River may represent one of the most important potential bull shark nurseries in the state of Florida under climate change. In this closed estuary, 99% of the freshwater supplied to Kings Bay is sourced from natural freshwater springs, unique from all other known bull shark nurseries. Thermal and saline stability of this natural spring-fed system may offer ideal nursery habitat and a potential thermal refuge for cold-sensitive bull sharks that use coastal low salinity estuaries to shelter young from predation, provide ample food resources, and reduce mortality.

Direct observation and anecdotal evidence from local residents and fishermen suggest Kings Bay/Crystal River is likely a growing, and yet uncategorized nursery for bull sharks. To date no studies have investigated shark presence, abundance, movement or habitat use within this region. Using acoustic monitoring, fisheries independent surveys, and citizen science, this project aims to establish bull shark movement and habitat use in the region, identify nursery habitat for this species, and quantify springs as thermal refugia for these key ecosystem contributors.

Genomic insights on the evolution of polyphenism in the soapberry bug, *Jadera haematoloma*

Dave Angelini

Polyphenism allows organisms to respond to varying environmental conditions by adopting alternative collections of morphological traits, often leading to different reproductive strategies. In many insects, polyphenism affecting the development of flight trades dispersal ability for increased fecundity. The soapberry bug *Jadera haematoloma* (Hemiptera: Rhopalidae) exhibits wing polyphenism in response to juvenile nutritional resources and cohort density. Development of full-length wings and flight-capable thoracic muscles occurs

more frequently in cohorts raised under low food density conditions, and these features are correlated to reduced female fecundity. Soapberry bugs represent an example of polyphenic dispersal-fecundity trade-off. Short-wing development is not sex-limited, and morphs can also differ in male fertility. Our lab has documented the evolution of polyphenic thresholds in different host-adapted populations, and the role of insulin and EGF signaling in wing morph determination and in the development of wing shape. Transcriptomic analysis has provided a functional view of this environmentally-dependent growth control. Assembly and annotation of a chromosome-level genome sequence for *J. haematoloma* allows insights into the evolution of heteropteran chromosomes and, with comparisons to two other rhopalid species lacking wing morphs, gene sets allow analysis of gene family expansion and reduction in this polyphenic species.

Rat superficial masseter operates differently during biting and chewing and across food hardness

Samuel Angelli-Nichols, Trushti Patel, Devin Jenness, Nicolai Konow

The vertebrate jaw apparatus supports diverse behaviors involving jaw motions generated by adductor and depressor muscles. Individual muscle function, with respect to food challenges, is adjusted through neural drive and governed by force-length-velocity effects. Despite this vexing system complexity, studies have typically focused on jaw kinematics and muscle EMG. Here, we compare activation, strain, force, and velocity of rat superficial masseter (SM) with respect to variations in food hardness during biting and chewing. We test the hypothesis that, with increasing food hardness (chocolate [6.9MPa] to chow [50.5MPa]) and the behavioral challenge of biting involving greater gape than chewing, SM undergoes greater activation, develops more force, and contracts slower across greater strains. However, we measure comparable force production across behaviors for a given food hardness despite significantly greater activation during chewing than biting, with significantly greater and faster fiber shortening strains during chewing than biting. Together, these results suggest alternative contractile strategies across behaviors for operation along the force-length-velocity relationships, as shown separately for rat zygomaticomandibularis. Our findings motivate studies that superimpose *in vivo* data onto the muscle's force-length-velocity curves. This approach can determine if chewing occurs on the ascending limb of the force-length relationship, as concluded by earlier twitch contraction studies, and if biting occurs closer to optimal length. Alternatively, slower contractions may facilitate comparable bite-force at lower activation. Funded by NSF IntBIO 2217246.

Food hardness, activation, and architecture influence *in vivo* operating lengths of rat jaw muscles

Samuel Angelli-Nichols, Trushti Patel, Devin Jenness, Nicolai Konow

Jaw muscles operate at lengths that are gape-dependent and determine critical feeding parameters, including bite force, according to the Force-Length (FL) relationship. Early twitch-contraction studies suggested that biting and chewing occur at muscle lengths below optimal (Lo), favoring stability but by necessity limiting occlusal force at larger gape. Newer studies demonstrate that Lo shifts to longer muscle lengths as activation increases, suggesting that jaw muscles might operate across the FL plateau, at longer, unstable lengths during high-force behaviors like biting. We used a carefully size-controlled food assay spanning different hardness to measure the influence of gape on jaw adductor force generation in rats. We measured fiber operating lengths of zygomaticomandibularis (ZM) and superficial masseter (SM) for each food type *in vivo* and determined the force-length-activation properties for the same muscle fibers *in situ* under twitch and tetanic stimulation. During biting on hard food, peak operating lengths reached the descending FL limb for both ZM ($< 1.1\% \pm 2\%$ Lo) and SM ($< 4.5\% \pm 0.5\%$ Lo). These findings align with limb muscle data by showing how a unipennate muscle architecture allows SM to operate at fiber strains just above Lo, whereas a parallel-fibered architecture can drive ZM to operate at long, unstable lengths. Our data provide new insight into the mechanical function of jaw muscles with potential implications for clinical interventions. Funded by NSF IntBIO 2217246.

Using virtual reality to teach biological concepts and transferrable skills to undergraduates

Michael Angilletta, Christofer Bang, Kiara Crawford, Madison Delaney, Tray Geiger, Rachel Griffin, Annie Hale, Alysha Hall, Christopher Pagliarulo, Liesel Sharabi, John VandenBrooks, Christian Wright

We present a new way of learning biology, called Dreamscape Learn, that combines immersive experiences in virtual reality with highly structured, student-centered activities in the classroom. This new curriculum enables students to enter a fictional world, where an artificial intelligence has created an intergalactic wildlife sanctuary to preserve endangered ecosystems from across the galaxy. In each learning module, students become the heroes of a story—discovering, investigating, and solving novel yet realistic problems. A narrative arc continues throughout experiences in VR and in the classroom, creating an immediate need to learn concepts and skills. Undergraduate students in this new curriculum were almost twice as likely to score between 90 and 100% on assignments than were students in the existing curriculum at Arizona State University. Students who participated in VR more frequently also felt more engaged in the course and performed better on assignments. Differences in student performance between the curricula were similar among demographic groups, suggesting that the new curriculum might help to reduce achievement gaps. This research underscores potential benefits of leveraging emerging technologies and narrative structure to enhance education and training in science.

Exploring Motion-Based Active Sensing in Relation to Predictive State-Estimation Uncertainty

Mustafa Mert Ankarali, Osman Kaan Karagoz, Aysegul Kilic, Emin Yusuf Aydin, Ismail Uyanik

Active sensing via movement is an intriguing phenomenon observed in various animals, wherein organisms deliberately move to enhance perception. This integrated link between sensory feedback and motor actions challenges traditional engineering practices, where sensing and actuator subsystems are often designed independently. To comprehend the underlying mechanisms through which animals exhibit motion-based active sensing behavior, we aim to model the active sensing behavior of *Eigenmannia virescens* observed in ill-light conditions. We hypothesize that the entire sensorimotor control system of the fish comprises three key components: state estimation, tracking controller, and active sensing generator. An Extended Kalman Filter fuses information from a nonlinear noisy sensor model and the fish's motion model to produce state estimates. State-feedback-based tracking controller processes the estimated states—particularly the relative position and velocity between the target and the fish—to generate motor commands. However, the structure of the sensory model renders the coupled system unobservable for near-zero velocities. This requires an active sensing component, as the coupled system becomes non-output-controllable in this regime. Our approach introduces an active sensing generator that produces an additional additive motor command to minimize the predictive estimation uncertainty. We demonstrate—on actual fish data collected with a custom-built setup in our lab—the successful predictive performance of our approach as compared to recent alternatives in the literature. Supported by TUBITAK (120E198).

Rising to the top: Animal evolution at the ocean's air-sea interface

Colin Anthony, Bastian Benthage, Rebecca Helm

Species that float freely at the ocean's surface include highly specialized taxa from distinct evolutionary lineages that convergently evolved floating morphologies. In 1958, Soviet scientist A. I. Savilov posited that floating species are derived from benthic ancestors, rather than species from the adjacent pelagic zone. To test Savilov's hypothesis, phylogenies and ancestral traits were inferred for all major floating animal lineages with molecular data. At least five animal clades have independently evolved to float at the ocean's surface, including two cnidarian lineages, two molluscan lineages, and one barnacle lineage. Our results revealed that four of these clades arose from an ancestor that attached to a substrate, though that substrate was not necessarily the benthos, and includes epibiotic and rafting species. Despite their

diverse evolutionary origins, all floating animals use gas-trapping mechanisms to remain at the surface, which appear homologous to substrate-attachment structures. Our results suggest that Savilov was partly correct to assume floating animals arose from species that could attach to a substrate, though not necessarily the benthos. The air-sea interface, despite its uniqueness, may in some ways be just another surface.

The importance of fluke geometry and stiffness on swimming efficiency in bottlenose dolphins

Gabriel Antoniak, Enric Xargay, Joaquin Gabaldon, Kira Barton, Bogdan-Ioan Popa, Alex Shorter

Efficient movement through water is important for biological and engineered systems alike. Lift-based propulsion used by cetaceans to swim is thought to be highly efficient, but forces generated during locomotion cannot be directly measured during unconstrained movement. To address this gap, we have created a first-principles, low-order model of the sagittal-plane swimming dynamics of bottlenose dolphins and developed a framework to generate estimates of locomotive costs directly from tag-based kinematic measurements. The modeling approach captures lift-based propulsion, unsteady hydrodynamics, fluke flexibility, and body posture. Kinematic data (depth, orientation, speed) were collected from six male bottle nose dolphins (mass: 187.3 ± 36.7 kg, length: 2.32 ± 0.12 m) during steady-state swimming over a range of swimming speeds (2.24–5.41 m/s). These data were combined with the model and used to estimate the swimming kinetics and propulsive efficiency. Animal specific morphometric parameters (length, girth, and fluke geometry) were used to tailor the model to each participant, with estimated propulsive efficiencies clustering for each individual. We investigate how fluke shape, fluke stiffness, and peduncle-fluke joint stiffness impact thrust production, the internal joint power necessary to drive the fluke through the water, as well as the resulting propulsive efficiency of locomotion. These methods can thus generate insights into the shape and properties of dolphin flukes in a simulation tied to the underlying physics of how dolphins move through the water.

Muscle twitch kinetics set differential performance limits for elements of a multicomponent display

Nicholas Antonson, John Capano, Matthew Fuxjager

Motor constraints significantly influence the performance of behavioral displays as the nexus through which the brain actualizes behavior. Behavioral displays are often multicomponent and rely on several individual display elements to generate a cohesive display. As such, determining whether constraints on behavioral displays are co-localized or partitioned across the motor apparatus has broad implications for how cohesive displays are produced, as independent constraints for each element may affect the variation with which each element can ultimately be performed. We investigated this idea in the multicomponent drumming display of woodpeckers. We applied in situ muscle stimulation and recording to determine how the longus colli ventralis (LCv), the main protracting neck muscle during bill strikes, imposes constraints on the speed (beats sec⁻¹) and length (total beats) of drum displays in downy woodpeckers (*Dryobates pubescens*). Additionally, we investigated if downy woodpeckers could match the rapid drumming patterns of two other species known to produce fast drum displays that vary in length. Our findings revealed that the LCv constrains the speed of drumming displays, but not drum length. Moreover, downy woodpeckers were unable to produce the faster drumming patterns of other species. These results provide evidence that two major components of drum displays are differentially constrained by the muscle physiology that actuates drumming and suggests that muscle constraint on drum speed is species-specific.

Assessing the biodiversity of a California desert ecosystem in an unusually wet year

Aniela Anuszczyk

Deserts and other arid habitats house many of the world's most biodiverse ecosystems and are known for high numbers of endemic species.

Although primary productivity levels are relatively low in deserts, abiotic factors such as precipitation can have a significant effect on year-to-year levels of biodiversity.

While some studies have shown a trend in dryland revegetation, the effects of recent warming and drought in the western United States on desert biodiversity are uncertain.

In this study, we assess angiosperm diversity in Anza-Borrego State Park, California across the years 2017 to 2023 with varying precipitation levels using data from iNaturalist.

We added complimentary data in March 2023 combined with previously documented biodiversity observations to assess correlations between precipitation rates and biodiversity.

Although we did not find a significant effect of precipitation rates on overall angiosperm biodiversity, we did find that plants listed as "threatened" showed decreased abundance in years with low rainfall.

Our data suggests that species of conservation concern may become more vulnerable if drought trends in the western United States continue as predicted by recent climate models.

Robotically Controlled Jellyfish: Biomechanics of Modified Bell Shapes

Simon Anuszczyk, John Dabiri

Jellyfish have the lowest cost of transport of all metazoans and live in a wide range of ocean temperature, salinity, pH, and depth, due to their evolutionary success and adaptability. By equipping live jellyfish with microelectronic swim controllers, we create a biohybrid jellyfish robot. This robot combines inexpensive electronics with jellyfish efficient swimming and self-powered locomotion via feeding. Previous work has demonstrated stimulated jellyfish vertical swimming speeds of 2.8 times baseline speeds without swim controllers. Here we explore mechanically modifying stimulated jellyfish bell shapes with added forebodies to reduce drag on swimming animals. We model the unsteady swimming dynamics of jellyfish with attached forebodies to study the impact of changes in drag and added mass on the animal dynamics. To inform this model, we conduct drop tests of various hemiellipsoid forebodies to empirically determine drag at terminal velocity. We validate the model by attaching 3D printed forebodies onto the jellyfish exumbrellar surface and testing swimming performance in a 6m tall water tank. These results help inform our understanding of the influence of bell shape on jellyfish biomechanics including power, locomotion, and feeding.

Roll stabilization of hummingbirds under external perturbation

Md Zafar Anwar, Bret Tobalske, Suyash Agrawal, Haoxiang Luo, Bo Cheng

Hummingbirds exhibit remarkable flight stability and can reject perturbations of large magnitude, including external ones such as turbulence, and internal ones such as those self-generated during rapid maneuvers. To investigate hummingbird flight stability, we attached a small magnet to their dorsal side and applied perturbation about the body roll-axis while they were hovering and feeding between a Helmholtz coil. The perturbation arrived at varied times during the wingbeat cycle, leading to the peak perturbed roll rate of 835 ± 167 deg/sec. Our analyses revealed that the hummingbirds can rapidly counter external torques and cease rotation within 50ms. To reject the perturbation, birds depressed the motion of the contralateral wing, which started consistently at the beginning of the downstroke. This resulted in a variable delay of 15ms to 35ms between the onset of perturbation and the active control. This limitation suggests that hummingbirds could also rely on passive mechanisms to achieve robust stabilization within 50ms. Further analysis confirmed there existed passive counter torque due to differential wing velocity caused

by body rotation that became effective in less than half of the wingbeat (i.e., ~ 10 ms). Such passive counter torque has both aerodynamic components arising from the flapping counter torque and inertial components from differential centrifugal forces on the wings. Combined, the aerodynamic and inertial components led to nearly equal torque during downstroke and upstroke. Our results show that hummingbirds can reject external perturbation in a robust fashion, which can be explained by a combination of passive and active stabilizing mechanisms of both aerodynamic and inertial contributions.

Identifying the pitch wing motion primitive in hummingbird flight using Principal Component Analysis

Md Zafar Anwar, Bret Tobalske, Suyash Agrawal, Haoxiang Luo, Bo Cheng

Hummingbirds can rapidly alter the patterns of their wing motion in a complex and flexible fashion to vector aerodynamic forces for flight control. Despite this complexity, we hypothesize that there exist low dimensional modes in the wing motions, as well as in the motor patterns and wing musculoskeletal process. In this work, we aimed to identify these modes, which we called the Wing Motion Primitives (WMPs) during the pitching maneuvers of escape flight using Principal Component Analysis (PCA). The results show that the first component captured WMP during hovering, while the second principal component is sufficient to explain pitch control, as it has a strong linear correlation with pitching torque, including those used for both pitch acceleration and deceleration. By decomposing the wing motion pattern into principal modes, we successfully transformed the pitching control into a simple linear mapping mechanism from the pitch control input (i.e., the degree of pitch WMP) to the pitch torque. We then applied this control mechanism in a simulated hummingbird model to stabilize the body pitch on a wingbeat-by-wingbeat basis. In summary, using PCA, we demonstrated there exist low dimensional modes in hummingbird wing motion and its linear mapping to body torque, thereby suggesting simplicity in hummingbird flight control.

Effects of the timeless gene's knockout in a new established mutant line of *Aedes aegypti*

Emilie Applebach, Lan Lou, Richard Rust, Nicole Wynne, Zhijian Tu, Chloe Lahondere, Clement Vinauger

Biological rhythms are essential to the fitness of many organisms, including mosquitoes. They permit the synchronization of physiological and behavioral processes to specific times of the day, in order to optimize chances of survival and minimize energy expenditures. These rhythms are sustained by molecular clocks comprising translational-transcriptional feedback loops, whose key molecular players have been well characterized in insect models such as *Drosophila melanogaster*. In mosquitoes, while the orthologs of the *Drosophila* clock genes are known, the functional analysis of these genes has been limited by a lack of available knockout lines. In this context, we used CRISPR/Cas9 to generate a line of *Aedes aegypti* mosquitoes that have a 91 bp deletion in the timeless gene. Next, we used locomotor activity monitoring assays to characterize the effect of the deletion on diel and circadian activity rhythms. Specifically, we compared the activity profiles of wild type mosquitoes from the Liverpool strain, to heterozygous and homozygous mutants. Our results show that the three genotypes differed in specific features of their activity rhythms. In addition, we found differences in the daily profiles of putative sleep, as well as lack of a startle response to light in the homozygous mutant. Altogether, these findings contribute to improving our understanding of the molecular mechanism underlying mosquito biological rhythms and could reveal targets of opportunity to disrupt their time-dependent host-seeking behaviors.

Comparative anatomy of the cranial musculature of darter fishes: insights from diceCT

Jessica Arbour

Fish skulls are often highly kinetic, with multiple linkage and lever systems powered by a diverse suite of muscles. Comparative analysis of the evolution of soft tissue structures in the fish skull is often limited under traditional approaches, however new imaging techniques like diceCT allow for high

resolution imaging of muscles in situ. Darters (Percidae; Etheostominae) are a diminutive and species-rich clade of lotic freshwater fishes, which show diverse head shapes believed to be associated with different foraging strategies. Previous analyses of ecomorphology in this clade have only focused on skeletal aspects of feeding morphology. We use diceCT to non-destructively sample all major cranial adductors and abductors responsible for movement of the jaw, hyoid and suspensorium, from >25 species ranging across Etheostominae. We applied comparative phylogenetic approaches to analyze the evolutionary trends in muscle size across the clade. We find two major patterns: 1) darter cranial muscles show fundamental trade-offs relating to investment in musculature, and buccal expansion vs. biting attributes. Early divergence in muscle size appears to be associated with shifts in habitat use and foraging. 2) Darter adductor mandibulae show high variation in architecture (especially considering their recent diversification), with shifts in features like the number of major divisions and fiber orientations. This study highlights how new imaging techniques can provide novel insights into the anatomy of even well sampled species.

Upright limb posture reduces muscle work during locomotion

Adrien Arias, Mary Pena, Manny Azizi

Scaling laws dictate that the force-generating capacity of muscle decreases as animals grow larger, requiring changes in limb joint and/or muscle function during locomotion to maintain performance across sizes. The relatively more upright posture used by large animals better align limb joints with external forces during locomotion, but it remains unclear whether changes in limb posture are associated with physiologically relevant shifts in underlying muscle function. American alligators (*A. mississippiensis*) encompass a large size range, have limbs that scale mostly isometrically throughout ontogeny, and use more upright postures at sizes above 5kg—offering a unique vertebrate animal system to test the hypothesis that an upright posture decreases in vivo muscle work during locomotion (while avoiding confounding factors like interspecific comparisons or allometric growth patterns inherent in comparing large vs. small mammals). Here we use sonomicrometry (to measure fascicle length), electromyography (activation timing), and leaf spring force transducers (muscle force) to measure gastrocnemius function during walking across a size range in alligators (0.4–12.0 kg). We found that alligators >7 kg experience less fascicle strain, slower fascicle velocities, and perform less mass-specific work during walking relative to smaller alligators (LME, $p < 0.05$). These results show that changes in limb posture associated with body size are complemented by shifts in muscle function during locomotion aimed at overcoming force deficiencies inherent in larger animals.

Tibial Bone Strains in the Green Iguana (*Iguana iguana*): In Vivo and Finite Element Analysis (FEA)

Timothy Arlowe, Russell Main, Worapat Sawatwong, Ruisen Fu, Haisheng Yang

Mechanical loading models are used to study adaptive skeletal mechanobiology mechanisms. Most studies have used mammal models, thus there is a knowledge gap for how these mechanisms differ among vertebrate groups. We evaluated the in vivo bone strain environment of the green iguana left tibia during locomotion, axial compressive loading, and finite element analysis (FEA). We examined subadult green iguanas ($n=7$) over a range of speeds (0.4–1.3 m/s) and axial load magnitudes (-25 to -100 N) to determine peak strains. Bone strains were measured using single element ($n=18$) and rosette strain gauges ($n=3$), which were surgically attached to the tibial anterior, posterior, and medial surfaces. At 1.3 m/s, peak strains observed were $646 \pm 699 \mu\epsilon$, $-448 \pm 464 \mu\epsilon$ and $206 \pm 168 \mu\epsilon$ at the anterior, posterior, and medial surfaces, respectively. Peak principal tensile and compressive strains on the medial surface were $199 \pm 113 \mu\epsilon$ and $-153 \pm 98 \mu\epsilon$ at 1.3 m/s. Peak strains during -100 N compressive loading were $403 \pm 277 \mu\epsilon$, $-506 \pm 459 \mu\epsilon$ and $-52 \pm 177 \mu\epsilon$ at the anterior, posterior, and medial surfaces, respectively. Our FEA model demonstrated a close correlation with experimentally measured strain values at the gauge sites (slope = 1.07, $R^2 = 0.7$). Our next objective is to evaluate the bone adaptive response following an axial compressive loading protocol.

Haltere mechanosensory input for social wing movements in the black scavenger fly, *S. punctum*

Lesley Armah, Jessica Fox

Sexual selection is a powerful evolutionary force that can produce distinctive features such as the eyes of a stalk-eyed fly. In these cases, selection gives a functional appendage a new evolutionary purpose that becomes a crucial factor in fitness. The haltere, another fly appendage, is a mechanosensory organ that provides fast sensory information to stabilize body rotations and maintain steady flight. Some flies move their halteres while their large indirect flight muscles are inactive. The black scavenger fly, *Sepsis punctum*, moves its halteres during walking and also when displaying their spotted wings to potential mates and rivals. We hypothesized that haltere sensory function is used to structure non-flight wing movements, specifically those involved in social interactions. We found *S. punctum* perform rapid, discrete wing sweeps at higher rates in the presence of conspecifics. In male flies paired with female flies, these sweeps were faster and larger. Immobilizing the halteres reduced the speed and amplitude of the wing flicks. This result suggests that *S. punctum* uses information from the haltere to structure specific movements of its wings that are unrelated to flight behavior, a novel function for this sensory organ.

My Snake Is Smaller Than Yours: Tracing Wide Gaped Feeding In The North American Snake Fossil Record

Roxanne Armfield

The evolution of wide gaped feeding is often cited as the driver to the rapid speciation and subsequent global success of modern alethinophidian snakes. Yet despite this posited panacea to dietary versatility alethinophidian taxa show significantly higher levels of cranial morphological disparity than most tetrapod clades. Macrostomy is a complex multistate trait designation – and accurate attribution requires combining evidence from morphological, functional, behavioural, and ecological perspectives. Our understanding of the timing, acquisition and origins of these numerous traits is hindered by a poor fossil record lacking diagnostic cranial material, and by conflicting phylogenetic topologies which present contrasting evolutionary scenarios for character acquisition. New fossil specimens containing cranial material, and a new fossil species of alethinophidian snake from the Palaeocene of North America contest the model of a linear stepwise gain of wide-gaped feeding traits suggested by morphological phylogenetics, and instead present a more complex scenario of losses and gains of palatomaxillary mobility across this clade throughout its early history. We combine new fossil data with musculoskeletal relationships from extant taxa (acquired through DiceCT techniques) to evaluate the potential ecological niches exploited by these prehistoric taxa and predict the possible diet and feasible prey for these snakes living in the wake of the K-Pg extinction event.

Morphology and mitogenomic insights into the evolution of the sea spider genus *Ammothea* (Pycnogonida)

Madeline Armstrong, Jessica Zehnpfennig, Kenneth Halanych, Andrew Mahon

Ammotheidae (Pycnogonida, Chelicerata) is one of the most abundant pycnogonid families in the Southern Ocean, with *Ammothea* being the most represented genus in collections from this region. *Ammothea* has a convoluted history, including erroneous collection records and multiple changes in taxonomic descriptions that have led to confusion surrounding this genus. Taxonomical challenges are compounded by pronounced intra- and interspecific morphological variability along with a lack of genetic references. These combined factors have prevented a realistic understanding of relationships within *Ammothea*. Thus, a holistic evaluation of *Ammothea* using morphological and molecular approaches is warranted. In this study, we present an analysis of specimens collected over four research cruises throughout the Southern Ocean. To date, we morphologically identified 19 of the >30 Antarctic and sub-Antarctic species. Recognizing the prevalence of morphological plasticity within and between species, we also present 13 novel *Ammothea* mitochondrial genomes that increases our genetic references for this genus. This allows us to gain insight into the mitochondrial variation and enables us to reconstruct and visualize the evolutionary histories of species in this complex

genus. By utilizing both morphological and molecular techniques, we greatly improve our understanding of this dominant genus of Southern Ocean sea spiders.

Identifying the source of Utah's invasive fox squirrel (*S. niger*) through population genetics

Noah Armstrong, Dylan Klure, Robert Greenhalgh, Tess Stapleton, M. Denise Dearing

Invasive species threaten global ecosystems and understanding the origins of invaders is critical to their management. One such successful invader is the fox squirrel (*Sciurus niger*), which has been introduced to much of western North America throughout the 20th century. This species was most recently introduced to Utah in 2011, and the origins of this introduction remain unknown. However, we hypothesize based on morphology that the source of Utah's fox squirrel population may be the Texas fox squirrel (*Sciurus niger limitis*). To test this hypothesis, we performed Sanger sequencing on the complete mitochondrial cytochrome B (mtCytB) gene for 16 individuals sampled in Utah and compared the resulting sequences to publicly available data for this species. We identified a single haplotype present in all individuals from Utah that was distinct from publicly available mtCytB sequences, confirming this fox squirrel population was introduced recently and is highly inbred. However, due to the unique haplotype present in Utah and the limited publicly available data for this species, this single locus dataset was insufficient to identify the origins of Utah's fox squirrel population. To further resolve the origins of this introduced population, we are performing whole genome sequencing of individuals from introduced and native portions of this species' range. This approach should generate a robust genome-wide marker dataset that can be used to identify the source of this introduction.

The Ecomorphology of Batoid Gills

Sarah Arnette, Stacy Farina, Matthew Kolmann, Lara Ferry

Elasmobranchii, the subclass of Chondrichthyes comprising sharks, skates, and rays, are so named for their bilaterally paired "plate-gills." The number of gill slits have been used for taxonomic purposes; additionally, gill slit characteristics like height, spacing, and placement on the body vary with ecomorphotype in selachians. Batoidea, the clade comprising skates and rays, has the most species diversity in Chondrichthyes and exhibits similar habitat diversity to sharks, with pelagic, benthopelagic, and benthic types. In addition, batoids include both fresh and saltwater species, as well as electrogenic species. These physiological and ecological differences suggest variation in the gill morphologies of batoids akin to that observed in sharks. The specific goals of this study are to 1) describe the variety of character states of gill slit morphologies in batoids, 2) investigate evolutionary trends in gill slit morphology, 3) assess the relationship between ecological parameters collected from literature, like diet or habitat, and gill slit morphology, and 4) quantify ontogenetic changes in gill slit morphology. We conducted a phylogenetically-informed, morphological survey of ventilatory traits using specimens from natural history collections. Variables of study included disc width, body depth, gill height, gill width (space between sequential gills), distance between gills and the body edge, spiracle radius, and oral width. Preliminary results indicate that pelagic rays have longer and more evenly spaced gills, and freshwater rays have shorter gills.

Auditory brainstem responses and the ontogeny of pup vocalizations in California mice

April Arquilla, Kerianne Wilson, Jamiela Kokash, Jeffrey Rumschlag, Khaleel Razak, Wendy Saltzman

In most mammalian species, infants emit vocalizations that elicit behavioral responses from their parents. The parents' ability to detect, discriminate, and respond to these calls is often critical for offspring survival. Although most studies of parent-offspring communication in mammals focus on mothers, some species exhibit extensive paternal care as well. While evidence exists for parenthood-induced auditory plasticity in mothers, it is not known whether fathers undergo similar changes in processing of infant calls, thus highlighting the need for additional study of fathers' responses to infant cries. We tested

the hypothesis that auditory processing in adults changes with parenthood, such that new parents become more sensitive to frequencies at which pups vocalize, compared to sexually naïve adults. Using the biparental California mouse (*Peromyscus californicus*), we first examined pup calling behavior by analyzing characteristics of pup vocalizations from birth until weaning age. We next exposed parents and virgins of both sexes to a series of tones and recorded the auditory brainstem response latency and detection threshold level for each frequency analyzed. We found that, while pup vocalizations all fell within the range of adult hearing, parents unexpectedly exhibited reduced sensitivity to 24-kHz acoustic stimuli compared to virgin adults. These findings indicate that in biparental species, fathers, in addition to mothers, undergo plasticity in their processing of pup vocalizations. Funded by NSF IOS-2118607 to W.S. and K.A.R.

Assessment of microbiota colonizing dormant Antarctic zooplankton embryos

Hunter Arrington, Joseph Covi

Zooplankton dormancy is a well-known concept; however, passive defense mechanisms to resist microbial attack while dormant are not. Active defense against microbes would require metabolic activity. Yet multiple species, included the species presented, are known to have minimal metabolic activity to support an immune response during embryonic dormancy. As a first step in evaluating microbial defenses in dormant zooplankton, colonization of the surface of the Antarctic freshwater copepod, *Boeckella poppei* was evaluated using scanning electron microscopy and genetic microbiome analysis. The data presented here demonstrate that embryos of *B. poppei* have a biofilm that is apparent immediately after isolation from Antarctic lake sediments. The biofilm varies from individual microbes to a complex biofilm covering the embryo completely. An extracellular polymeric substance of putative microbial origin was observed. Prokaryotes and diatoms with diverse physical structure were observed as well as putative fungal hyphae and amoeboid-like organisms. Bacterial fission in progress was captured, which suggests that the biofilm is viable upon isolation of embryos from stored sediments. There is no evidence of cuticular pitting, which suggests the biofilm may protect against chitinase-producing microbes. Preliminary microbiome data support the presence of diverse prokaryotes and fungal communities. In addition to the variable biofilm, two novel outer layers to the cyst wall were identified in *B. poppei*.

Markers of hippocampal plasticity vary with glucocorticoid levels in a rodent model (*Octodon degus*)

Kaja Arusha, Gurprince Attlas, Cassandra Carlson, Mallory Duprey, Paula Duarte-Guterman, Carolyn Bauer

In mammals, development of the endocrine stress response is plastic during the post-natal period. Brain development is also plastic during the post-natal period, especially in the dentate gyrus of the hippocampal region, as this is one of the few brain regions where neurogenesis can occur throughout the lifespan. Stress hormone exposure can regulate several facets of hippocampal development, including hippocampal volume, microglial morphology, and neurogenesis rates. We used a rodent model (*Octodon degus*) to test whether endocrine stress profiles at weaning were linked with several parameters of the dentate gyrus including volumes of separate regions, density of different microglial forms, and expression of doublecortin, a protein indicative of immature neurons. Baseline and stress-induced cortisol levels showed no significant relationships with dentate gyrus volumes or doublecortin expression. However, we found a positive trend between baseline cortisol levels and density of amoeboid microglia in the dorsal dentate gyrus. Furthermore, we found a significant negative correlation between stress-induced cortisol levels and density of ramified microglia in the dorsal dentate gyrus. As the amoeboid form is considered an active player in phagocytosis, this suggests that *degus* with high baseline cortisol levels and heightened cortisol responsiveness may have higher rates of cell turnover, an important part of neuroplasticity. Glucocorticoids could be directly impacting hippocampal development; alternatively, these systems could be undergoing separate developmental trajectories in response to similar environmental stimuli.

Morphology and sexual dimorphism of Longfin Smelt (*Spirinchus thaleichthys*) in San Francisco Estuary

Marzieh Asadi-Aghbolaghi, Md Moshir Rahman, Levi S Lewis, Tien-Chieh Hung

Longfin Smelt is a species of forage fish in coastal habitats in the north-east Pacific Ocean. This migratory species inhabits a diverse range of environments, including coastal marine, estuarine, and tidal freshwater systems. Despite much research into its spawning and population biology, little is known regarding the intraspecific morphological variation of this species. Here, we quantified spatial and sexual variation in the external morphology of mature Longfin Smelt that were collected from the San Francisco Estuary in 2021-2022. The dataset was comprised of digital images of a total of 225 specimens, with 157 from the Lower-Estuary (South-Bay) and 68 from the Upper-Estuary (Sacramento/San-Joaquin Delta). Of these specimens, 137 were identified as females and 88 as males. An assortment of morphological traits and geometric-morphometrics were quantified using ImageJ, and MorphoJ. The geometric-morphometrics analyses identified significant variation in body shape and centroid size between specimens from the Upper versus Lower-Estuary, with fish from the Delta exhibiting greater plumpness around the dorsal and anal regions. Furthermore, pre-anal fin length, pre-pectoral fin length, and pre-dorsal fin length were larger in females; and caudal peduncle depth, dorsal-region, and body height being larger in males. Males also exhibited more convex bodies around the dorsal-region, more downturned mouths, larger anal-fin, and greater plumpness around the anal-region. Further studies are needed to understand the causes and consequences of this sexual and morphological variation.

A Selective Plane Illumination Microscope to Study Form-Function Relationships in Biological Needles

Haaris Asghar, David Labonte, Christopher Rowlands

Many plants and animals have independently evolved needle-like structures for a diverse array of functions. The morphological “design” of each needle is a reflection of its functional requirements, but it is also subject to hard constraints – the physical laws involved in performing puncture. By comparing puncture performance across biological needles it may be possible to understand how these demands interact to define an overall ‘design space’ across needle scale and function. This is, however, not an easy task: needle puncture involves complex failure mechanisms within the punctured material, which involve deformation and stress distributions that are complex and depend directly on needle morphology. To visualise these complex 3D strain fields, we have prototyped a Selective Plane Illumination Microscope (SPIM) to image gold nanoparticles within a sample at high spatio-temporal resolution throughout an indentation. When illuminated by a light sheet, these gold nanoparticles scatter light. This light sheet can be rapidly moved through the sample using a galvo mirror, allowing imaging across multiple planes. Using two such orthogonal pathways, with light of distinct wavelengths, individual particles can be localised and tracked in 3D throughout the sample volume, to subsequently infer particle displacement fields resulting from indentation and puncture. We demonstrate a proof-of-concept by analysing strain fields underneath a spherical indenter.

Does Early Investment in Beak Growth Affect Circulating Nutrient Levels in House Sparrow Nestlings?

Olivia Asher, Michael Butler, Brooke Weiss, Mae Maddox

In avian species, nestlings exhibit begging behavior to signal hunger to their parents. During begging, nestlings open their mouths widely, frequently displaying colorful interiors. Nestlings vary in quality and, in many species – including house sparrows (*Passer domesticus*) – increased vividness of mouth coloration can increase food delivery to nestlings. These higher feeding rates can result in higher circulating nutrient levels (e.g., triglycerides, glucose) in those nestlings. However, it is currently unclear whether mouth size, and not just coloration, is an important component of signaling in house sparrows. During the summer of 2023, we collected data on 184 nestlings, measuring body mass and beak length, width, and depth throughout the nestling period. We collected blood samples and measured glucose levels toward the end

of the nestling period. During the fall of 2023, we will finalize our statistical analysis examining whether larger mouths during the early nestling period are correlated with larger body masses and higher circulating nutrient levels in full-grown nestlings. We predict that larger mouths in the early nestling period are correlated to greater body mass, triglyceride levels, and glucose levels in nestlings toward the end of the nestling period. We will discuss the possible functions of larger mouth sizes, including an increased ability to signal to parents, as well as increased competitive advantages relative to nestmates.

Geographic variation in immune gene expression in wild rodent hosts of *Borrelia burgdorferi*

Vania R Assis, Kailey McCain, Allison Brehm, John Orrock, Lynn Martin

Host-pathogen dynamics might be strongly affected by variation in host competence (the ability of one host to cause infection in another host). Thus, understanding factors driving variation in aspects of competence is essential for understanding disease dynamics. Although competence is well-known to vary among species, we lack information about intraspecific variation in competence, particularly how variation in habitat conditions across geographic regions alters host immunity and, hence, community-level disease risk. We are studying how habitat quality affects immune gene expression in *Peromyscus leucopus* and *P. maniculatus*, as both can be hosts of the causative agent of Lyme disease, *Borrelia burgdorferi* (Bb). Using tissue samples from the National Ecological Observatory Network (NEON), we made two comparisons: how gene expression varied between species and across space for the more broadly distributed species, *P. leucopus*. We focused on genes playing strong roles in Bb detection (Toll-like receptor 2), resistance/inflammation (interferon- γ and interleukin-6), and tolerance, anti-inflammation, and tissue repair (GATA-3, interleukin-10, and tumor growth factor- β). As *P. leucopus* is more competent for Bb and its blood is better at preventing Gram-negative bacterial growth than *P. maniculatus*, we predict that immune gene expression in *P. leucopus* will be more inflammatory (higher TLR-2, IFN- γ , IL-6) but more tolerant of Bb (higher GATA-3, IL-10, TGF- β) than *P. maniculatus*. We expect similar patterns in *P. leucopus* from higher versus lower-quality habitats.

Bad to the bone: sternal morphology and ecological radiation in bats

Jeanne Augustin, Andrew Orkney, Brandon Hedrick

Bats are an extremely diverse clade, consisting of over one thousand species, who exhibit among the greatest dietary diversity of any mammalian order. Additionally, bats represent the only origin of powered flight within mammals and many are powerful echolocators. Bats are thus a model system to investigate ecomorphological adaptation and the acquisition of novel innovations. Extensive study of bird and bat wing skeletons has found a strong correlation between wing proportions and flight style, and further research on bird sterna has also demonstrated a complex relationship between sternal shape and aerial ecology. However, similar morphological studies in bat sterna are lacking, motivating us to explore the intersection between morphological evolution of the sternum and ecological radiation in bats. In pursuit of this question, we have compiled 3D models of sternal shape across 28 species spanning 6 ecologically diverse bat families. We found extensive variation in sternal keel morphology between families, such as the double notched sternal keel of Pteropodidae and wide variation in the degrees of fusion of the manubrium with the ribs and xiphoid process, with total fusion of both in Hipposideros. We will apply advanced 3D geometric morphometrics analyses in a phylogenetic context to relate this variation to a comprehensive set of flight style variables that we are assembling to uncover how bats have diversified to rule the night.

Making bioinformatics more accessible via course-based undergraduate research experiences (CUREs)

Anthony Auletta, R Keating Godfrey, Peter DiGennaro

Bioinformatics is an increasingly important skillset in comparative biology. Yet, despite high demand for these skills, many undergraduates in the biological sciences gain little exposure to hands-on bioinformatics research. To address

this gap, we developed a course-based undergraduate research experience (CURE) that embeds authentic bioinformatics research into the Entomology & Nematology curriculum at the University of Florida. In this course, 32 students/semester work in small teams to assemble and analyze insect genomes or transcriptomes using bioinformatics tools; they then use those data to pose and answer relevant biological questions. Since this research is entirely novel, it can lead to publishable results and thus contribute new knowledge to our discipline. As they work on their project, students also engage in discussions on research ethics, the global nature of research, and science communication. Here we present qualitative and quantitative data from our CURE showing that students made substantial gains in bioinformatics skills, critical thinking, communication ability, and other transferrable skills. Our findings suggest that this CURE provides many of the same benefits as traditional research experiences, but on a larger scale—thus engaging and recruiting a more diverse array of students in bioinformatics research. Furthermore, this guided experience “demystifies” bioinformatics and genomics for students, thus making this research more approachable. In this presentation, we will also share practical strategies for instructors interested in developing their own CURES.

The butterfly(fish) effect: Using Chaetodontidae territories as bioindicators

Corrine Avidan, Roi Holzman

Coral reef fish from the family Chaetodontidae has been used in monitoring and citizen science plans as bioindicators for the health of coral reef ecosystem due to their close association with reef habitat and, for some, the dependence on coral as food. Particularly, the diversity of Chaetodon species and abundance, as well as territory size have been measured to estimate the health of the reef in question. Here, we studied the space use of two coral-feeding Red Sea butterflyfish species (Chaetodon austriacus and C. fasciatus) in the Gulf of Aqaba to determine the changes in butterfly fish territories starting 3 years before, during, and up to 3 years after a major reef destruction event. In 2020, a massive 75% reduction in coral cover at a depth of < 3 meters were caused by an exceptional summer storm. Throughout all observations, both species maintained small exclusive pair territories with clearly defined territory boundaries. While the abundance of both species decreased after the reef destruction, there were no discernable differences in territory size or diversity of species even 3 years after the event. This suggests that territory size in Chaetodon may not be the best indicator for monitoring reef health.

The role of cardiosensory feedback during fight or flight behaviors

Joana Avrami, Luis Hernandez-Nunez, Florian Engert

Research in the field of systems neuroscience in the past decade has primarily focused on explaining behavioral dynamics as a function of brain activity. Very few studies integrate the role of internal organs and the autonomic nervous system in the study of behavior. Through the sensory autonomic nervous system, viscera can modulate brain internal states and consequently behavior. In order to incorporate the role of viscerosensory feedback into the study of natural behaviors, we tracked the functional development of cardiosensory circuits in larval zebrafish. Using anatomical imaging, we discovered developmental landmarks for the formation of the vagal sensory ganglia that innervates the heart. We used aversive phototactic stimuli and calcium imaging to determine the onset of cardiac encoding properties of vagal sensory neurons; and laser ablations of the vagal sensory nerve to determine the onset of functional feedback to the brain. Our results establish the key stages for anatomical and functional development of the cardiac vagus nerve in zebrafish and set the stage for incorporating interoception into the study of natural behaviors.

How does variation of covariation vary? Intraspecific patterns of fish brain region covariation

Caleb Axelrod, Ellen Urquhart, Swanne Gordon, Bruce Carlson

Covariation between traits is critical to understand because it can constrain evolutionary and plastic change. Patterns of trait covariation have been shown at broad taxonomic levels and have also been demonstrated with individual

systems. However, the degree to which specific patterns of intraspecific trait covariation vary among closely related species has yet to be investigated. The size of different regions of the brain have been shown to impact specific cognitive functions and have been shown to covary with each other. The extent of this covariation though has yet to be formally evaluated at the intraspecific level. We test for patterns of covariation of brain regions within 10 fish species. This allows us to compare these patterns across species to test if brain region covariation within species may be conserved across species, and the degree to which these patterns differ depending on taxonomic relationship. These data provide novel insight into how brains evolve and change within species, as well as how general patterns of trait covariation can change across species.

The dynamics of aerial and aquatic feeding in largemouth bass (*Micropterus salmoides*)

Erik Axlid, Tim Higham

Despite a lack of any known morphological specializations for terrestrial prey capture, several teleost fishes have been observed leaping out of the water to capture flying or suspended prey. The kinematics underlying these feeding events, however, are understudied and warrant attention due to the challenges presented to fishes capturing prey outside of the aquatic environment. For example, comparisons of aerial and aquatic feeding events in the silver arowana found that aerial prey capture involved larger, faster movements than aquatic feeding. However, several questions remain: Can these kinematic differences between aerial and aquatic feeding be generalized to all fishes? Do fishes change their jump (and pre-jump) kinematics based on the height of the prey, and how are these changes achieved? We addressed these questions using largemouth bass (*Micropterus salmoides*), which occasionally feed on terrestrial insects, by obtaining 3D high-speed video of feeding events on mealworms suspended by wire under water and at two heights above the surface. We then measured several kinematic variables related to the jump, such as the angle, velocity, and acceleration of the fish throughout the behavior, as well as variables related to cranial motion, such as the time to maximum gape, maximum gape, and jaw closing dynamics. The results of this project will broaden our knowledge of how organisms can expand their ecological niches by exploiting prey in vastly different environments.

Microplastics ingestion and adhesion by reef-building corals under different flow rates

Jeremy Axworthy, Sicheng Wang, Michelle DiBenedetto, Allie Johnson, Ruth Sofield, Jacqueline Padilla-Gamino

Microplastics, plastic particles less than 5 mm in size, are increasing in marine environments worldwide but their fate is not fully understood. Reef-building corals are suggested to serve as sinks for microplastics via two processes: 1) active removal through ingestion and 2) passive removal by adhesion. However, it is not known which type of plastics are more likely to be ingested or adhered to corals and whether water flow rate or coral morphology affects these processes. To understand the mechanisms associated with microplastic removal, we exposed the corals, *Leptoseris* sp., *Montipora capitata*, *Montipora digitata*, and *Pocillopora damicornis* to weathered polyester fibers, acrylic fibers, and polystyrene fragments under three flow regimes (2.5, 5 and 7.5 cm s⁻¹). Results indicate that adhesion rates are higher than ingestion rates and that fibers are more likely to be ingested by and adhered to corals. Adhesion increased with water flow and was also affected by species. Additionally, we observed higher adhesion rates on non-living sections of coral fragments than live tissue suggesting that non-living sections of reefs may also serve as an important sink for microplastics pollution. These data are critical for determining which environments corals are more likely to interact with different types of microplastics and which corals remove more microplastics from the water, ultimately helping to understand the risk to corals and the fate of these pervasive pollutants.

Closed-loop Manipulation of Active Sensing Movements of Weakly Electric Fish During Refuge Tracking

Emin Yusuf Aydin, Onurcan Yilmaz, Sinan Topcakar, Ismail Uyanik

Animals benefit from auxiliary active sensing movements to better explore and perceive their environment. Despite the ubiquitous nature of this phenomenon across animal taxa, the underlying mechanisms that initiate these movements are not known. The models in the literature either consider active sensing as open-loop noise generator or stochastic resonance generator. We developed a model that characterizes active sensing as a part of an optimal control framework, where active sensing generator seeks to improve the state-estimation performance in closed-loop manner. In this work, we study active sensing within refuge tracking behavior of weakly electric fish. These fish perform ancillary whole-body oscillations to improve their sensing when the visual information is absent or limited. Our goal is to manipulate the impact of these movements on the tracking performance of the fish using a custom-built experimental setup. We track the movements of the fish online and in real time using custom-tailored image processing algorithm. The digitized movements of the fish are fed back to the refuge to manipulate the sensory consequences of fish's own movements. We experimented with $N=3$ fish to observe how the fish modulates active sensing movements when their effect is amplified or attenuated. Our results—supported by computational models—show that the active sensing movement generation is a closed-loop process, where the animal aims to improve its state-estimation performance. Supported by TUBITAK (120E198).

Odor Complexity: A Novel Paradigm for Quantifying Odor Discrimination in *Bombus impatiens*

Rebekah Ayre, Jordanna Sprayberry

Research documenting the negative effects of anthropogenic odor pollution on pollinator foraging behavior has proliferated in the last decade. Our lab has used a combination of free-moving proboscis extension reflex (FMPER) to test associative odor learning, along with a novel odor-quantification paradigm (Compounds Without Borders, CWB) to explore the impacts of odor pollution on bumblebee behavior. However, FMPER measures generalization rather than the perceptual threshold of discrimination. In order to probe the neural substrates of pollution-driven behavior changes, we need to test odor-discrimination - not generalization. In this study we present a method of differential conditioning that measures the discrimination ability of bumblebees. We will use this method to test the transition of odor blend encoding from elemental (typical with simple stimuli) to configural (found with complex stimuli). Once we have identified a complexity threshold for this encoding shift, we will be able to update our odor-representation paradigm. This in turn will improve our understanding of how odor-pollution disrupts bumblebee odor-driven foraging.

Evolution of oxygen-sensing genes parallels whole-animal hypoxia tolerance in fishes

Courtney Babin, Félix Leiva, Wilco Verberk, Bernard Rees

Aquatic hypoxia (low dissolved oxygen) is broadly recognized as one of the major threats to freshwater and marine ecosystems worldwide. Because energy metabolism relies largely on oxygen, understanding the functional and genetic drivers of whole-organism hypoxia tolerance is critical. Here, we explore the molecular evolution of central genes in the detection of and response to hypoxia in ray-finned fishes: the prolyl-hydroxylase domain (PHD)-hypoxia inducible factor alpha (HIFα) system. We searched fish genomes for HIFα and PHD genes, which were subjected to phylogenetic and positive selection analyses. Within each gene group, the physicochemical properties of the amino acids putatively under positive selection were summarized with linear discriminants. We investigated the relationships between these linear descriptors of functional variation for each gene and hypoxia tolerance of fishes as reflected by their critical oxygen tension (Pcrit). Variation in HIF2A explained more of the variation in Pcrit than other HIFα or PHD homologs. Specifically, the high hypoxia tolerance in common carp and related species was associated with variation at specific amino acid sites of HIF2Ab, a teleost-specific duplicate that is retained in this lineage. Due to its high expression in gills, a tissue at the interface between fish and environment, HIF2A may be

central to sensing ambient oxygen levels. Thus, the evolution of a single gene (HIF2A) shows clear parallels with whole-organism hypoxia tolerance (Pcrit) in ray-finned fishes.

Successful Rearing Methods for the Brooding Six-rayed Star *Leptasterias*

Berenice Baca-Ceballos, C. Sarah Cohen

The genus, *Leptasterias*, uses a remarkable mode of brood-fostering. These tiny intertidal stars produce a small number of large, yolky eggs which develop with maternal protection, hatching as non-feeding brachiolaria. For ~2 months, 50-1500 embryos develop below the cardiac stomach of the parent star while she forgoes feeding. After ~2 months, juvenile stars venture out of the brood and fend for themselves. Culturing the embryos of this genus is extremely difficult as studies on the development of brood-caring sea-stars are meager.

Leptasterias is a cryptic species complex with congeners that specialize in contrasting habitats. Here, we collected embryos at different early developmental stages from Pigeon Point in central CA. Collections ranged from wave-exposed and wave-protected habitats of the marine rocky intertidal. These contrasting sites are generally inhabited by two different species *Leptasterias pusilla* and *Leptasterias aequalis*, respectively.

This study focuses on the successful culturing methods that have produced juvenile stars from early developmental stages with the absence of parental care. The brooded indirect development, where embryos pass through the larval brachiolaria stage, is closely documented. Variation in timing of hatching compared to the developmental stage was observed. Juvenile *Leptasterias* ate frozen squid, copepods, mussel, juvenile littorines, juvenile *Lacuna*, and newly settled barnacles.

Tracking heavy metals during bleaching stress and recovery in Hawaiian reef-building corals

Callum Backstrom, Lisa Rodrigues, Jacqueline Padilla-Gamino

Ocean warming has increased rates of coral bleaching, causing corals to expel their algal symbionts, stunting growth and reproduction. Bleached corals can feed on zooplankton/detritus for energy. However, these food sources could also cause bleached corals to bioaccumulate heavy metals, which can be essential nutrients but can become toxic in elevated concentrations. It is not known how concentrations of heavy metals fluctuate in bleached corals, change during recovery, and/or affect coral gametes after colonies bleach. In a controlled experiment, we thermally stressed *Montipora capitata* colonies in Kāne'ohe Bay, Hawai'i and sampled colonies during recovery after bleaching. We also quantified metals in egg-sperm bundles spawned the year following bleaching. After bleaching, metals including arsenic, cadmium, manganese, and vanadium decreased by 25-57% in coral tissues, possibly through symbiont loss. Symbiont acquisition coincided with increases in depleted metals to pre-bleaching levels, suggesting these metals are acquired through the presence of symbionts, and not by increased heterotrophy. Concentrations of metals in egg-sperm bundles were at least ten times lower than in parent tissue and did not differ between bleached and non-bleached colonies. However, arsenic in bundles was exceptionally high (up to 70% of concentrations in parent tissue). Arsenic could be preferentially stored in coral gametes due to its high lipid affinity.

Effect of Breeding Site Characteristics on Gray Tree Frog (*Hyla versicolor*) Disease Dynamics

Rafael Baez-Segui, Kristin Winchell, Anthony Snead

Once widespread across the New York City metropolitan region, Gray Tree Frogs (*Hyla versicolor*) have largely disappeared within the city limits. As urbanization increased and freshwater green spaces began to disappear, this summer-breeding, wetland-dependent amphibian has become confined to an increasingly shrinking number of ponds and wetlands that are surrounded by high-intensity developed land. Transit infrastructure, impervious surface cover and building density act as barriers to population connectivity. These isolated urban populations are forced to use the same ponds every year for breeding in densities that may exceed that observed in non-urban sites

where more opportunities for habitat choice exist. Combined with the novel stressors of urban environments, this forced breeding site fidelity may result in chronic stress in the form of elevated leukocyte counts, altered corticosterone blood concentrations, and higher incidences of infection from highly transmissible diseases like chytridiomycosis or blood-borne trypanosomal infections. To evaluate the impacts of urbanization of breeding patterns and disease dynamics, we will examine the physiological profiles of Gray Tree Frogs throughout their breeding season in wetlands that exist within the urban to rural landscape of New York City. Our findings will provide insight into how urbanization impacts the health of these native northeastern frogs. Results will inform potential routes for conservation and will shed light on how these anurans may be adapting to increasing anthropogenic pressure.

Environmental perturbations initiate compaction in fire ant clusters but at an energetic cost

Hosain Bagheri, Michael Goodisman, Daniel Goldman

Collective behavior in social insects enables the accomplishment of tasks that challenge individuals. For example, fire ant rafting allows colony survival over long durations. Rafts are aggregates at the water surface where ants link via mandibles and tarsal claws. While steady-stage rafting dynamics have been studied over the last decade [Mlot et al, 2011], the effect of mechanical environmental perturbations (e.g., water waves) on raft morphology, dynamics, and energetics is less understood. Preliminary experiments revealed ant rafts self-compacted under controlled vertical oscillation to their housing. Similar effects were observed under dry conditions. Thus, to focus on clustering and compaction dynamics without hydrodynamic interactions, ~250-350 ants were monitored as their dry housing container was oscillated at different frequencies (10-40 Hz) and accelerations (0-15 g). At accelerations below 1 g, aggregates did not form, and ants walked freely. At 1-2 g, ants became static and cluster formation initiated. As acceleration increased, clusters compacted and began bouncing. Such compaction may aid the collective against mechanical assault but at an energetic cost. Cluster bouncing was associated with an increased CO₂ emission rate. For example, at 30 Hz and 6 g peak acceleration, the CO₂ emission rate increased by ~2.2. We posit this results from increased individual binding rearrangements and force generation. This study provides insight into how superorganisms, like fire ants, use collective dynamics to survive mechanical environmental perturbation.

Assessing the Scale of Deep-sea Benthos Impact and Recovery After the Deepwater Horizon Oil Spill

Jeffrey Baguley, Elisa Baldrighi

The Deepwater Horizon (DWH) oil spill resulted in widespread ecological impacts to Gulf of Mexico ecosystems. Research cruises in 2010, 2011, 2014, and 2022 were organized to assess the initial impact and recovery trajectories of deep-sea communities. Meiofaunal community composition was significantly impacted after the spill. Total meiofauna abundance peaked in 2010 and consistently decreased until 2022. Conversely, major taxa diversity was lowest in 2010 and peaked 2022. The data suggest signs of recovery in the meiofauna community from the combined effects of enrichment and toxicity that resulted from the DWH oil spill. However, a complete comparison of chemical and biological patterns across all years is needed to confirm community recovery is linked to reduced contaminant loads. Harpacticoid copepod family diversity increased the spatial scope of DWH deep-sea impacts. Fewer families were represented in most heavily impacted and moderately impacted areas. Diversity increased from 2010-2014 at impacted stations. Tolerant and opportunistic families such as Ameiridae and Tisbidae dominated in the impact zone in 2010 and 2011 but decreased in abundance in 2014. Conversely, sensitive families Argastidae, Canthocamptidae, Ectinosomatidae and Zosimeidae were largely absent in the impact zone in 2010-2011, but became more abundant in 2014. While we can appreciate some signs of recovery in the harpacticoid community, full recovery had not yet occurred by 2014.

Effects of red tide on sea turtle hatching success on the Gulf of Mexico

Andrew Bailey, Jacob Lasala

Exposure to red tide (*Karenia brevis*) in varying quantities has been known to affect health in a variety of ways in a large number of species. Sea turtles are often found impaired in the water during harmful algal blooms and can be subject to boating injuries or death if the levels become too high. However, less is known about how uptake can affect nesting behavior and nest success. Nesting success, hatching success, and emergence success rates of loggerhead (*Caretta caretta*) & green sea turtles (*Chelonia mydas*) from 5 beaches between 2005 and 2023 in Sarasota County, FL were compared with the county's *K. brevis* bloom data over the same period of time to determine if different levels of red tide had a significant effect on hatchling development.

Morphological Development and Propagation of Odontodes in Ancistrus Armored Catfish

Julia Bailey, Wesley Dillard, Gareth Fraser

Stem vertebrates possessed both dental and bony dermal armor encompassing their head and trunk. Throughout evolutionary history, many taxa have either reduced this armor or lost both components entirely. Cartilaginous fishes have retained the dental component in the form of dermal odontodes, tooth-like structures made of dentine and enamel with a pulp cavity. Contrastingly, bony fishes, including teleosts, have retained only the bony component in the form of scales. In this study, we investigate *Ancistrus* armored catfish, a teleost fish descending from a scaleless ancestor that regained dermal armor with both dental and bony components. *Ancistrus* have bony scutes with odontodes covering the head and trunk region, but the development of these structures is poorly understood. We use a combination of diaphonization, Computed Tomography (CT), and light microscopy to visualize the propagation of odontodes across the body throughout development. *Ancistrus* are an understudied non-model organism that can provide valuable insight on the evolution of dermal armor not otherwise found in model organisms. Gathering developmental data on this species will not only provide insights into propagation patterns but also establish a developmental baseline, serving as a visual reference for ensuring accuracy and consistency in future research on embryological development.

Micro CT of hawkmoths and silkmoths uncovers parallels between muscle morphology and flight strategy

Joanna Baker, Ethan Wold, Leo Wood, Brett Aiello, Simon Sponberg

Hawkmoths (Sphingidae) and wild silkmoths (Saturniidae) are moth sister families in the superfamily Bombycoidea which have evolved two distinct strategies for agile flight. Hawkmoths feed from flowers using high-frequency hovering flight. Silkmoths do not feed as adults and instead use erratic bobbing patterns to evade predators. While wing morphology and kinematics are well-studied across Bombycoidean moths, we lack a comparative understanding of how muscle morphology reflects the evolution of different flight strategies. Because silkmoths fly at a lower frequency with large maneuverable wings, they might have greater reliance on direct flight muscles to facilitate wing steering. Conversely, the higher flight frequency and wing loading of hawkmoths might place more relative demand on indirect flight muscles, leading to greater reliance on frequency modulation for maneuverability. To test our hypotheses, we segmented thoracic musculature from uCT scans of ten Bombycoidean species. We found the ratio of direct to indirect flight muscle volume is higher in silkmoths and inversely proportional to wingbeat frequency. The dorsal oblique muscles, which likely modulate exoskeleton stiffness and are present in almost all hovering insects, have a larger relative volume and width in hawkmoths. In silkmoths this muscle is either narrower, greatly diminished or absent entirely. This suggests that the dorsal oblique may have a functional role in frequency modulation which is important for hawkmoths' exceptional maneuverability during hovering flight.

Spatial Analysis of Gene Expression in *Lumbriculus variegatus* with Hybridization Chain Reaction

Zachary Baker, Kathy Gillen, Seryne Rafique

Many animals heal severe wounds and replace lost body parts via tissue regeneration. One such animal is the segmented worm *Lumbriculus variegatus* (phylum: Annelida), which can regenerate whole independent organisms from just a few amputated segments. What causes animals to differ in their regeneration abilities, however, is unclear. Many genes are thought to play a role in animal regeneration, including homeobox (hox) genes such as post-2. These genes code for transcription factors that help specify antero-posterior positionality during development and may help maintain positional identity in adults. To work towards determining the importance of these genes in the regeneration of *L. variegatus*, we hope to characterize basal patterns of post-2 expression in *L. variegatus* and compare these patterns to those seen in regenerating organisms. To begin this work, we used a Hybridization Chain Reaction (HCR) based fluorescence labeling experiment that does not currently have a published protocol in *L. variegatus*. To test our protocols, we stained for beta-actin mRNA. We observed increased fluorescence compared to the negative control, suggesting that staining was successful. Observing post-2 with HCR showed increased fluorescence just behind the head and just before the tail of the worm. This pattern was not consistent between all organisms, however.

Parasite removal alters thermal tolerance in a tropical lizard

Leah Bakewell, Carrie Alfonso, Karla Alujevic, Samantha Fontaine, Jaden Keller, Yanileth Lopez, Nathaly Ponce, Alejandro Vivas, Claire Williams, Kelly Wuthrich, W. Owen McMillan, Michael Logan, Christian Cox

Climate change can influence host-parasite dynamics by altering the abundance and distribution of parasites. These changes can lead to increased parasite loads or exposure to novel parasites, both of which can impact host performance and fitness. In particular, parasites can impact both the immune system and the response to thermal stress, and the impact of shifting climates and parasites are likely to be strong in tropical forest lizards that conform to environmental temperature variation. We used antiparasitic drugs (ivermectin and praziquantel) that target roundworms and flatworms, respectively, to test how removal of parasites would impact immune performance and thermal tolerance in the slender anole (*Anolis apletophallus*) from Panama. Treatment with ivermectin reduced nematode infection and praziquantel decreased the prevalence of cestodes. Immune function and liver mass were not impacted by antiparasitic treatment, but spleen size was positively associated with cestode infection intensity. Antiparasitic treatment also increased the heat tolerance (voluntary thermal maxima) of lizard hosts. Our results highlight that shifting rates of parasitism might compound the effects of climate change by reducing the heat tolerance of hosts, further decreasing host ability to tolerate increasing temperatures.

A hypothesis for the role of optic flow in controlling different flight modes in hummingbirds

Vikram Baliga, Roslyn Dakin, Douglas Wylie, Douglas Altshuler

Hummingbird flight is especially tractable to the study of how optic flow, the global visual signal caused by self-motion through the environment, can be used to guide locomotion. Hummingbirds possess a wide field of view along with a fine-scale ability to control position during flight, and previous studies have established the role of optic flow in hummingbirds' control of position during hovering or forward flight. How global visual signals inform the control of flight velocity, however, has been underexamined. To develop an integrated hypothesis for the role of optic flow in flight control, we examined the behavior of Anna's hummingbirds (*Calypte anna*) in a virtual reality tunnel where optic flow could be systematically manipulated. Hummingbirds flew fastest when they had a reliable signal of optic flow. All optic flow manipulations caused slower flight, suggesting that hummingbirds had an expected optic flow magnitude that was disrupted. Upward and downward optic flow drove optomotor responses for maintaining altitude during forward flight.

During hovering, optomotor responses occurred in all directions. Together, these results suggest that an internal model of optic flow is used to control forward flight velocity but that optomotor responses control forward flight altitude and hovering position.

Hunger effects on the signaling and cleaning behaviors of the cleaner shrimp, *Ancylomenes pedersoni*

Miya Ball, Benjamin Titus, Dan Exton, Eleanor Caves

Cleaner shrimp inhabit coral reefs and have a mutualistic relationship with reef fish (clients) in which they consume parasites from the bodies of these fish. Typically, cleaner shrimp live at cleaning stations that clients pose in front of, waiting to be cleaned. Usually, cleaner shrimp will also signal to clients to initiate cleaning. The cleaner shrimp, *Ancylomenes pedersoni*, signals its intent to clean by whipping its antennae. I annotated videos from Curacao and Honduras of *A. pedersoni* and found that the likelihood of antennae whipping and cleaning increases as time since last clean increases, suggesting that shrimp are more likely to signal and clean as they get hungrier. I then manipulated hunger levels of cleaner shrimp in the lab to test the hypothesis that hungrier shrimp are more likely to antennae whip and attempt to clean visual stimuli than those that are satiated. Using a cutout of a yellow goatfish, a common client of this shrimp, as a visual stimulus, I showed that the proportion of interactions in which a given shrimp antennae whips and the proportion of interactions in which a given shrimp cleans is greater in hungry versus satiated trials. These results reveal the information content of signals in *A. pedersoni*: cleaners display their hunger level to clients. Future work should examine if and how clients benefit from learning about cleaner hunger.

Nemertean predators evoke behavioral responses in interstitial annelids

William Ballentine, Kelly Dorgan, Elizabeth Murphy

Chemical cues mediate organismal behaviors at every scale in the marine environment, yet the role of chemical ecology in the interstitial spaces of marine sediments and the behavioral responses of the animals (meiofauna) that live there are unexplored. Meiofauna must contend with an opaque, complex habitat and variable porewater flows; chemical cues are likely especially important interstitially due to the limitations imposed on other sensory mechanisms. To investigate the role of chemical ecology in the interstitial environment, we hypothesized that chemical cues from predatory meiofauna (Nemertea) would affect the behaviors and locomotion patterns of their potential meiofaunal prey. We observed the behavioral responses of protodrilid annelids in a transparent analog for sand (cryolite) when they directly encountered live nemerteans and in dishes of seawater when protodrilids were exposed to droplets containing homogenized nemerteans. We also used a newly-developed flow chamber (the Meioflume) filled with cryolite to expose protodrilids to flowing porewater containing nemertean cues. Initial observations suggest that protodrilids respond in all three scenarios. Behavioral responses included rapid contractions of the head and tail and initiation of or increased rate of locomotion. These results provide some of the first empirical evidence that interstitial chemical cues affect the behavior of meiofauna and suggest that chemical ecology is an important factor in understanding the behaviors, strategies, and small-scale community distributions of interstitial organisms.

Can I automate this? Managing expectations from AI-based tools

Shir Bar, Roi Holzman, Shai Avidan

Applications of artificial intelligence (AI) for data analysis in biomechanics, morphology, and behavior are becoming increasingly popular. However, the initial cost of entering this fast-changing field is high, and with many AI tools under active development, there is no guarantee that this initial investment will pay off. Furthermore, estimating the effort, the required infrastructure, and even finding the right models/tools to solve your problem can be a daunting

task. In this talk, we will provide potential users with some intuition about the costs and benefits of using AI-based tools in the context of biomechanical and behavioral research.

We will tackle three questions: First, what do you need to have to start using AI-based tools in-house, and particularly, what should your data look like? Second, what properties of your data might complicate the application of such tools? And, importantly, when is AI an ill-fitting solution for your data/problem? Third, what can you expect to gain realistically? How to let go of the concept of automation and think about how to leverage imperfect AI tools to reduce, rather than eliminate, your workload. We will then review useful tools (e.g., for landmark detection and tracking, and behavior analysis) that can be integrated into research with relatively little overhead, and some newer methods that can provide an intuition for the developments yet to come (e.g., shape reconstruction and analysis).

Exploring how individual behavioral variation affects service quality in marine cleaning mutualisms

Bryce Barbee, Eleanor Caves

Marine cleaning mutualisms are interactions in which small fish and shrimp remove material from larger 'client' fish species. Small groups of the cleaner shrimp, *Ancylomenes pedersoni*, live at cleaning stations and interact with a large variety of client reef fish species. Given the benefits entailed in these interactions for cleaners (who receive a meal), extending cleaning services should theoretically be advantageous for all cleaners. However, in the field there is substantial variation in how individual shrimp behave during cleaning interactions, with some individuals cleaning much more often than others. Here, we explore behavioral variation among individual cleaners in both the lab and the field. In the lab, we found statistically repeatable differences in the boldness behaviors between individuals. In the field, we then linked this variation in boldness behavior to variation in the quality of cleaning services offered at a cleaning station, to test the hypothesis that a group's cleaning service quality is shaped by the behavioral composition of the individuals that comprise the group. These cleaning mutualisms play an important role in the health and diversity of coral reef communities. Exploring the role that individual behavioral variation has on a group's service quality will help us understand the mechanisms that shape the interspecies partner choices within these interactions.

Anti-bat ultrasound production in moths is globally and phylogenetically widespread

Jesse Barber, Akito Kawahara

Warning signals are well known in the visual system, but rare in other modalities. Some moths produce ultrasonic sounds to warn bats of noxious taste or to mimic unpalatable models. Here, we report results from a long-term study across the globe, assaying moth response to playback of bat echolocation. We tested 252 genera, spanning most families of large-bodied moths, and document anti-bat ultrasound production in 52 genera, with eight subfamily origins described. Based on acoustic analysis of ultrasonic emissions and palatability experiments with bats, it seems that acoustic warning and mimicry are the *raison d'être* for sound production in most moths. However, some moths use high-duty-cycle ultrasound capable of jamming bat sonar. In fact, we find preliminary evidence of independent origins of sonar jamming in at least six subfamilies. Palatability data indicate that jamming and warning are not mutually exclusive strategies. To explore the possible organization of anti-bat warning sounds into acoustic mimicry rings, we intensively studied a community of moths in Ecuador and, using machine-learning approaches, found five distinct acoustic clusters. While these data represent an early understanding of acoustic aposematism and mimicry across this megadiverse insect order, it is likely that ultrasonically signaling moths comprise one of the largest mimicry complexes on earth.

Individual Variation and Seasonal Repeatability of Egg Size in the European Starling

Raven Barbera, Tony Williams

Avian egg size varies widely within populations; in European starlings (*Sturnus vulgaris*) the largest mean egg size was 47% larger than that of the smallest. Many studies have found egg size to be a highly repeatable trait, suggesting low phenotypic plasticity; however, other studies continue to focus on adaptive plasticity of egg size. If egg size is a phenotypically plastic trait, we would predict it to vary systematically based on ecological context. We analyzed the egg size of nest box breeding starlings over five years at our study site in Langley, BC. Starlings typically double-brood so we compared egg size – as well as other life-history traits, e.g. lay date, clutch size, and number of chicks fledged – between 1st and 2nd broods with different ecological conditions and productivity outcomes. Birds did not adjust egg size between 1st and 2nd breeding attempts at the population level. We also found no significant co-variation of egg size with relative lay date, clutch size, or number of chicks fledged. Egg size was highly repeatable in females that double-brooded: egg size was largely maintained (not plastic) despite 'adaptive' adjustment of other life-history traits (e.g. clutch size) and seasonal variation in productivity. Individual variation in egg mass is still largely unexplained so we will expand our dataset and examine if egg size contributes to variation in lifetime reproductive success and survival.

Grizzly bears (*Ursus arctos horribilis*) alter amino acid kinetics during their winter fast

Perry Barboza, Nicolaas Deutz, Charles Robbins, Gabriella Ten-Have, John Thadden, Amelia Christian, Anthony Carnahan

We measured kinetics (95%CI) of 21 amino acids in post-absorptive adult grizzly bears (4♂ 268 – 278 kg peak mass; 5♀ 194 – 200 kg peak mass) to examine how bears reallocate body protein during the winter fast. Bears were studied during the hyperphagic period in August at 89 – 95 % of peak body mass. Measures were repeated at 86 – 93% peak mass in December (40 d fasted) and at 72 – 79% peak mass in March (127 d fasted). Taurine production declined by 27 – 40% from August, which was consistent with reduced bile synthesis through winter. Increased production of tau-methyl-histidine by 60 – 134% indicated mobilization of myofibrillar proteins through winter. In December, the production of glycine, glutamate, glutamine, isoleucine, leucine, valine, phenylalanine, tyrosine, tryptophan, histidine, proline, and methionine were only 60 – 74% of the August rate but production rose to 70 – 85% of that active rate in March. Production of the essential acids threonine and lysine were high in March at 82 – 100% and 130 – 160% of the respective rates in August. Slow protein turnover in early winter is consistent with declines in energy expenditure that conserve body fat stores for a prolonged fast. Rises in protein turnover in late winter are probably associated with the reactivation of digestive, absorptive, and intermediary processes as bears prepare to resume feeding and mass gains in spring.

Fish Out of Water: Effect of Substrate on Jumping Forces in the Blackspotted Rockskipper

Dan Bartlett, Kaylin Raffle, Hayley Pettit, Miranda Brainard, Paityn Houglan, Kaelyn Gamel, Zachary Nopper, Rebekah Harden, Henry Astley, Austin Garner, Richard Londraville

Escape responses are vital for the survival of prey. In water, fish display a highly stereotyped escape response called the C-start. However, some fish species have evolved to spend considerable time outside of the water in coastal habitats, necessitating a terrestrial escape response. Unlike the uniform properties of water, terrestrial habitats can vary in substrate friction, further complicated by wetting, requiring fish to either adjust their behavior in response or adopt robust performance mechanisms. We tested the terrestrial jumping of the blackspotted rockskipper (*Entomacrodus striatus*) to determine if substrate roughness and wetness influence their jumping performance. We predicted that substrate conditions could drastically change performance. Using a novel waterproof force plate capable of detecting millinewton loads, we collected ground reaction forces on wet and dry sandpapers of varying grits. We also conducted micro-CT scans to determine the proportion of muscle that makes

up the rockskipper to determine if power amplification was involved in this defensive response. Preliminary analysis of jumping forces revealed a significant difference in maximum horizontal forces between wet and dry substrates and no evidence for power amplification. This study serves as a field test for the new force plate and will yield information that furthers our understanding of defensive responses in amphibious fish.

Assessing Heat Resilience and Energy Allocation of Cryptic Coral Lineages in Palau

Shantelle Bartley, Carsten Grupstra, Matthew-James Bennett, Maikani Andres, Kirstin Meyer-Kaiser, Annabel Hughes, Aden Nagree, Sarah Davies

Coral bleaching can lead to colony starvation due to the loss of carbon sugars from the photosynthetic algal symbionts. Yet, some corals living in naturally high-temperature environments exhibit adaptations including increased energy stores that can facilitate survival during the nutritionally challenging conditions associated with bleaching. Semi-enclosed lagoon habitats in Palau's rock islands experience temperatures similar to those expected under future climate change; however, corals of the species *Porites lobata* that live in these lagoonal habitats experience less bleaching than adjacent colonies on cooler outer reef sites. Here, we identified three genetic distinct lineages (L1-L3) within these Palauan reefs and thermal challenge experiments revealed that the two lineages mainly inhabiting the warmer lagoonal reefs (L2 and L3) are more thermally tolerant than the lineage largely restricted to the cooler outer reefs (L1). We conducted a reciprocal transplant experiment and quantified host and symbiont energy reserves after 1 year to test if L2 and L3 exhibited increased energy stores. We found that transplantation did not significantly affect these energetic traits, and instead constitutive differences between the lineages were observed. Specifically, L2, but not L3, had higher energy stores than L1. These data suggest that these lineages are likely adapted to higher temperatures in distinct ways: increased energy reserves facilitate L2 survival in high-temperature environments, but L3 likely employs a different, yet undetermined, adaptive strategy.

Exploring Correlations Between Habitat Complexity and Biodiversity Through Quantitative Analysis

Dkaria Bascom

This project aimed to quantify data from live footage from dive sites in and around Hubbard Springs visited by our scientific divers. Three sites were selected. "Drop 180/181", "Sandy Drop 159", and Hubbard Springs, each with distinct bottom compositions. These substrates significantly influenced fish biodiversity. Our scientific divers characterized the presence of species using the following categories: some (< 1), few (2-10), many (11-99), and abundant (100+). Prompt reporting of data occurred after every dive, coupled with immediate processing and review of video footage upon surfacing. Results unveiled Hubbard Springs (nearly 100% natural reef with a 10 ft max relief) as the most biodiverse of the three sites and hosted species like goliath grouper, white fin remora, leopard toadfish, and mangrove snapper. Drop 180/181 (80% natural reef with a 2-foot max relief). This site featured species like white fin remora, black seabass, polkadot batfish, and sand perch. Conversely, Drop 159 (90% sandy bottom and approximately max ½ ft relief) exhibited the lowest presence of species among the visited sites. Drop 159 featured limited quantities of white fin remora and white grunt. Statistical analyses were conducted to understand the relationships between species diversity and habitat, showcasing the data from each site.

Paddling against the waves: how varying wavelength kinematics affect tomopteris locomotion

Nick Battista

The soft-bodied, midwater polychaete *Tomopteris* is an interesting swimmer. Not only do *Tomopteris* swim continuously throughout their life, they also perform two modes of locomotion simultaneously: metachronal paddling and bodily undulation. They have two rows of flexible parapodia positioned on opposite sides of its body, which beat out of phase to one another. Their

metachronal paddling behavior occurs in concert with their lateral bodily undulation. A notable feature of their bodywave is that it moves in the direction of travel, unlike eels or smooth-bodied polychaetes who use a rearward-directed wave for forward swimming. In this work we used a computational fluid dynamics model to explore the effects of varying wavelength kinematics, morphology, and scale on tomopteris swimming performance.

How Does Squid Skin Shine? Investigating reflectins with *D. pealeii* structural color and iridescence

Eden Anne Bauer, Jennifer McCarthy-Taylor, Stephen Senft, Carrie Albertin, Roger Hanlon

Octopuses, squids and cuttlefish are noted for their complex neural networks, sensory capabilities, limb dexterity and flexibility, and highly dynamic camouflage abilities. Camouflage patterns are rapidly generated by manipulating pigment-containing organs called chromatophores in concert with white leucophores and iridescent, reflective iridophores, which house specialized proteins named reflectins. Reflectins can self-organize into a wide variety of intracellular structures, including spheres and Bragg stack-like plates, to produce white diffusion and tunable iridescence. We investigated how reflectin diversity contributes to structural colors in *Doryteuthis pealeii*, the longfin inshore squid. *D. pealeii* was chosen due to its sequenced genome, accessibility, translucent body, and simpler skin anatomy compared to octopus and cuttlefish. Phylogenetic approaches identify 17 candidate *D. pealeii* reflectin sequences. We employed in-situ hybridization chain reaction (HCR) for eight of these sequences in fixed *D. pealeii* adult tissue, hatchlings, and embryos. Additionally, we examined morphological development of iridescence in these animals using light microscopy. Preliminary HCR images illustrate overlapping but unique patterns of reflectin expression in surface layers of the eye, ink sac, arms, and mantle of late-stage embryos and hatchlings. These results provide insight into where, when, and how different reflectins are deployed in *D. pealeii*, informing ongoing efforts to understand fundamental mechanisms of coloration and camouflage in cephalopods.

Bone distribution in the avian humerus and its correlation with flight style

Stephanie Baumgart, Andrew Moore, Emma Schachner

In vertebrate powered fliers, the forelimbs are specialized to navigate low-density fluids (air) and resist the stress and strain of the flight stroke. With over 10,000 species occupying many ecological niches, birds provide a powerful system for exploring the relationship between bone structure and ecology, in both volant and non-volant taxa. Here we evaluate the relationship between internal structure and external shape of the humerus and how bone distribution specifically relates to flight style. Our taxonomic sampling incorporates birds across four orders of magnitude of body mass (8g–11300g) assigned to 28 orders and 51 families to determine how bone distribution changes with flight style. We used microCT data from 15 evenly spaced slices from each avian humerus; some taxa were also evaluated with functionally homologous slice locations compared against eight evenly spaced slices to investigate how slice selection method affects results. The data show that birds with continuous flapping have the largest variation in bone distribution, whereas soarers or short-burst fliers are clustered together in bone structure morphospace. Diving and swimming birds have a broad range in bone morphospace. These results indicate that bone distribution correlates with flight style in extant birds. Further exploration of bone distribution in vertebrate fliers will elucidate how both external and internal bone morphology is related to behavior, and may provide useful for interpreting the ecologies of fossil taxa.

Trophic discrimination of compound-specific stable isotopes in raptor nestlings

Anna Bautista, Devin Johnson, Michael Henderson, David Anderson, Cory Williams

Bulk stable isotope analysis (BSIA) of carbon and nitrogen has been commonly used for assessing trophic relationships among organisms in a wide variety of ecosystems. However, compound-specific stable isotope analysis of

individual amino acids (CSIA-AA) may be a more accurate approach for resolving food web structure. We conducted BSIA and CSIA-AA of red blood cells and muscle samples from nestling raptors and their potential prey in an Arctic tundra ecosystem to compare the applicability of both methods. We subsequently estimated bulk and amino-acid specific trophic discrimination factors (TDFs) for nitrogen in gyrfalcons (*Falco rusticolus*) using high-precision diet estimates from nest cameras to compare against TDFs in the literature. Although bulk $\delta^{15}\text{N}$ values of secondary consumers were enriched relative to primary consumers, overlap occurred across groups and greater separation was apparent using CSIA-AA. Bulk TDFs and trophic amino acid TDFs between nestling gyrfalcons and their prey were lower than values from the literature that have typically been used to estimate trophic position. Our findings suggest that while CSIA-AA has the potential to improve our assessment of trophic relationships, the assumption that bulk and amino acid specific TDFs remain constant across all consumers and diet types is violated for juvenile raptors, such that consumer-specific TDFs must be calculated to accurately estimate trophic position.

Metabolic Variability Across Altitudinal Gradients: Exploring the Energetic Dynamics of Grasshoppers

Simran Bawa, Lauren Buckley, Sean Schoville, Caroline Williams

Locally adapted insects are being negatively impacted by warming temperatures, leading to range contractions and declines in population size. Metabolically cold-adapted species, who typically have higher metabolic rates (short-term energy usage), are more vulnerable to warming temperatures and exhibit variations in developmental time due to shorter growing seasons. To address the metabolic consequences of local adaptation, my work focuses on the Rocky Mountains grasshopper, *Melanoplus boulderensis*. It is a short-winged, early-season species with low dispersal, which now emerges from diapause half a month earlier than it did in the 1950s as a result of warming average temperatures. We performed a reciprocal transplant experiment to determine the extent to which grasshoppers are locally adapted to their collection site, in terms of their metabolic rates and energy balance. This experiment compared transplant grasshoppers with wild-caught ones, distinguishing between plasticity and population origin effects. We hypothesized that the higher elevation origin grasshoppers would have higher metabolic rates than lower elevation grasshoppers, which could be deleterious when transplanted to lower elevations. We found that metabolic rates and energy stores were primarily influenced by rearing environment rather than origin of population, suggesting that local adaptation will not impact the responses of this species to future climate change.

Energy Landscapes of Large Neotropical Bats: The Relationship Between Heart Rate and Accelerometry

Travis Bayer, María Camila Calderón-Capote, James Lee, Margaret Crofoot, Dina Dechmann, Teague O'Mara

Accelerometry-derived energy estimates based on dynamic body acceleration have been shown to represent quality measures of energy expenditure in birds and some terrestrial mammal species. However, the complex kinematics of bat flight may make these proxies for energy expenditure less accurate than in other taxa. For example, to increase travel speed, animals can increase the frequency that their limbs cycle or exert more force. Bats will instead often alter the kinematics of the flight such as the angle at which their wings are positioned during wingbeats to modulate their flight speed. This leaves uncertainty about the accuracy of acceleration-based estimates of energy expenditure. To validate relationships of energy expenditure and accelerometer-based movement in bats, we deployed both heart rate loggers along with GPS and triaxial accelerometers on individual greater spear-nosed bats. We used heart rate estimates of energy expenditure to develop relationships between dynamic body acceleration metrics and estimated energy expenditure. These relationships are then used to show movement-based energy landscapes – or the distribution of energy expenditure over space and time. Calibrated energy landscape perspectives allow the exploration of how and why individual bats make movement decisions, as well as more accurate estimates of the costs of their high-energy lifestyles.

Loggerhead (*Caretta caretta*) Hatchling Disorientations on Gulf of Mexico Beaches

Isabella Beasley, Jacob Lasala, Melissa Macksey

Sea turtles find the ocean by crawling towards the brightest horizon and moving away from sand dunes and large shadows. Their ability to find the ocean can be impacted by artificial light sources that illuminate the beach, removing natural visual cues. The Sea Turtle Conservation and Research Program (STCRP) at Mote Marine Lab has documented loggerhead (*Caretta caretta*) hatchling disorientations in Sarasota and Manatee counties, Florida, USA from 1989 – 2023 across five beaches that vary in size, building sizes, and population. Disorientation data were used to determine the primary hatchling disorientation direction, to quantify what caused the most disorientation events, to determine how moon phase impacts the number of disorientation events, and to identify how many hatchlings disorient from those that emerge from their nests. When disoriented, hatchlings crawled southeast across all beaches, with most attracted toward condominium building lighting. Moon phase significantly affected the number of disorientations that occurred, with the most events occurring during the fourth quarter of the lunar cycle. While the percent of hatchlings from each nest is roughly the same on every beach, the number of disorientation events is dominated by Longboat Key. Additionally, the number of events on every beach is increasing independent of the increase in number of nests on each beach.

Neophobia, but not perch hopping or corticosterone, is sensitive to the intensity of chronic stress

Ursula Beattie, Lily Mikolajczak, Nina Fefferman, L. Michael Romero

To further elucidate the role that wear-and-tear plays in the transition from acute to chronic stress, we manipulated the intensity and duration of applied chronic stress to determine if physiology and behavior would respond proportionately. We brought wild house sparrows into captivity and subjected them to high stress, medium stress, low stress, or captivity-only over the course of six months. We varied the number of stressors per day and the duration of stress periods to vary wear-and-tear, and thus the potential to exhibit chronic stress symptoms. We assessed corticosterone (baseline, stress induced, and negative feedback) neophobia (the fear of the new; assessed via food approach latency), and perch hopping (activity). We predicted that birds would show proportionate decreases in neophobia and activity throughout a long-term chronic stress paradigm and that if the hypothalamic-pituitary-adrenal (HPA) axis was causing these changes in behavior, similar proportionate changes in corticosterone would be measured. Our results indicate that neophobia is sensitive to the intensity of chronic stress, however the birds became more neophobic – the opposite of what we expected. Conversely, perch hopping and corticosterone did not differ across treatment groups and are thus not sensitive to the intensity of chronic stress in this paradigm. Together, these data show that different aspects of behavior are impacted differently by long-term chronic stress and can be driven by pathways other than the HPA axis.

Investigating metrics of individual repeatability of the stress response

Ursula Beattie, David Harris, L. Michael Romero, Michael Reed

To date, there is no consensus on the best way to calculate individual repeatability of stress response curves. The most common technique for assessing repeatability is to use linear mixed-effect models, which can be used only at a population level and only on discrete timepoints. In the current study, we compared three metrics of individual repeatability that incorporate the whole stress response curve: profile repeatability, Kullback-Leibler (KL) divergence, and hypothalamic-pituitary-adrenal (HPA) flexibility. Using three datasets (one synthetic and two corticosterone datasets from live birds), our objectives were to compare how these metrics correlate with one another and to determine how representative repeatability scores of fewer replicates were to the “consensus” score (i.e., the score of the full dataset). We found that (1) these three individual repeatability metrics do not consistently correlate with one another; (2) KL divergence and HPA flexibility are poor at distinguishing individuals from each other; and (3) that profile repeatability tends to overestimate repeatability when fewer replicates are available and the consensus score

is low. Despite this drawback of profile repeatability, we suggest that it may be the most well-suited metric for assessing individual repeatability of stress response curves as the scores generated using KL divergence and HPA flexibility may not be biologically meaningful. We also introduce a new R package, *profrep*, for calculating profile repeatability.

A planar omnidirectional treadmill to study insect locomotion

Hendrik Beck, Fabian Plum, David Labonte

Insects seem to move effortlessly in a variety of habitats. They can walk upright, climb up vertical walls, stick to inverted surfaces, and are robust to the loss of one or even multiple limbs. Studying these fascinating locomotor skills has been a long-standing source of inspiration for six-legged walking robots. However, obtaining 3D kinematics data of insects remains difficult and at best tedious - insects generally do not cooperate. Previous work utilized spherical treadmills that were either passively or actively actuated to tackle these issues. However, passive actuation requires to tether insects, and active actuation still relies on either large spheres or involves a restricted recording area, requiring rapid feedback loops that may perturb natural locomotion.

To circumvent these limitations, we developed a planar omnidirectional treadmill, which perpetually keeps walking insects in a central region of interest. In brief, a set of small treadmills is mounted on a large treadmill; the small treadmills and the large treadmill move in perpendicular directions, and are actuated by independent motors; the insect's position on the treadmill is tracked in real-time, and serves as the control input.

The treadmill design allows observation of natural locomotion on a flat surface; insects can freely select speed and walking direction over long periods of continuous gait cycles. By deploying our treadmill to study the 3D kinematics of insect locomotion, we hope to provide a better understanding of how insects adapt to different mechanical conditions.

Patterns of arm use during walking in young *Octopus bimaculoides*

Ivan Beck, Melina Hale

Octopuses can use any of their eight arms to walk and can generate movement in any direction in relation to their anteroposterior body axis. The neural control underpinning this flexibility in locomotion is only beginning to be understood. This study takes a behavioral approach to investigating arm coordination and deriving underlying principles of neural control. We recorded locomotion in young *Octopus bimaculoides* in an open arena environment (N=5 animals). Consistent with a previous study (Levy et. al., 2015), we did not find locomotor gaits. However, further analysis showed consistency in other aspects of the movement pattern. We found that the likelihood of a given arm to be planted after the previous one was based on proximity. For backwards movements, the closer the arm to the currently planted arm, the greater its likelihood to be planted next. For movements to the right/left sides of the animal, the arms directly adjacent or two arms-away all were roughly equally likely to be recruited next. Over the course of several cycles of arm planting, we recorded a sequence of recruitment that generally alternates between recruitment of the next arm in a positive (clockwise) and negative (counter-clockwise) position in relation to the arm currently planted. We suggest that this pattern helps to maintain the direction of travel and use of the preferred set of arms for that behavioral context.

Impacts of marine heatwaves on coral environmental memory and cross-generational acclimatization

Danielle Becker, Ariana Huffmyer, Lauren Zane, Pierrick Harnay, Terava Atger, Hollie Putnam

Environmental challenges such as increasing temperature can result in either beneficial or negative legacy effects in reef-building corals. However, our understanding of the impact of marine heatwaves on environmental memory is not well investigated. In this study, we hypothesized that sublethal exposure of *Acropora pulchra* in Mo'orea, French Polynesia to a simulated bleaching event during gametogenesis would increase offspring thermal performance. Colonies (n=12) were split in half and exposed to diel fluctuations in ambient

temperature or a marine heatwave for four weeks, resulting in decreased symbiont density, chlorophyll content, photosynthesis, and respiration in exposed colonies. Such responses indicate a reduction in energetic capacity that could limit parental provisioning to offspring or trigger epigenetic effects. Six months following exposure, high spawning success rates were observed in both the ambient (100%) and heatwave parental histories (87.5%). Gametes from the heatwave history had significantly larger egg sizes (38%) and higher fertilization success (25%) than the ambient history. When offspring were subjected to a reciprocal thermal stress experiment, those from parents who had experienced a marine heatwave had significantly greater survivorship (20%) under thermal stress compared to offspring from ambient colonies. These results demonstrate that the offspring of parents challenged by thermal stress exhibit enhanced fitness through environmental memory. As climate change continues to threaten reef persistence, cross-generational acclimatization may allow early developmental stages to survive stress events.

Production of Reactive Oxygen Species Post-Amputation in *L. variegatus* Suggests Role in Regeneration

Freya Beinart, Kathy Gillen, Emily Banthin, Amanda Carroll, Sydney Buchman

Many animals can replace body parts lost to injury, an ability that varies depending on the species, specific organ, and developmental period. In animals studied to date, a burst of reactive oxygen species (ROS) occurs after injury and this ROS upregulation is required for proper regeneration. Many annelids show remarkable regeneration ability, but little is known about ROS production post-amputation in these animals. We investigated ROS levels in the widely available freshwater annelid *Lumbricus variegatus*, which can regenerate both head and tail ends from as few as four internal segments. Using the ROS activated fluorescent dye H2DCFDA, we observed a transient ROS increase at the wound site after amputation. By decreasing the ROS burst with various chemical inhibitors, such as the NADPH-Oxidase inhibitors diphenyleneiodonium (DPI) and apocynin (APO), we are testing if inhibiting ROS production limits regeneration in this model annelid. Determining the necessity of ROS generation for annelid regeneration will help reveal how phylogenetically conserved this phenomenon is, and thus whether it may be a requirement for regeneration in all species. Furthermore, if ROS are required for regeneration in *L. variegatus*, it supports their use as model organisms for uncovering tissue regrowth mechanisms that may be applicable to other animals.

I-RISE with U-RISE at Detroit Mercy: Preparing Underrepresented Trainees for Research Careers

Rachelle Belanger, Elizabeth Roberts-Kirchhoff, Kendra Evans, Eric Krukonis, Jahzara Mayes, Tommie Smith

The purpose of the NIH Undergraduate Research Training Initiative for Student Enhancement (U-RISE) program is to foster the development of underrepresented research trainees for research-focused careers (e.g., Ph.D. or M.D./Ph.D.). Detroit Mercy's program will recruit, train, and mentor underrepresented undergraduates for three years (undergraduate years 2-4) per student with a curriculum emphasizing scientific literacy, development, and research. Trainees will be introduced to both course-based undergraduate research experiences (CUREs) and mentor-engaged intensive research experiences. The I-RISE with U-RISE program provides trainees with early entry into authentic research experiences, student success support, research seminars, a scientific writing course, big data and data reproducibility training, repeated Responsible Conduct of Research (RCR) training, and a required external summer research experience at a research-intensive institution. Overall, we seek to provide trainees with 1) a sense of belonging, comfort, and connectedness in their environment, including their research laboratories, 2) support to earn a bachelor's degree in a biomedical science-related discipline, and 3) assistance with applications to and matriculating into graduate programs (master's or doctoral) in the biomedical sciences. To achieve these goals, we seek to develop scientific identity, support cohort building, provide advising and professional development, model careers in biomedical sciences, and provide extensive summer research experiences.

Epigenetic Patterns in Juvenile Mussels: DNA Methylation and How it Drives Thermal Tolerance

Riya Belani, Wes Dowd, Lani Gleason, Richelle Tanner

Climate change is influencing the frequency and intensity of extreme temperatures for organisms worldwide, but the effects are especially felt in the marine intertidal zone where organisms like *Mytilus* mussels already live near their thermal limits. The thermal plasticity of physiological traits like growth is well-understood in *Mytilus* mussels, but we understand far less of the epigenetic mechanisms underlying these traits and their inheritance. We explored whether DNA methylation levels in juvenile mussels varied between sites with different thermal variances in an intertidal zone at the Hopkins Marine Station in Monterey, CA. Juvenile mussels were exposed to protected (warm) and exposed (cool) wave conditions in a field acclimation experiment. Previous studies from this experimental design showed higher growth rates and survival in exposed (cool) juvenile mussels after one month. In this study, gill tissue was collected from these same individuals to undergo reduced representation bisulfite sequencing (RRBS) to evaluate methylation at CpG islands in the genome. We quantified, visualized, and compared DNA methylation profiles between the treatment conditions using Bismark and MethylKit. Global methylation levels were not significantly different between the cool and warm sites, however, we found some patterns related to genes involved in key cellular stress pathways and growth. This research is a first investigation into the role of epigenetic modifications that influence physiological tolerance under heat stress in highly dynamic environments.

Combining molecular analyses with reef surveys: Monitoring benthic components on reefs located at To

Stanton Belford

Annual coral reef surveys continue to monitor abundance and distribution of benthic organisms in marine ecosystems. Recent surveys recorded cnidarian and invertebrate abundance off the northeastern coast of Toco, Trinidad, specifically for scleractinian corals, zoantharians, and sea urchins. However, some species are difficult to accurately identify due to morphological similarities. One such cnidarian species are zoantharians, which have differences in oral disc and tentacle colors making in situ identification of these marine cnidarians challenging. Additionally, invertebrates such as, sea urchin color morphotypes occur, therefore molecular and phylogenetic analyses in this study gained a more comprehensive understanding of species diversity in this region. This study used the line intercept transect and quadrat methods for abundance data, species morphology and molecular analyses for species identification in reef survey data. Benthic surveys at 3 sites: Salybia Bay (SB), Pequelle Bay (PB), and Grande L'Anse (GLA) recorded mean cover 47%, 12%, and 22% respectively. Zoantharian mean cover was 32%, 70%, and 51% respectively, with *Palythoa* and *Zoanthus* spp. being most widely distributed. Molecular analyses using the mitochondrial cytochrome oxidase subunit I (mtCOI) identified specimens as *Z. pulchellus*, *Z. sociatus*, *P. caribaeorum* and *P. grandiflora*. Specimens sequenced for the mitochondrial 16S rDNA marker added another species *Z. aff. pulchellus*. Mitochondrial COI identified the sea urchin *E. lucunter*. Sequences were deposited in NCBI, and GenBank accession numbers were obtained for specimens.

Rhinoceros Beetles Carry Informational Chemicals About Body Size and Sex

Micah Bell

Japanese rhinoceros beetle (*Trypoxylus dichotomus*) males have exaggerated horns used to compete for feeding territories. Larger males with larger horns generally win competitions, providing them the potential to mate with female beetles. However, agonistic interactions between males appear to begin with an initial assessment ritual, which often results in one beetle retreating without escalating to physical combat. It is unknown what information competing beetles may be able to communicate to each other during the assessment ritual. In many insect species, chemical signals can carry a range of information, including social position, nutritional state, morphology, and sex. Specifically, cuticular hydrocarbons (CHCs), which are waxes excreted on the surface of insect exoskeletons, are responsible for diverse forms of

chemical communication in insects. Here, we asked whether CHCs in rhinoceros beetles carry information about body size and sex that males could use during assessment behavior. The CHCs of male and female Japanese rhinoceros beetles were extracted by washing the elytra of deceased beetles in hexanes. Samples were then analyzed through gas chromatography-mass spectroscopy (GCMS). Multivariate analysis of the composition of hydrocarbons observed in GCMS spectra revealed patterns associated with sex and multiple body size components in males (horn length, pronotum width, elytra length). We suggest that male rhinoceros beetles could communicate body size information through CHCs, explaining the decision-making behind escalating to combat and retreating behaviors after the initial assessment. We also suggest that male rhinoceros beetles could identify a conspecific's sex through analysis of CHCs.

Decadal variation in ocean productivity mediates life history trade-offs in an ecosystem sentinel

Roxanne Beltran, Allison Payne, A. Marm Kilpatrick, Richard Condit, Conner Hale, Madison Reed, Elliott Hazen, Steven Bograd, Joffrey Jouma'a, Patrick Robinson, Emma Houle, Wade Matern, Alea Sabah, Kathryn Lewis, Samantha Sebandal, Allison Coughlin, Natalia Valdes Heredia, Francesca Penny, Sophie Dalrymple, Heather Penny, Meghan Sherrier, Ben Peterson, Joanne Reiter, Burney LeBoeuf, Dan Costa

Rapid environmental changes are visibly altering terrestrial food webs, but open ocean ecosystems are far less understood due to the difficulties of observation. We examined decadal-scale oceanographic drivers of demographic traits in elephant seals, which integrate resources over thousands of kilometers in the open ocean. We then estimated the impacts of observed demographic variation on predator-prey interactions. Productive oceanographic conditions increased maternal mass gain and allocation to offspring size, resulting in six-fold higher first-year survival and four-fold higher recruitment success for offspring. As a result, seal population growth rates fluctuated dramatically with ocean conditions; the strongest cohort produced three-fold more pups than the weakest cohort. A population pulse under the strongest cohort demography would result in an additional 26,000 seals consuming 15 million more kilograms of mesopelagic fishes compared to the weakest cohorts. Reduced oceanographic productivity could shift elephant seal population trajectories from 4% growth to a 14% decline each year. Our results show how environmental changes in the ocean cascade through the food web to mediate the magnitude of trade-offs faced by organisms. Therefore, top predator populations may be more sensitive to climate perturbations than previously thought; future climate change could threaten population viability of large marine vertebrates and the ecosystems that depend on them.

Ex vivo and in situ work loops capture function of rat medial gastrocnemius during locomotion

Caitlin Bemis, Nicolai Konow, Praneeth Abburi, Andrew Biewener, Monica Daley, Kiisa Nishikawa

Time-varying muscle force remains challenging to predict despite our understanding of muscle function. Some challenges owe to differences in approaches for measuring muscle function in vivo and ex vivo / in situ approaches to measure muscle mechanics under isometric or isotonic conditions. New methodology is required to integrate these approaches. We used scaled in vivo muscle fascicle strains from sonomicrometry and electromyographic activation of rat medial gastrocnemius (MG) as inputs for mouse work loop experiments, ex vivo for extensor digitorum longus and in situ for MG. Work loops from both preparations strongly resembled those from rat MG during in vivo treadmill locomotion at varying speeds (ex vivo $r^2 = 0.796 - 0.959$; in situ $r^2 = 0.638 - 0.961$). Both preparations best replicated force at fast locomotion (ex vivo $r^2 = 0.796 - 0.923$; in situ $r^2 = 0.862 - 0.961$), which exhibit larger strain and velocity transients compared to slower locomotion, thus possibly demonstrating the importance of strain perturbations during muscle force production. Optimization of strain and activation inputs successfully accommodated differences across species and muscles in size, activation dynamics, fiber types, and strain excursion. The ability to scale and optimize ex vivo, in situ, and in vivo approaches provide a powerful tool for building our understanding of muscle properties and function during locomotion.

BUMP: a Benthic Underwater Microscope with Pulse Amplitude Modulation Capabilities

Or Ben-Zvi, Paul Roberts, Devin Ratelle, Joseph Snider, Pichaya Lertvilai, Daniel Wangpraseurt, Dimitri Deheyn, Jennifer Smith, Jules Jaffe

The central driver facilitating life on earth is the abundance of marine photosynthetic micro-organisms which utilize sunlight energy to assimilate atmospheric carbon while releasing oxygen. Despite this significant function, the means of measuring photosynthetic efficiency in-situ of these microalgae have been limited due to their aquatic habitat, small size and their occasional occurrence as endosymbionts. To address this challenge, we have developed the BUMP, a submersible, diver-operated microscope that provides high-quality micro-imaging and variable chlorophyll-a fluorescence-based measurements of photosynthetic efficiency (Fv/Fm). An important subject of such studies is the symbiotic partnership between photosynthetic dinoflagellates and their coral hosts.

Results, to date, have consisted of system calibration followed by laboratory and field testing. Preliminary data indicates that the system can reliably measure maximal and effective photosynthetic efficiency as well as capture useful micro-images. In laboratory experiments, we acquired parallel images of symbiont abundance and Fv/Fm of Red Sea corals, demonstrating depth-dependent photophysiological differences. During field deployments in both the Red Sea and Hawaii, we documented morphological differences between taxonomically-close coral species that were otherwise difficult to discriminate and recorded high spatio-temporal resolution chlorophyll fluorescence dynamics under ambient irradiances and natural conditions. The BUMP therefore shows promising attributes that allow, in-situ and in real-time, imaging and measurements of photosynthetic capacities of critical systems.

Comparative analyses of long non-coding RNA activity in three coral species

Zachary Bengtsson, Hollie Putnam, Steven Roberts, Sam White, Danielle Becker

Long non-coding RNAs (lncRNAs) represent a diverse class of transcripts exceeding 200 nucleotides in length. While they do not encode proteins, they are implicated in a variety of regulatory processes impacting gene expression—such as guiding chromatin-modifying enzymes, modulating transcription, influencing post-transcriptional processes, and interacting with both proteins and micro RNAs (miRNAs) to alter their activities. In this study, we use RNA-seq data from three coral species (*Acropora pulchra*, *Porites evermanni*, and *Pocillopora meandrina*) to characterize lncRNAs and evaluate activity with respect to gene and miRNA activity. Over 1000 lncRNAs were characterized in each species, and differential expression analysis identified a small subset of lncRNAs unique to each taxa. Furthermore, distinct patterns in the genomic landscape were revealed, showing a clear relationship with other transcript types. Studying lncRNAs in corals provides insight into an important player in physiological responses and acclimatization, allowing researchers to better anticipate how regulatory mechanisms will respond to environmental stressors and to compare the resilience of different coral species.

Integrative approaches to studying intraspecific physiological adaptation to environmental change

Phred Benham

Analysis of geographic variation has long been a cornerstone of evolutionary research, yet studies comparing physiological variation across two or more populations of the same species remain rare. The increasing ease of generating genome-scale datasets now provides novel opportunities to integrate intraspecific variation from genomic variants to whole-organism performance that promises to advance our understanding of the proximate and evolutionary mechanisms shaping avian adaptation to changing environments. To highlight the power of this approach, I present work on salt marsh adaptation within the Savannah sparrow. High salinity makes salt marshes a challenging environment for songbirds, yet multiple populations of Savannah sparrows derived from a freshwater-adapted ancestor have established populations in this habitat. Taking advantage of these replicated tidal marsh populations, I integrate genomic and physiological data to investigate: (1) how demographic and

selective forces interact to shape physiological divergence, (2) the genomic basis of physiological divergence across salinity gradients; and (3) how a focus on intra-specific variation allows for the incorporation of time-series data of phenotypic and genomic responses to climate change in salt marsh systems. This work highlights the need to account for evolutionary processes beyond selection (e.g. gene flow) in shaping patterns of physiological divergence and the power of genomic data for generating novel insights and hypotheses about the physiological mechanisms contributing to adaptation in extreme environments.

SEA Scholars: Communicating Research through Science, Education, and the Arts

Chelsea Bennice, Jeanette Wyneken

The mission of the Florida Atlantic University Marine Science Laboratory (FAU Marine Lab) is to conduct novel and necessary scientific studies on marine life, train the next generation of scientists, and share discoveries with the public to increase understanding of marine science, conservation awareness, and ocean stewardship. Scientific outreach is an important educational activity for students and faculty. FAU established a visitors' gallery overlooking the Marine Lab to engage visitors from children to seniors in science. In the past year, more than 244,000 visitors engaged with scientists/ scientists-in-training. This commitment to scientific outreach sparked the need for additional formal and informal education. To foster this vision, the first cross-disciplinary program, The Glenn W. and Cornelia T. Bailey Marine SEA (Science, Education, and Arts) Scholars program was created. SEA Scholars "get their hands wet" by (i) participating in research, (ii) engaging in public education and outreach activities, and (iii) becoming effective science communicators to broad audiences. This program capitalizes on the students' diverse backgrounds and talents spanning science, education, and arts. Students joining the program share their skills to develop both static and interactive ways to communicate the science that is fun, at STEM events (reaching over 23,000 people). Additionally, we create free virtual resources for use on multiple platforms. SEA Scholars gain valuable training as they help bridge the gap between scientists and the community.

Octopus Skincare: Insights to Understanding the Microbiome for Two Tropical Octopuses

Chelsea Bennice, Lauren Krausfeldt, W. Brooks, Jose Lopez

Coastal marine ecosystems are among the most important providers of biodiversity and ecosystem services, but at the same time are heavily impacted by threats that could risk the health of key species to marine food webs. Despite their small size, microbes play a critical role in the physiology of animal hosts; however, little is known about bacterial symbionts with octopus and whether such associations aid in octopus health. The skin microbiomes of two octopuses (*Octopus vulgaris* and *Macrotritopus defilippi*) along with environmental samples (sediment and seawater) were analyzed to determine if octopus have a unique microbiome. High throughput sequencing of the bacterial 16S rRNA gene (V3-V4 region) amplicons was performed using an Illumina MiSeq. Linear Discriminant Analysis Effect Size identified taxa and their relative abundances. Microbial communities differed between octopus, sediment, and seawater samples. The octopus microbiome had significant representation from phylum Bacteroidetes while there was significant representation from phyla Actinobacteria and Proteobacteria for sediment and seawater, respectively. Flavobacteria was the major class representing the octopus microbiome, which contains pathogen-suppressing members. Octopus species-specific microbial compositions were detected at the genus and species level. This was the first study to characterize the octopus skin microbiome for these wild octopus species. Understanding the importance of bacterial symbionts can provide insight to the physiology and ultimately the health of these important animals inhabiting many marine environments.

Rapid behavioral effects of endogenous & exogenous corticosterone vary by sex in sibling chicks

Morgan Benowitz-Fredericks, Sierra Pete, Stephanie Walsh, Alexis Will, Shannon Whelan, Alexander Kitaysky

Glucocorticoid responses have presumably been selected to redirect physiology and behavior in ways that help maintain homeostasis, but the function of drastic corticosterone elevations in young chicks is not always clear. We tested the hypothesis that in facultatively sibling kittiwake chicks, elevated corticosterone increases mobilization of endogenous stores and promotes short-term behavioral changes that facilitate resource acquisition. We experimentally elevated corticosterone in 5 day old chicks in 2-chick broods, measured ketone levels, and recorded behavior for 60 min before and after experimental manipulation. To decouple effects of corticosterone from other components of the stress response, we evoked both endogenous (via restraint) and exogenous (via topical corticosterone application) increases in corticosterone. Restraint substantially elevated corticosterone, which was correlated with increased ketone levels and subsequent sex-specific behavioral changes. Post-restraint corticosterone was positively correlated with increases in male aggression, female begging, and the number of feeds regardless of sex. In the exogenous corticosterone experiment, 60 min post-application, corticosterone was positively correlated with ketones and increased male aggression, but had no effect on female behavior or number of feeds. Different behavioral outcomes for endogenous and exogenous corticosterone elevations indicate that other hypothalamus-pituitary-adrenal axis components play a role in redirecting chick behaviors. We conclude that rapid corticosterone elevations in young kittiwakes increase use of endogenous energy stores and may shift chicks' behavioral resource acquisition strategy in sex-dependent ways.

Effects of dehydration on oxidative stress and osmolality in a desert-adapted rattlesnake, *Crotalus atrox*

Derek Benson, Dale DeNardo

Paramount to an organism's survival is its ability to maintain homeostasis in response to environmental and ecological challenges, such as those predicted to result from climate change. While temperature dominates the focus on climate change impacts, an often-underappreciated component of climate-change models is the decrease in rainfall predicted for many xeric environments that (for some locations) is expected to be both rapid and drastic. Due to the short timescale at which climate change is anticipated to occur, many organisms may not be able to evolutionarily adapt quickly enough to mitigate impacts. Therefore, understanding how organisms maintain homeostasis in response to environmental challenges through fast-acting plastic mechanisms such as physiological modulation and transcriptional changes is of great interest. A key aspect of homeostasis across vertebrates and a potential organismal vulnerability to climate change is the regulation and balance of reactive oxygen species to antioxidants. Reactive oxygen species are naturally generated by mitochondrial dysfunction, but many ecological factors can increase their production. Reactive oxygen species are highly volatile and cause both cellular and DNA damage. Organisms can either consume or produce antioxidants to counteract the negative effects of reactive oxygen species. When the amount of reactive oxygen species exceeds the antioxidant capacity of an organism fitness can be compromised. To better understand the dynamics between dehydration and oxidative balance I examined osmolality, reactive oxygen metabolites, and antioxidant concentrations initially, after 13 weeks of water deprivation, one week after receiving water again, and one month post-rehydration in western diamond-backed rattlesnakes, *Crotalus atrox*.

Effects of thermophily-relevant temperature variation and sex on digestive performance in pythons

Derek Benson, Dale DeNardo

Ectothermic animals utilize their environment to achieve body temperatures that maximize the performance of the most critical physiological process at a given time. Though unable to generate their own body heat, ectotherms have remarkable control over their body temperature. For example, during digestion snakes often elevate their body temperature by 1-3°C depending on

their hydration state. Despite knowing the existence of performance-specific thermophily, it is not well understood to what extent such small changes in body temperature affect performance. Accordingly, we determined whether small, thermophily-relevant changes in body temperature impact digestion efficiency or passage time. Eighteen (9 female and 9 male) adult Children's pythons (*Antaresia childreni*) were fed a meal at each of three temperatures (29°C, 30°C, and 31°C). We found that neither digestion efficiency nor passage time were significantly affected by temperature within this range. However, digestion efficiency was significantly impacted by the interaction between sex and temperature, in that males had significantly lower digestive efficiency than females at 31°C, but not at 29°C or 30°C.

The Methyl farnesoate mystery: investigating its role in crustacean ecdysteroidogenesis and molting

Vanessa Bentley, Donald Mykles

Methyl farnesoate (MF), a crustacean juvenile hormone (JH) produced by the mandibular organ, controls metamorphosis, reproduction, and molting in decapods. Molting is stimulated through the increased production of 20-hydroxyecdysteroid (20-E) by the Y-organs (YOs), which are antagonistically regulated by molt-inhibiting hormone (MIH) produced by the X-organ/sinus gland complex (XO). Furthermore, MF impacts ecdysteroidogenesis, although the effects may depend on the ecdysteroid titers. In vitro assays showed that YOs were responsive to JH mimics, but not to MF. It is hypothesized that MF and JH analog action is mediated by the Methoprene tolerant (Met)/Krüppel homolog 1 (Kr-h1)/E93 transcriptional cascade in the YO. Transcriptomic analysis of the YOs from the blackback land crab (*Gecarcinus lateralis*) and green shore crab (*Carcinus maenas*) revealed MF signaling and metabolic genes, including Met, Kr-h1, E93, Steroid receptor coactivator (Src), CREB-binding protein (CBP), Farnesoic acid O-methyltransferase (FAMeT), and MF esterase(s). These components were differentially expressed over the molt cycle indicating the YO response is determined by the hemolymph ecdysteroid titers. MF stimulates ecdysteroidogenesis at low titers (intermolt stage) but is inhibited with high 20-E levels (pre-molt). MF may mediate the YO transitioning from the committed to repressed state in late pre-molt when 20-E peaks and drops prior to ecdysis. Supported by NSF IOS-1456942 and IOS-1922701.

A Molting Mystery: LGRs Role in Limb Regeneration

Kendal Berasley, Jorge Perez-Moreno, David Durica, Donald Mykles

Crustacean fisheries are one of the most profitable and damaging industries to the ocean, prompting researchers to study various ways to reduce the need for wild caught animals. This study examines a signaling pathway for molting in *Gecarcinus lateralis* (black-backed land crab), which is an essential process for growth, development, and reproduction of crustaceans and thus has significant implications for aquaculture, fisheries, and conservation. The aim of this study is to identify the receptor for the Limb Autotomy Factor (LAFpro), which allows for the regeneration of new limbs through the regulation of limb bud growth. LGR3s or leucine-rich repeat G-protein coupled receptors are a group of LAFpro receptor candidates that putatively bind to insulin-like peptides, which in turn delays crabs from entering pre-molt and allows them to regenerate damaged limb buds before continuing with the molting process. Similarly, LGR3s have been shown to bind to insulin-like peptides to delay molting in *Drosophila* from larva to adult when imaginal discs are damaged. Bioinformatic and phylogenetic analyses were conducted using the CrustTome database to identify and characterize the LGR3 repertoire across crustaceans with emphasis on *G. lateralis*. Candidate receptors were then evaluated for differential gene expression in the Y-organ, and endpoint PCR was performed across tissue types and molt stages to further determine expression patterns and possible roles across the molt cycle. Supported by NSF IOS-1922701.

High-resolution tracking of zooplankton reveals metabolic control of diel vertical migration

Cory Berger, Ann Tarrant

Diel vertical migration (DVM) of aquatic animals is arguably the largest migration on Earth, and occurs each day in most marine and freshwater ecosystems.

DVM is influenced directly by factors such as light, food, and predator abundance, but is also regulated by internal circadian clocks. Untangling the mechanistic controls of DVM, and the relative importance of direct vs. endogenous cues, has been hampered by a lack of experimental systems. Here, we leverage advances in animal tracking software to develop an imaging system allowing us to quantify the positions of dozens of individual copepods (*Acartia tonsa*) at sub-second resolution over multiple days. We use this rich dataset to describe diel behavioral patterns over small spatial scales (~30cm) that entrain to light cycles and regular feeding, and which persist in constant conditions. This demonstrates DVM-like behavior in this species controlled by a circadian clock. Using feeding experiments, we also find that daytime-restricted feeding appears to weaken circadian behavioral rhythms compared to nighttime-restricted feeding. Our results 1) illustrate that food availability can impact DVM indirectly via effects on internal clocks in zooplankton; and 2) provide a novel methodology for future studies of circadian zooplankton behavior. More generally, our tracking data provide a high-resolution view of copepod movement ecology, allowing us to interrogate how population-level patterns emerge from the behaviors of individual animals.

Conserved mechanisms of vertebral evolution mediate clade-specific body proportions in dinosaurs

Philip Bergmann, Emma McLellan

Variation in body region proportions along the anterior-posterior axis is a primary axis of diversity in vertebrates. The evolution of regional proportions can be mediated by changes in the number and/or length of vertebrae, and how the vertebrae evolve is clade-specific. Dinosaurs have incredible variation in body proportions, and this is also clade-specific, including the long-necked sauropods to the long-bodied theropods, and short-necked ceratopsians. We use models of trait evolution to understand the mode and tempo of the relative proportions and number of vertebrae of 133 species of Dinosauria. We find different rates of evolution of the neck, tail and cervical vertebrae between the Ornithischia, Sauropodomorpha, and Theropoda. These three clades evolved different proportions of the thoracic region and vertebrae and the sacral region. We then used PGLS regression to test whether the relative proportions of each region were mediated by changes in the number or the relative length of their vertebrae, and whether these patterns also differed by clade. Surprisingly, across all clades, relative elongation of the neck, thorax and tail was mediated through both an increase in vertebral number and a lengthening of the vertebrae, demonstrating conservatism of mechanism. This differs from mammals, which evolve body proportions almost exclusively through changes in vertebral length, and from lizards and snakes, which do so almost exclusively through changes in vertebral number.

Plant genotype drives rhizosphere microbial community assembly during invasion

Mae Berlow, Katrina Dlugosch

Introduced plants that proliferate in their new environments due to a complex of mechanisms that can be challenging to disentangle, but soil microbiota likely play a role through both enemy release and enhanced mutualisms, and other mechanisms. What is less understood is the role that evolution of invaders plays in exploiting the novel microbiota of their new environments. We combine field soil sampling and a factorial greenhouse experiment with native and invader *C. solstitialis* genotypes and soil microbial communities to better understand the relationship between rapid evolution of an invasive plant and soil microbial communities. We hypothesize that native and invaded range soils present different bacterial communities from which plants draw their root bacterial communities, and that native and invader *C. solstitialis* genotypes will associate with bacteria differently. We found that field soil samples differ in both diversity and specific bacterial taxa between native and invaded ranges. We also found differences between these measures of rhizosphere microbial communities between plant genotypes, but only when grown in invaded range microbial communities, but not in native range communities. Altogether, our research provides insight into the role of microbial communities in the consequences of plant evolution during an invasion.

To eat or not to eat sugar: A bat dilemma

Andrea Bernal, Jasmin Camacho, Xexi Yi, Oscar Murillo-García, Nicolas Rohner

Among mammals, bats show the greatest trophic diversity, exhibiting diets based on insects, meat, blood, fruits and/or nectar. The dietary evolution from an insectivorous ancestor to organisms with diets based on sugar, instead of proteins or lipids, imposed physiological, anatomical and morphological changes. With the aim of elucidating the dietary evolution in Neotropical bats we evaluated sugar assimilation in chiropterans with different diets. We performed oral glucose tolerance tests with three sugars (glucose, sucrose and trehalose), looked for positive selection in genes coding for enzymes, and calculated the absorptive area of the duodenum. We found phylogenetic signal for disaccharide assimilation, and differences on sugar metabolism between diets after controlling for evolutionary relationships. We also found higher trehalose (sugar on insect's hemolymph) assimilation in omnivorous and insectivorous bats, while *treh*, the gene coding for trehalase enzyme, is a pseudogene in fruit and nectar bats, the ones who oppositely showed higher glucose and sucrose assimilation. Bats with rich-sugar diets showed higher villi surface exposed to nutrients and higher number of enterocytes and microvilli in the duodenum, suggesting a higher capability of sugar absorption. This result matches the extreme capability of glucose absorption of frugivorous and nectarivorous bats, (blood glucose levels above 600-750 mg/dL) and their accelerated absorption capability (glucose peak 10 min after glucose ingestion) compared to insectivorous or hematophagous bats (glucose peak 60 min after glucose ingestion).

Total vs Free CORT in predicting nest abandonment across 6 passerine species

Hannah Beyl, Creagh Breuner

In conservation physiology, hormones have the potential to predict changes in reproductive success as disturbance alters habitat quality. There is evidence that glucocorticoid (GC) physiology predicts reproductive success across vertebrate classes, but patterns are not consistent. To further explore possible wide-spread utility of GC physiology as a conservation tool in passerines we measured GC reactivity in late-incubation females across 6 species and evaluated the relationship between GCs and subsequent nest abandonment. GC physiology did not predict abandonment across species (95 nests); in individual species' analyses, elevated baseline GCs predicted greater abandonment propensity in incubating Brewer's blackbirds, but not in American Robins, gray catbirds or mountain bluebirds (the remaining two species did not have adequate sample size for species-level analysis). These results do not support GCs as a useful tool generally in predicting abandonment propensity. However, there is evidence that binding globulins may clarify the relationship. Plasma corticosteroid binding globulins (CBG) regulate access of GCs to tissues. A previous study found that free (unbound to CBG) GCs predicted nest abandonment in European starlings where total GCs did not. We are currently running CBG assays from these same individuals to further investigate the utility of GC measures in predictions of reproductive success.

Variation in heat shock elements and its role in the evolution of Cnidarian heat stress response

Janki Bhalodi, Adam Reitzel

Heat shock proteins (HSPs) are vital chaperones that prevent proteotoxic conditions. HSP transcription is regulated by heat shock factors (HSFs) which bind to heat shock elements (HSEs) in gene promoters. Despite participating in this highly conserved system, HSEs vary in sequence and number among HSP promoters of related species and populations. *Nematostella vectensis* is a model Cnidarian with naturally-occurring populations along a temperature gradient, ranging from Nova Scotia to Florida. Here, we explored the role of HSEs in the evolution of Cnidarian heat stress response. We compared HSE number and sequence variation in the promoters of various HSP70 and HSP90 genes, common biomarkers of heat stress, between *N. vectensis* and other Cnidarians. Our results show that the number of HSEs differ among the various HSPs and species. Additionally, our results indicate that some nucleotide positions in canonical HSE sequences may be more flexible compared

to others. Together, these findings suggest an adaptive role of HSE variation in Cnidarian heat stress response. Future work will characterize HSE variation among different *N. vectensis* populations and its effect on the expression of downstream HSPs. This research provides a unique perspective on the role of regulatory elements in the evolution of Cnidarian heat stress response and highlights the opportunity for similar approaches in other species.

Towards a general model for legged locomotion

Vikas Bhandawat, Tirthabir Biswas, Ali Tehrani-Safa

Despite the overall complexity of legged locomotion, the motion of the center of mass (COM) itself is relatively simple and can be qualitatively described by simple mechanical models. In particular, walking can be qualitatively modeled by a simple model in which each leg is described by a spring-loaded inverted pendulum (SLIP). Here, we explore SLIP as a quantitative model for locomotion. Using numerical simulations, analytical approximation, and fitting to locomotion data collected using flies and humans, we have come to the following three conclusions. First, we show why SLIP can explain many features of gait and gait transitions. Second, we show that one limitation of SLIP comes from two constraints in human walking: the need to synchronize vertical and horizontal motion and the need to redirect the velocity vector. These two constraints limit the ability of SLIP to capture human walking at high speeds. Third, another limitation of SLIP is its inability to produce tangential forces. These tangential forces can be produced through an angular spring. We show that adding this angular spring improves its ability to model animal locomotion. We show that this new model is able to model a fly's locomotion.

The Effect of Ground on Perching Maneuver

Samik Bhattacharya

Birds employ rapid pitch-up motions close to the ground. Perching birds use this motion to decelerate and come to a complete stop while hunting birds, such as bald eagles, employ it to catch prey and fly away. Motivated by these observations, our study investigates how natural flyers accomplish diverse flying objectives by rapidly pitching their wings while decelerating near ground. We conducted experimental investigations focusing on rapidly pitching plates in close proximity to the ground. Initially, we executed synchronous pitch-up motion, where both pitching and deceleration have the same motion duration, at different ground heights. Experimental results demonstrate that as the pitching wing approaches the ground, the instantaneous lift increases by approximately 38% compared to a far-from-ground case, while the initial peak drag force remains relatively unchanged. Initiating the wing pitch early in the deceleration leads to the formation of larger counter-rotating vortices at the early stage of the maneuver. These vortices generate stronger dipole jets that orient backward in the later stages of the maneuver after impinging with the ground surface, which hunting birds utilize to accelerate after catching prey. Conversely, when the wing pitch is delayed, smaller vortices form, but their growth is postponed until late in the maneuver. This delayed vortex growth produces lift and drag force at the end phase of the maneuver that facilitates a smooth landing or perching.

Does epigenetic aging underlie trade-offs between development rate and aging rate?

Abigail B. Bickle McKittrick, Benjamin Parrott

Across vertebrate taxa, the rate of development is fundamentally linked to subsequent rates of aging - slower development is associated with slower aging and vice versa. This trend has been observed between species, populations, and individuals. Despite the apparent universality of this relationship, the underlying biological mechanisms that connect development and aging are unresolved. We hypothesize epigenetic aging serves as a molecular bridge connecting developmental rate to aging rate on individual, population, and species levels. Using newly developed epigenetic clocks for medaka fish, we aim to investigate the effect of individual developmental rates on epigenetic age and other life-history traits. We induced disparate developmental rates in medaka (*Oryzias latipes*) embryos using three temperature treatments: 31.0°C, 25.0°C, and 18.5°C. Individual embryo stages were tracked daily until hatch. Hatched fry were kept individually housed at

room temperature, measured monthly, and checked weekly for markers of sexual maturity until sacrifice at approx. 5 months post-hatch. Here we report the effects of temperature treatments on rates of development, and also identify a significant relationship between days-to-hatch (developmental rate) and age at maturity in females. Hepatic DNA was isolated from the tissue and DNA methylation patterns are currently being resolved for each individual. Future work will assess measures of epigenetic aging to test the hypothesis that faster developing individuals will demonstrate higher epigenetic age than expected for their chronological age.

The nervous system is important for cell division during regeneration of rhinophores in *Berghia*

Haleigh Bilodeau, James Newcomb, William Scala

The nervous system may be important in the early stages of regeneration in axolotls and other animals, with stem cell proliferation being important for the regrowth of appendages. The nudibranch, *Berghia stephanieae*, can regenerate its chemosensory rhinophores. Here, the aim was to determine if inhibition of the nervous system would impact this cell division during early stages of regeneration. One of the rhinophores was amputated in 28 *Berghia*. Half of these animals were not exposed to anesthetic. The other 14 animals were anesthetized with 0.1M magnesium chloride during amputation of the rhinophore and for the duration of the experiment. Select animals in each group were sacrificed at 4, 24 or 48 hours. Immunohistochemistry was then performed on each animal with an antibody to phosphorylated histone 3, a marker for cell division. It was found that anesthetic did not influence the baseline level of cell division occurring in non-regenerating tissues, at all timepoints tested. Inhibition of the nervous system via anesthetic significantly decreased the level of cell division in regenerating tissues 4 hours after amputation ($p = 0.028$). At 48 hours, anesthetic decreased the number of dividing cells at a level that approached significance ($p = 0.053$). These results suggest that the nervous system may be important in early stages of regeneration in both nudibranchs and amphibians, and thus be an ancestral mechanism related to regeneration.

Flipping Fins & Feeding Curiosity: Unconventional Marine Science Communication, Indian Fish Markets

Meghana Binraj

This study presents an innovative paradigm in marine science outreach, focusing on Indian fish markets. Endeavors to pioneer an unconventional marine science communication and partnership approach. Central to this initiative is the meticulously systematic data collection from elasmobranchs as part of baseline study for elasmobranchs. However, this scientific endeavor inadvertently unveiled a distinctive sociocultural tapestry. Intriguingly, the mere presence of researchers meticulously documenting these elasmobranchs post-harvest kindled a remarkable curiosity amongst fishers, visitors, and traders, leading to spontaneous and enlightening dialogues. These interactions facilitated a knowledge exchange concerning the rationale and significance of the marine resources by the researchers. Of profound significance, these exchanges shed light on a prevailing deficiency in awareness pertaining to India's rich marine biodiversity within the broader public. Notably, a captivating dimension emerged as local fishermen emerged as custodians of experiential acumen, possessing a treasure trove of narratives encompassing encounters with a diverse array of marine species within their native habitats. Yet, paradoxically, the fishermen's inclination to share their insights remained restrained by prevailing concerns rooted in established conservation policies and societal norms. Consequently, this study undertakes a nuanced exploration, navigating the intricate interplay between entrenched traditional knowledge, evolving conservation paradigms, and dynamic societal perceptions. Synthesizing rigorous scientific inquiry with the intricate tapestry of socioecological dynamics emerges as the crux of this study. Advocating a departure from conventional didactic outreach, the study extols the virtues of compassionate and unrestricted engagement. This transformative approach precipitates a remarkable shift—from initial skepticism and resistance to an environment characterized by openness and receptivity. This metamorphosis stands as a potent testament to the latent power of unbridled dialogues.

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Connecting copy number variation to microbial and viral diversity in *Nematostella vectensis*

Sydney Birch, Adam Reitzel, Yehu Moran

The estuarine environment has a plethora of microorganisms that allow for diverse animal-microbe interactions which may impact the behavior and physiology of host animals. The sea anemone *Nematostella vectensis*, is a model cnidarian found along the east coast of North America, ranging from Nova Scotia to Florida. Previous research has shown that anemones from different locations have unique microbial communities and that some of these differences are maintained in the laboratory for long periods of time. However, how the genome varies for distantly related *N. vectensis* individuals remains unknown, which could inform why the microbiome may differ across locations. Here we sequenced and assembled genomes for individuals from four locations for comparison with the currently published reference genome produced by individuals from Maryland. We found numerous structural variants, including copy number variation of genes. Several genes that vary in copy number are involved in a hypothesized antiviral pathway and other immunity-related gene families. We will present current investigations into copy number variation of immunity-related genes of individuals from different locations using qPCR. Additionally, we completed a mesocosm experiment examining how anemones from different locations respond to shared microbial and viral communities. We will present our current findings and future plans for this research which is focused on characterizing how genomic variation can help explain the diversity of the associated microbes and viruses of coastal invertebrates.

Lucky Break: Comparative biomechanics of insect antennae

Megan Bishoff, Kostya Kornev, Adam Puchalski

In response to various environmental stressors, insects have undergone adaptations in their antenna morphology. Antennae are multifunctional hemolymph-filled fibers where of an external chitinous cuticular wall provides rigidity and protection for nerves and tracheae. Muscles are present only in the initial two segments of the antenna. We recently discovered that insects

could control the antenna movement by changing hemolymph pressure. The capacity of the inner fluid core to distribute stress and subsequently revert to its original form varies across species, influenced by the specific stressors experienced by the insect. Certain antennae exhibit characteristics akin to brittle fibers, whereas others exhibit stiffening behavior when subjected to stretching.

This study undertakes a comparative analysis of the tensile properties exhibited by distinct insect species. The investigation encompasses well-documented aerial Lepidoptera, namely *Manduca sexta* (Carolina hawkmoth) and *Vanessa cardui* (painted lady butterfly), and extends to previously untested terrestrial organisms, including *Aedes domesticus* (house cricket) and *Periplaneta americana* (American cockroach). Furthermore, the research covers creatures with heightened tactile sensitivity, exemplified by *Peruphasma schultei* (stick insect).

Employing tensile testing and high-speed imaging to capture antenna breakage, in conjunction with an assessment of structural attributes, enables us to establish a correlation between the distinct properties of fiber shapes and the mechanical traits of the antenna. This foundational understanding can subsequently inform the development of multifunctional fluid-filled fibers with diverse applications.

Is bigger better? Effect of tadpole size on responsivity of different tissues to thyroid hormone

Noelle Black, Julia Gosling, Alexander Schreiber

The primary hormone that mediates virtually all aspects of metamorphosis in amphibians is thyroid hormone (TH). In order to determine the influence of tadpole size on the relative responsivity of different developmental programs to TH, we treated Nieuwkoop-Faber (NF) 54 stage prometamorphic tadpoles with triiodothyronine (T₃, 10 nM) for 5 days. We measured the sizes of various morphological parameters of individual tadpoles (snout-vent length, tail length, hindlimb length, gill width, and brain width) before and after treatment. We then conducted a correlation analysis for sizes of initial tadpole morphological parameters against the rates of development (% change in magnitude) of each parameter following TH treatment. Significant ($p < 0.05$) positive correlations as measured by Pearson correlation were observed for parameter size prior to TH treatment versus % change in parameter magnitude after TH treatment among the following variables: snout-vent length reduction, gill width reduction, and interocular distance reduction. Significant negative correlations were observed for increases in leg length and brain width. No significant correlations were observed for reduction in tail length. These findings suggest that compared with larger tadpoles of the same stage, smaller tadpoles have a higher responsivity to TH in some developmental programs involving cell proliferation (brain and leg growth) compared with larger tadpoles. By contrast, larger tadpoles have higher sensitivity to TH in some developmental programs involving craniofacial remodeling.

Looking for Lungs in All the Wrong Places: diceCT reveals lungs in the only “lungless” frog

David Blackburn, Jaimi Gray, Edward Stanley

The only examples of lungless tetrapods come from modern amphibians, with examples reported from salamanders (all Plethodontidae and one species of Hynobiidae), one or more caecilians (e.g., *Atretochoana*) and one frog species. In 2008, Bickford et al. (Current Biology) reported that the Bornean frog *Barbourula kalimantanensis* lacks both lungs and a glottis, making it the only reported example of lunglessness in an adult anuran. As part of our efforts to generate contrast-enhanced CT datasets for representatives across all extant amphibians as well as notable phenotypes, we created datasets for *B. kalimantanensis* and its sister taxon *B. busuangensis* from the island of Palawan. Through cursory examination of these datasets, we discovered evidence of both a trachea and diminutive lungs in *B. kalimantanensis*. Here we present our investigation from contrast-enhanced (diceCT) datasets of the respiratory and cardiovascular system of both species in the genus *Barbourula* and other early-diverging extant frog lineages. Though this reveals that the “lungless” frog *B. kalimantanensis* is, in fact, lunged, it also supports that this poorly known species may have other surprising aspects to its biology.

Temperature variability and salt pollution interact to alter parasite susceptibility in tadpoles

Paradyse Blackwood, Emily Martin, Grace Schumacher, Catherine Searle

Wild populations face anthropogenic environmental changes and parasites simultaneously. We sought to understand how host-parasite interactions are affected by the interactive effects of multiple environmental stressors. We focused on American bullfrog tadpoles (*Lithobates catesbeianus*) that can become infected by multiple parasites (e.g., *Echinostoma* sp. trematodes and *Batrachochytrium dendrobatidis*; “Bd”) and are affected by abiotic stressors including road salt and variable temperatures. In a multi-phase laboratory experiment, we exposed tadpoles to 2 sublethal salt treatments (0 and 1.5g/L NaCl) and 2 temperature treatments (constant 23°C and fluctuating between 20°C/25°C). We then exposed tadpoles to one of four parasite treatments (none, trematodes only, Bd only, and Bd & trematodes together). We recorded morphological measurements and quantified infection prevalence.

With trematode infection, we found significant salt x parasite and salt x temperature interactions where tadpoles exposed to fluctuating temperatures, Bd and trematodes, and salt together were infected with more trematodes. With Bd infection, we found significant temperature x salt and temperature x parasite interactions. Tadpoles exposed to Bd and trematodes, fluctuating temperatures, and no salt had higher Bd infection prevalence than those exposed to Bd only. Those exposed to fluctuating temperatures, 1.5 g/L salt, and both parasites had lower Bd infection severity than tadpoles exposed to no salt. Together, these results indicate that salt pollution in freshwater systems and variable temperatures can interact to influence infection in tadpoles.

Interplay of visual and olfactory cues in mosquitos

Adam Blake, Jeff Riffell

Vision underlies many important behaviors in mosquitos, and recent work has shown visual responses are color dependant. Despite the medical importance of mosquito born illness and the importance of visual cues in vertebrate host finding, the spectral sensitivities of their photoreceptors remain uncharacterized, and little is known about how olfactory stimuli can modulate visual responses. Depending on the behavioral context, mosquitos use visual cues to locate and select flowers, vertebrate hosts, or oviposition sites, however the spectral reflectance of these resources differs substantially. To investigate the interplay of olfactory and visual cues, we adapted previously used wind tunnel bioassays to use targets created with a novel LED synth. We coupled these visual targets with CO₂ and the odors of either vertebrate hosts or floral resources and assessed their response to these quasi-monochromatic targets (390-740 nm) with real time 3D tracking of the flight path of female *Aedes aegypti*. When CO₂, human foot odor or their combination is present, we observe a preference for wavelengths above 600 nm as with previous bioassays using paper targets, however unlike in previous work we also observe a preference for stimuli at or around 400 nm. We expect the presence of floral odor. In contrast we expect floral odors to shift mosquito preference more towards the green portion of the visible spectrum, better matching the reflectance of most flowers.

Lead exposure is associated with limited physiological effects in urban lizards

Annelise Blanchette, Alex Gunderson

Lead contamination can have serious negative health implications for humans and wildlife. That said, there is a gap in our understanding on how natural chronic lead exposure affects wildlife beyond key taxa like birds of prey. We tested tissue lead levels and physiological performance across an urban lead contamination gradient in the brown anole lizard (*Anolis sagrei*) in New Orleans, LA. We predicted tissue-lead would correlate with environmental-lead, and that tissue-lead would negatively affect physiological performance, measured as sprint speed, balance, and endurance. Anoles from the high-lead neighborhoods had on average 8.5x higher bone-lead and 25.5x higher blood-lead levels than those from the low-lead neighborhoods. Despite the extreme tissue lead levels, and contrary to our predictions, we found little association between lead level and performance. On average, males but not females from high-lead neighborhoods had worse balance. However,

neighborhood lead level was not associated with sprint speed or endurance. Furthermore, individual lead level was not associated with individual performance for any performance metric. Lead exposure had a limited effect on the physiological performance of the anoles in the current study. Lizards are underrepresented in studies on the effects of heavy metal pollutants, but the brown anole is a model system in urban ecology and physiological ecology, amenable to field and experimental approaches that will continue to elucidate their physiological tolerance of lead exposure.

Grazing kinematics and the evolution of waterfall climbing in gobiid fishes

Richard Blob, Kelly Diamond, Elpidio Buelecope-Sepa, Joshua Cullen, Takashi Maie, Heiko Schoenfuss

Of the gobiid fishes that climb waterfalls, most species employ “powerbursts” in which pectoral fin adduction is followed by cycles of axial undulation. In contrast, species in the genus *Sicyopterus* climb via “inching”, in which the body advances by alternating attachment of the pelvic sucker with a novel, oral sucker. *Sicyopterus* species are also obligate herbivores, grazing diatoms off benthic substrates. *Sicyopterus* cranial kinematics are similar between grazing and climbing, with both behaviors including extreme premaxilla extension. Because inching likely evolved from powerburst-climbing ancestors, jaw movements for inching may have been an exaptation, in which grazing kinematics were enhanced and incorporated into climbing mechanics. To test this hypothesis, we recorded high-speed video of feeding and climbing from three species of *Sicydium* (the sister lineage to *Sicyopterus*) from islands on opposite sides of the Atlantic Ocean: Dominica in the Caribbean, and Bioko in Western Africa. All three *Sicydium* species use premaxillary extension to graze diatoms off substrates; moreover, *S. bustimante* from Bioko climbs via inching, the first example of this mechanism outside the genus *Sicyopterus*. However, *S. bustimante* showed less premaxilla extension during grazing than the powerburst climber *S. brevifile* from Bioko. Thus, although the evolution of inching may be correlated with specific feeding behaviors, the steps that led to inching cannot solely be explained by the elaboration of grazing kinematics into a new functional role.

Surveying cell type diversity in the ctenophore *Mnemiopsis*, one cell at a time

Yuriy Bobkov, Natalia Padillo-Anthemides, Alexandra Hernandez, Allison Edgar, Joseph Ryan

Combining patch-clamp and/or imaging recordings with single-cell RNA sequencing (scRNA-seq) in the same cells is becoming an indispensable tool for capturing cell type diversity and identifying specific cell types/subtypes. In this study, we also employ functional sequencing, which integrates electrophysiological characterization and calcium imaging with deep scRNA-seq and transcriptome analysis, to define the cell types present in the comb jelly *Mnemiopsis* (Ctenophora). Our approach combines gene expression profiles of individual cells with in vivo, in situ and in vitro preparations to establish correlations between spatial phenotypes, biophysical properties like membrane current and calcium signaling parameters, with the expression of key components including enzymes, channels, and receptors. We used this approach to assess a range of ctenophore cell types including phagocytic, glial, neuronal, muscular, sensory, and glandular cells, as well as relatively rare, otherwise elusive cell types such as lithocytes and balancer cells. These results provide unprecedented resolution of cell types in a ctenophore (the sister lineage to the rest of animals) and as such, provide key data towards reconstructing the cellular makeup of the last common ancestor of all animals.

Development of DNA methylation-based age predictors in an elasmobranch

Samantha Bock, Kady Lyons, Lei Yang, Jennifer Wyffles, Gavin Naylor, Benjamin Parrott

All organisms undergo some form of aging. Yet, despite the near universality of this process, it is not yet understood how the proximate cellular processes associated with aging interact with environmental factors to shape

the distribution of lifespans, both within species and across the tree of life. The recent development of DNA methylation (DNAm)-based aging models ("epigenetic clocks") has opened an opportunity to better understand the forces that shape aging, species-specific lifespans, and the evolution of various life histories across a diversity of taxa. Characterization of epigenetic aging signals in elasmobranchs (sharks and rays) holds unique potential to advance both conservation and evolutionary biology as this group is long-lived, contains a large fraction of endangered species, and occupies a basal phylogenetic position which can be leveraged to inform our understanding of life history evolution across vertebrates. Here, we describe current efforts to develop a DNAm-based age estimator in the zebra shark (*Stegostoma tigrinum*), a species of conservation concern. Genome-wide methylation data are used to identify age-associated epigenetic patterns in whole blood from samples collected across aquariums. Subsequent work aims to characterize conserved DNAm aging signals across multiple elasmobranch species.

Avian community nestedness and turnover differ between habitats in a complex urban-agroecosystem

Rachel Bockrath, Erin Questad, Eric Wood, Elizabeth Scordato

Agricultural expansion and urbanization, the dominant forms of land use globally, are expected to expand and intensify, contributing to declines in avian biodiversity and avian community simplification. While avian community structure and composition are known to vary between natural and anthropogenic habitats, the differences in community assembly and ecological value between different anthropogenic habitats, specifically agriculture and urban habitats, are unclear. We conducted year-round point count surveys of avian communities at 64 sites split between natural, agricultural, and urbanized habitats in Southern California. We asked whether avian community composition and diversity varied between habitats and across seasons, and if potential differences in community composition were due primarily to nestedness or turnover. We found that avian communities were distinct among habitats, though species richness and abundance were generally lower in agricultural and urbanized habitats. However, agricultural habitats shared many species with the natural habitats (nestedness), while most birds found in urbanized habitats were novel species (turnover). The differences between agricultural and urbanized communities were due to both nestedness and turnover, indicating that although both these habitats supported small and simple avian communities, their species compositions were distinct. There was minor seasonal variation. Thus, to maintain high biodiversity and ecosystem functions in urban-agroecosystems, it is critical to conserve natural habitats, while also maintaining agricultural and urbanized environments, as all three landscapes provide distinct ecological opportunities for birds.

The evolution thermal and hydric physiology of Hispaniolan anoles.

Brooke Bodensteiner, Nathalie Alomar, Martha Munoz, Isabela Hernandez-Rodriguez, Miguel Landestoy, Saúl Domínguez-Guerrero

Behavior is a factor that can directly shape evolution: regulatory behaviors like thermo- and hydoregulation, for example, shield organisms from environmental selection, resulting in slower rates of evolution and limited physiological divergence across environmental gradients (a phenomenon known the Bogert effect). The Bogert effect has most frequently been investigated by examining the effect of thermoregulatory behavior on the rate and pattern of thermal physiological evolution. Nevertheless, thermal and hydric physiology are tightly intertwined: a more holistic understanding on the Bogert effect – and its limitations – urges a shared investigation of thermal and hydric physiology. Here we examine the patterns and rates of evolution of hydric and thermal physiologies in a model system of ectotherms, *Anolis* lizards. We focused on a group of closely related anole species from the Caribbean island of Hispaniola that are found in a variety of habitat types and across an elevational gradient to investigate if the Bogert effect impacts various physiological traits in similar or contrasting ways.

Flying songbirds: tracking wing movement and sensory signals in the zebra finch

Clémentine Bodin, Katherine Chadwick, Sarah Woolley

Although flying is a fundamental behavior in birds, we know surprisingly little about the kinematics or neural circuitry underlying sensory perception during flight. Here, we tested how flight components change according to a constrained trajectory in zebra finches. Using a social reward, we trained birds to fly linearly in a transparent corridor between two perches that were gradually separated up to 160cm. We processed high-speed videos using DeepLabCut to track the position of the wingtips, beak, back, and tail. While all the zebra finches tested displayed flap-bounding behavior, there was individual variation in the position and number of bounds. The ratio of bounding over flapping increased with speed and distance between perches. By placing a vertical obstacle at various positions along the corridor, we found that these birds can flexibly shift the moment they bound to pass through it. Only obstacles placed close to the landing perch generated U-turns and more trials. Taken together, these results highlight the individual variability and adaptability of zebra finch flight. We are currently using single and multiunit electrophysiology to study how sensory signals are transmitted from feathers to the brain by mapping the somatosensory responses to air puffs manipulating individual feathers along the surface of the wing. Preliminary data indicate that the rostral hyperpallium (HA) could be the pallial entry point for somatosensory inputs, as previously suggested in pigeons.

The role for gill morphology in adaptation to low oxygen in the blind Mexican cavefish.

Tyler Boggs, Joshua Gross

Detecting and acquiring oxygen from the ambient environment is crucial to survival. Although much evidence has shown varying degrees of efficiency across aquatic species, the role of environmental shifts on oxygen uptake potential is unknown. One exceptional model to investigate this role is the blind Mexican cavefish, *Astyanax mexicanus*. This species is comprised of surface- and obligate cave-dwelling morphotypes. Differences in the cave environment, from surface waters, include the absence of light, limited nutrition, and low dissolved oxygen. Cavefish are found in over thirty different caves throughout northeastern Mexico, many of which are geographically distinct with likely variable levels of dissolved oxygen. Here, we examined gill morphology of a surface population and three cave populations; Molino, Tinaja, and Pachón. *Astyanax* gills are comprised of four cartilaginous arches anchoring many filaments with perpendicular lamellae wherein oxygen change occurs. We quantified the density of serotonin-positive oxygen sensors (neuroepithelial cells) within gill filaments of the first arch to estimate differences in oxygen detection. Further, we measured the number and length of filaments and the length of lamella to estimate area for potential oxygen exchange. Preliminary evidence suggests a higher density of oxygen sensors, and greater area for potential oxygen exchange in cavefish compared to surface fish. This phenotypic divergence may permit lower detection thresholds and greater uptake efficiency of oxygen to adapt to the hypoxic cave environment.

Humpback whale (*Megaptera novaeangliae*) visual acuity and the detection of anthropogenic threats

Jacob Bolin, Vanessa Moreno, Lorian Schweikert

Like other large whales, humpback whales (*Megaptera novaeangliae*) are vulnerable to anthropogenic threats, such as ship strikes and entanglements in commercial fishing gear. One strategy employed by conservation biologists is to study the visual capabilities of such animals to better understand, anticipate, and potentially mitigate their interactions with these threats. Visual acuity is the capacity of an animal to resolve static spatial detail, indicating how blurry objects may appear at differing distances. Here, we estimated the limits of humpback whale visual acuity by completing a morphological analysis of a subadult eye. Cresyl violet histological stain was applied to a whole-mounted retina for visualization and density estimation of the retinal ganglion cell (RGC) field. Taken with an estimation of the eye's focal length, the value of peak RGC density was used to calculate a threshold of humpback whale visual acuity

at 4.6 cycles per degree (CPD) – a value that is an order of magnitude lower than what might be predicted based on absolute eye size. We then used the R package, *AcuityView*, to transform an image of a commercial fishing net as it would be viewed by a humpback whale over a range of distances based on their visual acuity. Analyzing these images then allowed us to gain insights into the threat of entanglement over distance.

Is there an urban-tolerant endocrine phenotype?

Fran Bonier, Emma Sinclair, Paul Robert Martin

Species vary markedly in their responses to urban environments. Most species cannot persist, some can, and a few even appear to thrive in cities. What distinguishes species that tolerate urban habitat from those that do not? Past studies have identified ecological, behavioural, and life history traits that appear to increase urban tolerance, and many of these traits are regulated by hormones. As such, among-species differences in endocrine traits might predict urban tolerance. We used two large datasets to test this hypothesis, finding complex and context-dependent relationships between baseline corticosterone, stress-induced corticosterone, testosterone, and urban tolerance among hundreds of species of birds. These findings suggest that mean circulating hormone concentrations might influence urban tolerance. We expect that other aspects of the endocrine phenotype, such as the ability to mount an adaptive plastic response to urban challenges, might more strongly influence success in cities.

Effect of Embryonic Maternal Investment on Elasmobranch Physiology

Annais Bonilla-Johnson

Elasmobranchii's diverse reproductive strategies pose an unknown amount of risk to pregnant females from the level of embryonic maternal investment. This study aims to investigate the frequency and nutritional content of intrauterine histotroph secretions of Matrotrophic stingrays in Eastern Gulf of Mexico and assess the effects of embryonic maternal investment on the physical performance of gravid females throughout gestation.

Mature female *Hypanus sabinus* and *Rhinoptera bonasus* will be collected throughout all stages of gestation with seines, gillnets, and otter-trawls independently and in collaboration with Fish and Wildlife Research Institute's Fishery-Independent Monitoring Program. Organic dry weight procedures will quantify embryonic maternal investment at all stages of gestation. Histotroph will be assayed for total energetic content, carbohydrates, fatty acids, proteins, and lipids.

Lastly, 12 *H. sabinus* will be collected at early gestation and transported to Florida State University Coastal and Marine Laboratory facilities for respirometry trials. The individual's average oxygen consumption rate will be measured over a six-hour trial period and repeated every three weeks for six months to identify changes in physical performance as gestation progresses.

Identifying intraspecific and interspecific variation in fluids that provide embryonic nourishment is integral to understanding how these reproductive strategies contribute to elasmobranch fitness. Physiological differences throughout gestation between species of varying reproductive modes and modes of embryonic nourishment may further our understanding of reproductive trade-offs in elasmobranchs.

Resource allocation trade-offs in male leaf-footed bugs

James Boothroyd, Christine Miller

Males in many species invest in sexually selected weapons that are used in competition over access to females. Weapons can be costly, and investment in these structures may limit the ability to allocate resources to other traits. In mating systems where females mate multiply, males may face heavy sperm competition after mating, and can find themselves pulled in two different phenotypic directions. Experimental manipulations are powerful tools in elucidating the specifics of these allocation relationships, and leaf-footed bugs (Coreidae: Hemiptera) have been an invaluable in this respect. Here I summarize the work in this system and provide key future directions. Leaf-footed bugs are able to voluntarily drop their weaponized hind legs, which

they do not regrow, in a process called autotomy. Males induced to drop their weapons have consistently shown an increase in testes mass across studies and species. As a result, males have increased sperm production and father more offspring, at least in some contexts. Interestingly, we have recently discovered that males that drop a limb have increased dispersal capacity. Further work should focus on how sexual selection has shaped resource allocation trade-offs, by investigating changes in traits involved in female mate choice, as well as lifetime fitness consequences to males.

A multi-omics approach investigating regulation of symbiont state in a facultatively symbiotic coral

Erin Borbee, Louis Oviedo, Lauren Fuess

Many species of corals are dependent on relationships with symbiotic algae of the family Symbiodinaceae to meet their nutritional needs. This relationship is often characterized as a mutualism. The symbiont receives inorganic nutrients necessary for photosynthesis and the host receives organic nutrients essential for energy production. In tropical corals, this relationship is most often obligate, the coral must maintain the symbiosis to survive. However, other corals display facultative relationships with their algal symbiont and can exist with high and low densities of the symbiont with no apparent cost to their fitness, and even can exhibit variation in symbiotic state within a single colony. These facultative species provide an excellent model for investigating the dynamics of coral-algal symbiosis, particularly as it pertains to regulation of this relationship. Here we leverage the facultative coral, *Astrangia poculata*, to determine the causes and consequences of variation in symbiotic state within individual colonies using a multi-omics approach. Using an integrative approach, we will explore the genetic and physiological factors which contribute to this variation. The transcriptomic data will be used for differential expression analyses to investigate how symbiont density affects gene expression across a single colony. Combined, these results will provide important new insight regarding the mechanisms of symbiosis regulation in cnidarians, and the nature of these associations (i.e. parasitic vs. mutualistic).

Nestling size and ornamentation interact to shape early development in house sparrow families

Shana Border, Matthew Dugas

In many nuclear families, dependent offspring receive unequal shares of parental investment. Initial overproduction can be adaptive from the perspective of parents, but parents must be able to identify appropriate candidates for favorite status. We studied early nestling development in house sparrows (*Passer domesticus*), a species in which the loss of some brood members is common, testing the prediction that body mass and carotenoid-rich flange colors are important to nestling success. There was substantial variation in both traits within broods, even only one day after hatching. Nestlings low in the within-brood mass hierarchy gained more mass if they displayed more carotenoid-rich flanges than broodmates. Position in the color hierarchy did not, however, predict mass gain for individuals that were heavier than their broodmates. Nestlings that were heavier or had more carotenoid-rich mouths were also less likely to be the victim of brood reduction. Our results suggest that house sparrow parents use both nestling body size and mouth color when making allocation decisions. Understanding both how and when offspring traits and parental preferences function is key to understanding how selective pressures act on offspring-parent communication.

What's in a 'Game'? The Effects of a Video Game on Evolution Comprehension Among Undergraduates

Jacob Botello, Matthew Wolak

Evolution can be a difficult subject to learn and teach owing to its broad scope and widespread cultural misconceptions. One possible solution to this problem may involve gamification, an approach that has been shown to be especially effective when integrating video games into the classroom. We developed a browser-based, 2D platformer game that allows students to interact with the process of evolution by placing the player in control of an entire population of individuals subject to selection within each game level.

Player-controlled individuals who survive a level (i.e. those selected for) are randomly paired for mating to produce offspring (playable in the next level) with phenotypes generated based on the genetic makeup of their parents. We manage generation-to-generation changes in phenotypes by tracking the alleles of all parents and offspring in a multi-locus model and generating environmental deviations based on player-selected heritability. To test the efficacy of the game as a teaching tool we recruited >1400 students over two semesters from classes in the biology department at Auburn University and surveyed their knowledge of and attitudes about evolution before and after 30 minutes of gameplay. We present our analyses of students' understanding of evolution before and after gameplay and advocate for the development of more freely-available video games designed to improve academic outcomes in STEM.

Changes in sex ratio and morphology of adult Diamondback Terrapin in a commercially crabbed system

Claire Boudreaux, Timothy Clay

The Diamondback Terrapin (*Malaclemys terrapin*) is an estuarine turtle native to the Atlantic and Gulf coasts of the United States. Terrapin were overharvested from the mid-1800s to early 1900s, and the IUCN currently lists them as vulnerable, describing the species' population trend as decreasing. Modern threats driving this trend include blue crab pot entrapment, road mortality, and habitat loss. Terrapin are sexually dimorphic, with males smaller than females, and consequently the sexes may experience different selection pressures. To better understand the pressures affecting terrapin, a mark-recapture study began in 2012 within southeast Louisiana. From this data we are examining changes in population demographics, focusing on sex ratios and shell morphology. Through un-baited sampling, we have found a shift in adult sex ratios toward female dominant. With the use of commercial blue crab traps, we have concluded that despite previous studies in Louisiana, terrapin are at risk of drowning due to entrapment. We have also found that adult female terrapin become too large for capture in crab pots, but adult males do not attain sizes that prevent their capture. Our data suggests that differential selection pressure on adult terrapin by the commercial blue crab fishery may be pushing populations to be skewed toward females. Continued crabbing pressure may increasingly push reproductive responsibility onto older individuals, eventually leading to recruitment declines and genetic bottlenecks in local populations.

Jerboa Jump, But Can a Camera Catch it? Machine Vision for Behavioral Labeling in Captive Jerboa

Matthew Boulanger, Talia Moore, Juri Miyamae, Gerry Hish

Jerboas (family Dipodidae) present a unique model to study biomechanics, as they are obligately bipedal hopping rodents whose ricochet escape responses involve unpredictable three-dimensional trajectories. However, because little is known regarding the natural history, activity budgets, and behaviors of jerboas, it is unclear whether and how captivity affects their locomotor performance. For example, how do jerboas maintain their rapid acceleration ability when their movement is restricted in captivity? Previous work has shown that immobilization quickly alters the fiber type of muscles. We have begun to address this question by building an activity budget for captive jerboas. We first built an ethogram of singly-housed captive adult lesser Egyptian jerboas (*Jaculus jaculus*; N=2 age-matched males) using a machine vision classifier, DeepAction, to automatically classify behaviors. Ethogram states identified include grooming/eating/foraging, jumping, locomotion, wall interactions, dust bath interaction, rolling, sedentary, and obstructed behaviors with an overall, combined accuracy of 85.9%. In particular, we found that jerboas spend approximately 5-20% of their active time jumping vertically, which has been identified in historical descriptions of free-living jerboas and may prevent muscular atrophy and maintain predator evasion ability. This type of automated ethogram assessment might also be used to identify repetitive behaviors with stereotypic potential, and aid in the evaluation of novel enrichments as they pertain to animal welfare.

In Utero Developmental Effects of Sodium Perchlorate Exposure in Mice

Kyle Bouten, Michael Minicozzi

Perchlorate is a ubiquitous endocrine disrupting chemical capable of inducing hypothyroidism in vertebrate animals including humans. Previous studies in stickleback and zebrafish have elucidated some effects of perchlorate exposure on the thyroid, liver and gonads but disparate results across these two models have been shown. Because of these findings, we expanded these experiments to mice to better understand perchlorate's effects on mammals. Previous research in our lab has shown post-natal exposure to perchlorate causes histological effects in the thyroid, liver and gonads of exposed mice. This study analyzes the histological effects of early perchlorate exposure on the same organs during in-utero development through sexual maturity at post-natal day (P)85. Mice were exposed to perchlorate in-utero through the mother and then through drinking water to two environmentally relevant concentrations and a control (10ppm, 100ppm, and 0ppm). At P85, mice were euthanized and organs were prepared and stained using hematoxylin and eosin. Based on the post-natal exposure study, we predict that perchlorate exposed mice will have smaller and more numerous thyroid follicles, and lipid accumulation in the tissues surrounding the thyroid, increased steatosis and inflammatory cell clusters in liver tissue. Increases in disorganization and vacuolization in testes and fewer late stage ovarian follicles in ovaries. This study will elucidate if chronically exposed mice can remediate the effects on specific tissues or is chronic exposure exaggerates these effects.

Environmental drivers of coral-associated algal and microbial communities across multiple scales

Colleen Bove, Annabel Hughes, Alexa Huzar, Karl Castillo, Daniel Segrè, Sarah Davies

Rising ocean temperatures pose the greatest threat to coral reef persistence, as thermal stress can lead to the breakdown of coral symbioses. Studies suggest that prior exposure to thermal variability can enhance coral performance under thermal stress and microbial and algal associations may further shape tolerance. However, the mechanisms shaping these networks remain largely unexplored. Here, we assess the diversity and community composition of coral-associated algal and microbial communities across four ecologically relevant scales. We collected corals from four paired inshore-offshore reefs across the Caribbean, covering a 15° latitudinal gradient that span environments that vary in their environmental conditions. We leverage algal and microbial community metabarcoding across multiple spatial scales to explore how patterns of environmental variation shape community composition and diversity of symbiotic partners. Our hypothesis is that corals from more variable environments (e.g., inshore, higher latitude) will host lower algal symbiont and higher microbial community diversities compared to less variable sites.

The organization of retinal projections to the pretectum of the Anna's Hummingbird (*Calypte anna*).

Julia Bowen, Cristian Andres Gutierrez-Ibanez, Andrea Gaede, Douglas Altshuler, Douglas Wylie

Hummingbirds (family Trochilidae) have unique flight capabilities which include the ability to sustain hovering flight. Optic flow, the motion across the retina due to self-motion, has been shown to be essential for the control of hovering in hummingbirds. In birds, a key nucleus involved in the processing of optic flow is the retinorecipient nucleus lentiformis mesencephali (LM), in the pretectum. The LM, but not other visual nuclei, is hypertrophied in hummingbirds compared to other birds, and this enlargement may represent a neural specialization related to hovering flight. Given the hypertrophy of LM in hummingbirds, it is possible that other specializations exist in the organization of this nucleus related to hovering. In this study we used fluorescent tracers injected into the eye to investigate the organization of retinal projections to the LM of the Anna's Hummingbird (*Calypte anna*). We found that projections to LM are not uniform. The lateral subdivision of LM (LMl) receives few projections to its lateral part, while that at the same time it receives dense terminals in its more medial region. In contrast, the medial subdivision of LM (LMm) receives uniform and less dense terminals. The dense terminals in LMI

resemble closely those found in the nucleus of the basal optic root (nBOR), which receives projections from displaced ganglion cells (DGCs). This suggests that LMI may also receive projections from DGCs.

When hunters become the hunted: an investigation of chemical alarm signaling in a cartilaginous fish

Joshua Bowman, Jamie Cornelius, Mauricio Cantor, Jonny Armstrong, Taylor Chapple

Phylogenetically distant species of teleost fishes as well as lampreys have been found to warn conspecifics of nearby danger by releasing chemical signals, suggesting ancient evolutionary origins of alarm signaling behaviors in fish. However, no studies have looked directly at alarm signaling and recognition in elasmobranchs, though they are among the oldest extant groups of fishes. Many elasmobranch species are both predator and prey, thus identifying the mechanism of communication is important for understanding the broader evolution of alarm signaling in the context of their complex trophic interactions. Here we present machine-learned and observational ethogram analysis of video data collected from California bat rays (*Myliobatis californica*) in a captive experiment. We measured changes in locomotor behavior as well as metabolite levels to assess whether captive bat rays respond behaviorally or physiologically to chemical cues released by a stressed conspecific in an upstream holding tank. These experimental data contribute to our understanding of intraspecific communication in an understudied group of marine organisms and may help inform the evolutionary origins of chemical alarm signaling.

Diapause initiation in the solitary bee *Megachile rotundata*

Julia Bowsher, Gagandeep Brar, Joshua Rinehart, Alex Torson, Sarah Signor, George Yocum, Joseph Rinehart

Environmental cues regulate diapause initiation in facultatively diapausing insects. In the alfalfa leafcutting bee, *Megachile rotundata*, diapause has a maternal component to its regulation. We explored multiple environmental cues to determine which one regulates diapause. We also used whole genome bisulfite sequencing and RNAseq to determine the molecular mechanisms underlying the decision to diapause. We found that daylength when the mother was laying the egg influences diapause status in the offspring. We found that methylation rates were low across the genome regardless of diapause status. We identified differentially expressed genes involved in oxidative stress and other diapause-related pathways in diapause-destined individuals. There was a significant overlap between genes that were differentially expressed and genes that were differentially methylated, but the direction of differential expression did not always correspond with methylation status. We confirmed that methylation is related to gene expression in general, and we also explored methylation rates in field caught mothers. We are still exploring the maternal component of diapause. However, in the offspring, methylation does not seem to regulate gene expression prior to the onset of the diapause state.

Comparative brain morphology among Musteloids

Angela Boyer, Dominik Valdez, Christopher Heesy, Leigha Lynch

Brain morphology of relatively large-brained mammals has been linked to variation in diet, environment, sociality, and body size. This data, however, is biased toward primates with little data on the comparative anatomy of other large-brained clades, such as carnivorans. The purpose of this study is to identify and compare endocast morphology among musteloids, providing a foundation to further explore the evolutionary relationship between these species and to interpret their complex behaviors. We studied the following species: *Procyon lotor* (raccoon), *Neovison vison* (mink), *Mustela erminea* (ermine), *Pekania pennanti* (fisher), *Martes americana* (North American pine marten), *Taxidea taxus* (North American badger). We generated endocast models from micro-CT scans of skulls of each species using Avizo, a software application for data visualization and analysis. We then labeled 3D anatomical images and published them onto Sketchfab, which is an open-access 3D modeling platform. We identified gyri and sulci by following previously published brain atlases from *Mustela putorius furo* (ferret). We found that the larger the species,

the more profound gyri and sulci they have compared to smaller species. We also found that some species, such as the *Pekania pennanti* and *Procyon lotor* have an additional gyrus, the post cruciate gyrus. The post cruciate gyrus was not evident in the *Mustela erminea*, *Martes americana*, or *Neovison vison*. Asymmetry of the ectosylvian gyrus was noted in *Neovison vison*. This data now enables future studies analyzing per- and polyfluoroalkyl substances (PFAS) and heavy metals in the areas where these species inhabit and how it affects brain development.

It's About Time: Unraveling the Molecular Clocks of Fiddler Crab Embryos and Larvae

Caitlin BrabbleRose, Quinton Krueger, Paola López-Duarte

Organisms can tune biological processes to geophysical cycles of the Earth, including day and night (circadian) and the tide (circatidal). The circatidal clock is attributed to cyclic and rhythmic behaviors of intertidal organisms, providing adaptive advantages to synchronize activity to the tide, while the circadian rhythm modulates behavior to a day:night cycle. Entrainment of the circatidal clock during development allows newly-hatched larvae to use tidal rhythms for transport into developmental areas; however, the mechanism of entrainment is unknown. The Atlantic fiddler crab, *Leptuca pugnator*, expresses both a circadian and a circatidal rhythm, making it an ideal model for studying these clocks. Here, we identify circadian and candidate circatidal gene expression in embryos and larvae to determine if gene regulation is synchronized to a diel or tidal cycle. While behavioral characterization of the circatidal rhythm is well-documented in many species, we seek to identify and describe the molecular mechanisms underlying it. We performed TagSeq and gene expression analysis using embryos 72-hr pre-hatching to larvae 48-hr post-hatching from different tidal periods. We observed 1,933 differentially expressed genes in embryos 24 hours before hatching and a maximum of 466 genes at both 24 and 40 hours post-hatch. Elucidation of the molecular mechanisms of the circatidal clock is key to understanding the tidally timed behaviors in all stages of the fiddler crab's life cycle.

Persistence of the small Neotropical cats under climate change scenarios

Jonathan Branco, Fabio Nascimento, Fabio Machado, Erika Hingst-Zaher

The ongoing climate change has widespread effects on Earth's biodiversity. Felids, in particular the Neotropical small cats of the *Leopardus* genus, have been consistently declining over the past years. Habitat loss and poaching are among the most impactful factors contributing to their decline. Furthermore, recent revisions in taxonomy have revealed that some previously identified species in the *Leopardus* genus are, in fact, comprised of multiple distinct species. This implies a reduced geographic range for certain members. In our study we modeled the distributions of all 13 recognized *Leopardus* species using their known ranges and climate data under a pessimistic climate projection for 2041-2060. Results indicate that the Eastern tigrina (*Leopardus emiliae*) could lose suitable habitats due to temperature changes. Additionally, the Southern tigrina (*L. guttulus*) faces impact from altered precipitation patterns. Similarly, the Uruguayan pampas cat (*L. munoai*), restricted to pampas vegetation, is susceptible to precipitation shifts. High-elevation species like the Northern pampas cat (*L. garleppi*) and Andean cat (*L. jacobita*) show lower vulnerability to abiotic changes, but prey shifts and isolation on mountaintops might threaten them. The ocelot (*Leopardus pardalis*), more adaptable in habitat and diet, is less affected in the projections, though the model's accuracy varies with wide distributions. This study underscores the varied climate change impacts on different members of the *Leopardus* genus and highlights the urgent need for conservation strategies.

"Sick" of stress? Interactions between neuroendocrine and immune response to social stress in finch

Emily Brandow, Austin Swallow, Jason Davis, Hunter Rogers

Zebra finches are a highly social species. Social interactions seem to be a necessary factor for the regulation of their hormonal, neurological, metabolic, behavioral, and immunologic balance. This study seeks to explore if short term

social isolation increases corticosteroids and so impairs aspects of immune reactivity. To explore this, birds were either isolated for 24 hours or housed socially, then exposed to a brief cognitive stressor. Blood samples were taken immediately after the test and compared to baseline metrics taken previously. ELISA was used to measure corticosterone, and we assayed heterophil/lymphocyte ratio using blood slides, and measured various behaviors and cognitive performance. We present our findings in relation to correlations between metrics, age and sex differences.

A baffling conundrum: why don't more crickets make acoustic tools?

Erin Brandt, Sarah Duke, Honglin Wang, Natasha Mhatre

Male crickets produce mate attraction calls by rubbing their wings together. Loud calls travel further and are clearer against background noise and thus loudness has clear fitness implications. Unfortunately, the small size of crickets means their calls are produced inefficiently. Tree crickets make tools known as acoustic baffles to overcome this acoustic inefficiency, but no other crickets do. We hypothesize that baffling may be rare, because like other tools they offer insufficient advantage for most species. To test this, we quantified the utility of cricket baffles by investigating the increase in efficiency available to crickets if they used a baffle. We measured wing sizes and calling frequencies for 112 cricket species, across 7 clades. We used finite element analysis to model sound fields generated by cricket wings within natural wing size and call frequency ranges. We also modeled more realistic scenarios incorporating the effect of reflective and scattering surfaces like the ground and vegetation. We found that all sampled crickets, in all conditions, could gain efficiency from tool use. Surprisingly, however, we also found that calling from the ground significantly increased efficiency, with or without a baffle. Indeed, theory suggests that this increase in efficiency is accessible not just to crickets, but to all acoustically communicating animals whether they are dipole or monopole sound sources.

Microbial changes in a northeast Florida salt marsh-mangrove forest ecotone

Pamela Brannock, Gabriela Canas, Nikki Dix, Zoe O'Malley-Pearson, Tiffany Rojas, Samantha Chapman

Microbial community composition can impact important wetland soil processes such as sulfate reduction and nutrient cycling, which may influence overall ecosystem functioning. Along the Florida's northeastern coast, *Avicennia germinans* (black mangrove) has been encroaching on *Spartina alterniflora* (smooth cordgrass) dominated salt marsh ecotone. This encroachment is attributed to in part a decline in severe freeze events. To investigate the effect of vegetation shifts and temperature increases on benthic microbial community composition, the top 0.5cm sediment layer was collected and examined from an established three-year in situ experimental field setup. Environmental DNA was extracted and the V3-V4 region of the 16S small ribosomal subunit was amplified. Resulting amplicons were sequenced on an Illumina MiSeq platform and reads were clustered into amplicon sequence variants (ASV) using the Quantitative Insights into Microbial Community (QIIME) 2 pipeline. Alpha and beta diversity will be determined and used to explore the differences within and between vegetation and temperature treatments. Community composition will also be examined between the treatments. Based on previous studies, microbial communities are expected to differ based on vegetation and temperature changes. Understanding shifts in benthic microbial communities, especially in this ecotone, can have potential implications for soil carbon storage and food webs.

Duplication of and variation in succinate dehydrogenase affects response to stress in willow beetles

Justin Brasil, Nathan Rank, Elizabeth Dahlhoff

Montane environments pose unique challenges to animals living there, including elevation-induced hypoxia and variable temperatures, which exert strong selective pressures on metabolic genes. Succinate dehydrogenase (SDH) is a multisubunit enzyme complex that plays a central role in aerobic

cellular metabolism and a secondary role in hypoxia signaling. In the willow leaf beetle *Chrysomela aeneicollis*, we discovered a duplication in the gene coding for the SDH-b subunit and characterized polymorphisms at introns and exons for both loci. A phylogenetic analysis of close BLAST matches to these SDH-b loci indicates that the duplication occurred within Insecta. Preliminary evidence suggests that interactions among the two SDH-b gene loci affect recovery of running speed after heat exposure. To further examine the role of duplicated SDH-b gene products in response to stress, we performed an RNA-seq experiment in which beetle hatchlings collected from an introgressed population were reared at high and low elevation in the laboratory before heat treatment. Analysis of resulting RNA-seq transcripts, including 16 genes involved in the hypoxia signaling pathway, revealed effects of rearing elevation, temperature and mitonuclear genotype on transcript expression, including those of the duplicated SDH-b loci. These results contribute to our understanding of the relationship between nuclear and mitochondrial genetic variation and ability to cope with a challenging and changing climate.

Cockroaches bend antennae by pumping blood

Artis Brasovs, Kevin Nguyen, Griffin Donley, Kostya Kornev

Insect antennae are hollow, blood-filled fibers with complex shapes. Muscles in the two basal segments control antennal movement, but the rest (flagellum) is muscle-free. Surprisingly, the insect can controllably flex, twist, and maneuver its antennae laterally. The working hypothesis is that the primary mechanism for controlling antennal bending is the blood pressure–cuticle coupling. We question whether the insect could generate the necessary bending moment by pumping blood in the antennal lumen. To answer this question, we studied the behavior of antennae of live cockroaches *Periplaneta americana*. The antenna was taped at the base to eliminate any muscular action while the rest was laid on a stage. The antenna was subject to compression at its proximal, middle, and distal parts so that the cross-section of the antennal lumen changed, hence the blood pressure. The free end reacted to outstretch the antenna. The outstretched part formed an angle with the substrate. The angle depended on the applied load: the angle formed during loading did not reversibly recover after unloading, while no damage to cuticles was observed. The visualization of hemolymph flow revealed an intricate flow between the antennal vessel and hemocoel. We model this flow to show that the pressure gradient along the antenna is not constant. Analyzing the cross-sectional morphology of hemocoel, it was shown that the centroid and the pressure center of cross-sections are shifted one from the other. The resulting bending moment due to pressure was estimated to confirm that the blood pressure can bend antenna.

Nest Box Orientation in Cavity-Nesting Birds

Kamau Braxton-Hall, Mackenzie Alderson, Jared Gladbach, Camilo Alfonso, Miguel Moreno-Palacios, Amalia Moore

Previous studies suggest that orientation plays a role in nest site selection, and cavity-nesting birds may prefer specific natural cavity or nest box orientations. It is thought that nest orientation preferences could stem from thermoregulatory benefits, offered by sun and wind exposure. However, not all studies have found significant effects. In our research, we first investigated nest box orientation preference in Tree swallows (*Tachycineta bicolor*), a species with a wide latitudinal distribution, and found a preference for north and east facing nest boxes. This result contrasts with some other studies of this same species as well as other species. To explain these species and population differences, we subsequently conducted a meta-analysis to investigate if geography is related to nest box orientation preference. We hypothesized that there is variation in a bird's preferred orientation based on latitude and elevation of the population. We investigated latitude and elevation due to their relation to climate and thus nest box temperature and potentially reproductive success. This study may demonstrate the importance of climate in relation to breeding behavior. In our findings, we hope to raise concern about the effects climate change can have on the success of migratory species.

A spreading, multi-tissue wound signal initiates regeneration in the acoei *Hofstenia miamia*

Catriona Breen, Mansi Srivastava

Whole-body regeneration (WBR)—the ability to replace any missing structure or cell type—is widespread across animals. In cnidarians and bilaterians, Wnt signaling drives correct patterning of new structures along the primary body axis; high Wnt activity is established at wounds that must form posterior (bilaterians) or oral (cnidarians) structures. In *Hydra* and planarians, rapid, wound-induced Erk signaling drives expression of Wnt pathway components at wounds. To understand whether a wound - Erk - Wnt cascade could be a shared regulatory network for pattern establishment during animal WBR, we examined Erk dynamics and function in the acoei *Hofstenia miamia*, which robustly regenerates missing anterior and posterior structures. We found that within 10 minutes of amputation, Erk is activated in multiple cell types at both anterior- and posterior-facing wounds. We show using a chemical inhibitor that Erk signaling within the first three hours of amputation is required for head and tail regeneration. Erk activity begins at the wound edge and spreads away over time; we are asking what cell signaling events (e.g. EGF signaling, required for Erk activation in planarians) mediate this spread. We are also asking whether Erk drives Wnt ligand expression to initiate posterior patterning. Our goal is a thorough understanding of how distantly-related animals meet the shared challenge of linking wounding to the particular patterning process called for by the missing body part.

Effect of Tail Prehensibility on Perturbation Resistance in Veiled Chameleons (*Chamaeleo calytratus*)

Trevor Brewington, Victor Munteanu, Savannah Swisher, Richard Blob

Animals that inhabit high-risk habitats often evolve morphological and behavioral adaptations to contend with environmental inconsistencies. In arboreal (tree-based) habitats, such adaptations can include accessory structures to increase stability, such as prehensile tails, to avoid risk of injury from falling. This study evaluated the use of prehensile tails by *Chamaeleo calytratus* (the veiled chameleon) for resisting unexpected arboreal perturbations. Animals were placed on a custom-made laterally sliding perch apparatus, with non-toxic, high-contrast paint applied to a point representative of the location of their center of mass (CoM proxy). Effects of tail prehensibility were evaluated by comparing perturbations with unrestricted tail use versus perturbations where the tail was immobilized by the attachment of lightweight splints. Data indicates that chameleons with splinted tails show larger position deviations from their perch during perturbations, and restabilize further from their starting perch position. This suggests that, although not required to prevent a fall, prehensile tail use contributes quantifiably to stability in arboreal environments, and that it is a component of the suite of anatomical and behavioral specializations for arboreality that help to ensure stability.

Differential Gene Expression of Crown Gall Formation and NoGall Treatment in Roses

Sophia Brice, Zaphillia Yost, Jarrett Mattson, Spencer Horn, June Kelly, Malcolm Manners, Jason Macrander

Crown gall formation (CGF) in roses is a tumor-like symptom of soil-borne bacterium (*Agrobacterium tumefaciens*) infection. The severity of CGF can vary among species, cultivars, exposure backgrounds, soils, and tissue types. To combat this, NoGall has been widely used as a preventative measure for CGF, however, very little is known about the molecular signals involved with defending against the agrobacterium and the overall influence of NoGall treatment. Using a comparative transcriptomics approach, we have been able to identify differences in gene expression among roots from various cultivars that have been reared from propagules of varying CGF symptoms. To explore this, a “cultivated rose” transcriptome was assembled, from which various rose (Leonie Lamesch, Red Cascade, Tausendschoen, among others) raw reads were mapped using a RNAseq TagSeq approach. Across each treatment type (NoGall and DI water) we compared changes in gene expression profiles prior to *A. tumefaciens* exposure and 9 days after. The number of differentially expressed (DE) genes varied considerably among cultivars and treatments, but found no correlation with gene expression profiles and CGF symptoms.

Many upregulated DE genes that had some roles in plant pathogen response or immune defense were upregulated in both the DI water and NoGall exposure. Overall, these results indicate that regardless of preventative measures, the complex interactions between plant pathogens and their hybrid hosts may mimic gene expression profiles of infection.

Relating whole-animal oxygen consumption to tissue-specific mitochondrial respiration in killifish

Samantha Brieske, Brian Irving, Bernard Rees

The energetic costs of maintenance, growth, reproduction, and sustained activity of animals are largely met by mitochondrial oxidative phosphorylation. This study examines whether variation among individual Gulf killifish, *Fundulus grandis*, in rates of whole animal oxygen consumption ($\dot{M}O_2$) during exercise and at rest are related to variation in the maximum capacity for mitochondrial oxidative phosphorylation (OXPHOS) by their tissues. Because sustained aerobic swimming requires heart to circulate blood and contraction of oxidative skeletal muscle to power swimming, we hypothesized that the maximum $\dot{M}O_2$ of an individual during sustained swimming correlates with OXPHOS of these tissues. Conversely, previous studies suggest that the mitochondrial oxygen consumption required to offset the dissipation of the transmembrane proton gradient in the absence of ATP production (LEAK) may account for a significant fraction of an individual's metabolic rate when measured in a post-absorptive, resting state (standard metabolic rate, SMR). Thus, our second hypothesis was that SMR is correlated with LEAK in tissues that represent a large proportion of the total body mass (white muscle) or contribute disproportionately to metabolism at rest (liver and brain). The results support the first hypothesis by showing that cardiac mitochondrial OXPHOS is positively related to maximum $\dot{M}O_2$ during swimming. However, support for the second hypothesis was weaker. The latter result suggests that mitochondrial may not be in LEAK state, even in tissues of an animal at rest.

Resilience of amphibian systems in the face of disease

Cheryl Briggs, Michel Ohmer, Emily Le-Sage, Mark Wilber, Corinne Richards-Zawacki, Joe DeMarchi, Louise Rollins-Smith, Jamie Voyles

Amphibians have existed for hundreds of millions of years, persisting through multiple ice ages and extinction events. Recent amphibian species losses, however, dwarf background extinction rates. The emerging infectious disease, chytridiomycosis, caused by the chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), has contributed to the population declines and extinctions of many amphibian species, worldwide. However, recent evidence is showing that populations of some species that previously declined due to Bd epizootics are now not only persisting with the pathogen, but also rebounding in abundance after epizootic events. Interestingly, Bd is still present in these populations, which suggest changes in host-pathogen dynamics since declines. In this talk, we will explore the mechanisms of resilience by which some amphibians are persisting and recovering in the presence of Bd. These mechanisms include mechanisms at the individual level (components of the innate or adaptive immune response and the amphibian skin microbiome), population level (evolutionary rescue, host reservoirs, density-dependent transmission), and community-level (community reorganization, pathogen-mediated indirect interactions). By uncovering the drivers of resilience to disease across scales of biological organization, we can better understand which species, populations, and communities are most at risk, and better focus management actions.

Resource allocation costs of melanin pigmentation

Sarah Britton, Goggy Davidowitz

Melanin is the most common pigment type in animals and plays numerous adaptive roles. However, the effect of diet on melanin pigmentation is not well explored. In particular, understanding how diet influences the cost of melanin production and melanin plasticity remains to be tested. Melanin production can be costly when it leads to resource allocation trade-offs. In this study, we use *Hyles lineata*, the white-lined sphinx moth, to test whether diet can constrain melanin production and influence potential melanin trade-offs. In this species, melanin pigmentation is plastic during the late larval instars, and individuals display a wide range of melanin phenotypes. As in most folivores,

amino acids are likely to be a limiting dietary factor. Specifically, we test how the abundance of the amino acid tyrosine, the precursor to melanin synthesis, influences melanin production. We find that diets low in tyrosine can constrain melanin production, even in conditions where melanin is prioritized for thermoregulation (low temperatures and short photoperiods). Larvae raised on low tyrosine diets were both lighter and had less melanin coverage than those raised on high tyrosine diets. Since dietary availability of tyrosine can be a limiting factor in melanin production, diet may also influence whether trade-offs exist between pigmentation and other traits that use this precursor, including the immune response and muscle building. We test whether there are resource allocation trade-offs by raising larvae in conditions that either induce a melanic form or a non-melanic form and on diets either low or high in tyrosine. We then measure melanization immune response during the last larval stage and amount of muscle during the adult stage. Constraints imposed by diet and resource allocation tradeoffs can explain plasticity

Offshore vagrancy in migratory passerines

William Brooks

Decades of study have uncovered stunning navigational adaptations in migratory birds including specialized proteins for sensing the Earth's magnetic field and genes encoding migratory routes. However, the exact mechanisms behind navigation remain elusive, in part due to the difficulty of studying social route learning. A particularly revealing approach may be to study individuals that migrate to incorrect destinations, called vagrants. I studied vagrant passerines flying over the Pacific Ocean using eBird, a community science dataset comprising millions of bird sightings. I assessed the probability of offshore vagrancy under varying external conditions like weather, geomagnetic disturbance, and solar activity. Furthermore, I examined how vagrancy varied between species due to differences in morphology, migration distance, and phylogenetics. Geomagnetic disturbance and reduced visibility emerged as primary external drivers of offshore vagrancy. This suggests that external factors primarily induce vagrancy by interfering with magnetic and visual senses, rather than physically diverting birds off course. Most variation in offshore vagrancy was explained by species-specific differences. Notably, the Brown-headed Cowbird occurred offshore significantly more frequently than other passerines. I contend that as a brood parasite, solitary juvenile Brown-headed Cowbirds experience more difficulty in joining migratory flocks, thus missing out on social route learning opportunities.

Ranavirus-Bd coinfections and immunogenetic diversity in native and invasive American bullfrogs

Erin Brosnan, Milagros Rincon-Paz, Jacob LaFond, Anna Savage

The infectious pathogens *Batrachochytrium dendrobatidis* (Bd) and Ranavirus (Rv) cause widespread amphibian declines and are of conservation concern. Genes of the major histocompatibility complex (MHC) mediate the adaptive immune response in vertebrates, and MHC allelic diversity is associated with amphibian susceptibility to Bd and Rv in multiple host species. However, understanding simultaneously occurring infection from multiple pathogens, or co-infections, is an understudied area of immunogenetics that is important given the high rate of co-infections in amphibians. Across its native and invasive range in North America, the American bullfrog (*Rana catesbeiana*) varies in Bd infection prevalence and infection intensity, and across its range, specific MHC alleles are associated with the risk of Bd infection. In this study we quantified Rv prevalence and infection intensity within the same populations of *R. catesbeiana*, measured Bd-Rv co-infections, and assessed whether infection with one pathogen increased the likelihood of infection with the other. Additionally, we tested for relationships between MHC diversity, MHC alleles, Rv infection, and Bd-Rv co-infection, and we compare these relationships between native and invasive populations. Overall this study contributes to our knowledge on the interplay of immune diversity and co-infection in an ecologically and economically relevant invasive species.

Leg-spur morphology predicts foraging behaviors in adult dragonflies (Odonata: Anisoptera)

Christofer Brothers, Stacey Combes

Dragonflies are predatory flying insects that forage in one of two discrete modes: sit-and-wait foragers that hunt from perches (perchers), and active, constantly flying foragers (fliers). In either mode, dragonflies capture their prey in mid-air with their long spiny legs, which come together to form a "capture basket" to grasp or trap aerial prey. Each leg has two rows of chitinous projections (spurs), which differ among species in shape, angle, and spacing, particularly on the tibiae. However, the range of variation in leg spur morphology and the functional implications of these differences for prey capture remain largely unexplored. We compiled information from previously published behavioral studies and naturalist observations on the foraging mode and typical prey size for dragonfly species in the superfamily Libellulidae, which contains both perchers and fliers. We used light microscopy and microCT scans to quantify leg spur morphology (spur length and number, row angles and spacing) between species to determine whether particular patterns of spur morphology can predict foraging behaviors. We found that fliers had more spines per leg on average than perchers, especially on the mesothoracic leg, and had lower (more acute) average angles between the spur rows. These results support the idea that leg spurs play an important role in prey capture and niche partitioning in dragonflies, paving the way for future research on functional significance of spur morphology.

The Effect of Temperature and Stress on Blowfly Abundance and Development in Tree Swallow Nests

Gracey Brouillard, Maren Vitousek, Conor Taff, David Chang-van-Oord

Climate change threatens bird populations around the globe, influencing aspects of life history, survival, and interspecies relationships, such as parasite-host interactions. Although global temperatures are rising, climate change is also causing more variable and intense weather conditions, including cold snaps. This study seeks to understand how extreme temperatures and the resulting hormonal responses affect the interaction between blowflies (*Protocalliphora* spp.) and tree swallows (*Tachycineta bicolor*). Specifically, we examine how cold snaps and the hormone corticosterone affect blowfly parasitic load and development in tree swallow nests, and whether they influence fledging success. We measured the abundance and size of blowfly larvae and pupae in nests that were artificially cooled or whose females had experimentally elevated corticosterone during incubation, and the number of nestlings that successfully fledged. We hypothesize that: 1) lower environmental and nest temperatures inhibit blowfly abundance and development, 2) blowfly abundance negatively predicts fledging success, and 3) increased corticosterone positively predicts blowfly abundance. Thus, we expect that experimentally cooled nests have less blowfly abundance than control nests, while corticosterone-treated nests have greater blowfly abundance than control nests. Within treatment groups, we expect that nests with more blowfly pupae and larvae have lower fledging success. These results will illuminate whether changing environmental conditions are having direct or indirect effects on the interactions between hosts and their parasites.

Encoding and Processing of Visual Information in the Leech

Belle Brown, Krista Todd

The medicinal leech, *Hirudo verbana*, possesses a dual visual system consisting of five pairs of cephalic eyes and numerous photoreceptors located in segmental sensilla along its body. In this study, we employed microdissection, extracellular electrophysiology recordings, iontophoretic dye injections, and intracellular recordings to explore the visual processing mechanisms in the leech, focusing on cellular responses to ultraviolet (UV) light stimuli. Our investigations revealed that the leech exhibits robust behavioral motor responses when its head eyes are exposed to UV light, consistent with previous findings of reactions observed with sensilla exposed to UV light. In addition, the behavioral response from the nerve cord showed significantly more activity than the simple encoding of the sensory information of the light from the eye nerve. A novel technique was also developed for dual recording of sensory and behavioral information, allowing action potentials

from the eye to be matched with resulting action potentials from the nerve cord. This research significantly enhances our comprehension of the leech's visual system and sensory processing, while also providing valuable insights into broader visual neuroscience. The leech's visual capabilities represent a valuable model for investigating the visual mechanisms in simpler organisms, and it may also inspire innovative approaches in the development of artificial vision systems.

Blue Catfish (*Ictalurus furcatus*) Skull Morphology Over Ontogeny in the Nanticoke River

Blake Brown, Noah Bressman

Blue Catfish (*Ictalurus furcatus*) are an invasive species that entered the Chesapeake Bay in the 1970's for recreational fishing. Now, after only a few decades, millions of blue catfish have taken over the watershed. Although these large fish are caught in high concentrations, their detrimental effects to the species around them have not gone unnoticed. Species including shad, perch, bass, clams, and blue crabs among others are experiencing a decline in their population directly resulting from the surging number of blue catfish present within the bay. To determine the dieting habits of blue catfish over ontogeny, I have taken 11 skull morphological measurements of many blue catfish samples covering a broad range of sizes. These measurements will help pinpoint when it is that a blue catfish experiences a shift in its diet from mainly plant-based meals to more carnivorous ones. Based on my current findings, it seems that blue catfish heads grow exponentially larger, unlike most other species, allowing them to switch from a plant-based diet to a fish/crab diet earlier than if their heads grew at the same rate as their bodies. These findings indicate that to limit the harm done by this invasive species, blue catfish must be caught early during ontogeny in order to limit their detrimental effect on the Chesapeake Bay ecosystem.

Jumping mechanics of desert kangaroo rats: the role of the midfoot joint

Christian Brown, Craig McGowan, David Lin, Marie Schwaner

Desert kangaroo rats (*Dipodomys deserti*) are renowned jumpers, capable of vertically jumping to over 10-times their standing hip height to evade rattlesnake strikes. A previous study in our laboratory found that net work increased with jump height but was not equally divided across individual hindlimb joints, such that the ankle contributed 50-70% of the total work done. Here, we examine vertical jumping in *D. deserti* more closely by incorporating the previously overlooked hindlimb joint, the midfoot joint, located distal to the ankle and proximal to the metatarsalphalangeal joint (MTP). We use inverse dynamics to quantify mechanical output from all five hindlimb joints during vertical jumping and ask: 1) what is the moment at the midfoot joint, and 2) how does this affect the moments and work done at the other joints? We found moment and net work done at the midfoot to be ~10% and ~70% lower than the moment and net work at the ankle. Addition of the midfoot decreases the moments at the MTP and ankle and reduces the work contribution of both by ~10% relative to not including the midfoot joint. Thus, adding the midfoot joint to the inverse dynamics analysis causes some changes in estimates of net work across joints, suggesting adding the midfoot joint will improve the accuracy of a biomechanical model of the kangaroo rat used to study jumping.

A Global Comparison of Sciurid Thermoregulatory Phenotypes

Eric Brown, Claudia Saldaña-DeCamillis, Danielle Levesque

The rodents of Sciuridae constitute one of the most speciose mammal families and have a near cosmopolitan distribution in most terrestrial habitats. From the Arctic to the Kalahari and Mojave deserts to the rainforests of Southeast Asia, the Sciuridae exhibit a great variety of locomotory modes, diets, and thermoregulatory phenotypes. They are consequently the focus of many investigations into the physiology of endothermy. Holarctic squirrels (including tree squirrels, chipmunks, and marmots) especially have earned much attention due to their diversity of torpor types and use of hibernation, though little is known about how their phenotypes compare with sciurids of other

zoogeographic regions. Here we use sciurid body temperatures to explore how thermoregulatory phenotypes compare across zoogeographic regions and locomotory modes. We also construct thermoneutral zones (ambient temperature range over which body temperature is maintained at negligible energy costs) with existing data using mechanistic niche modeling to generalize thermoregulatory costs between sciurids. We expect that species from relatively aseasonal, warm environments such as tropical rainforests will exhibit broader thermoneutral zones than their cold-adapted relatives and therefore bear fewer thermoregulatory costs. A holistic understanding of the costs of thermoregulation in Sciuridae will better prepare us to predict how they might adapt to a warming climate.

Distinct Physiological Responses to Water Temperature and Flow in Three Marine Mussel Species

Ruairi Brown, Mike Nishizaki

Many marine organisms must contend with the physiological challenges associated with warming water temperatures. This is especially true in nearshore ecosystems where organisms often live near their thermal limits. However, physiological responses to thermal stress may vary depending on local flow conditions that can influence rates of gas exchange with surrounding waters. As such, we examined the influence of both water temperature and flow on respiration rate in three congeneric mussels, *Mytilus californianus*, *M. trossulus*, and *M. galloprovincialis*. Aquatic respiration rates were quantified at five different temperatures (5, 11, 17, 23, and 29 °C) and water velocities (2, 4, 6, 10, 20 cm s⁻¹) in a fully factorial design. In general, respiration was highest in *M. californianus*, followed by *M. trossulus* and the lowest rates were measured in *M. galloprovincialis*. Whereas, respiration was affected by both temperature and flow in *M. trossulus* and *M. galloprovincialis*, rates in *M. californianus* were largely unaffected by environmental conditions. We discuss these physiological patterns in context of their different ecologies.

Characterizing ctenophore phagocytes and their innate immune function role in *Mnemiopsis leidyi*

William Browne, Lauren Vandepas, Kevin Wong, Nikki Traylor-Knowles

Understanding the evolution of metazoan cell types that mediate self/non-self recognition is essential for understanding the origins of metazoan multicellularity. Ctenophora are one of the earliest diverging extant metazoan phyla and are uniquely positioned to provide insights regarding the early evolution of immune cell types in metazoans. Our current work characterizing *Mnemiopsis leidyi* phagocytes seeks to inform several basic questions relevant to the evolution of metazoan innate immunity. Do *Mnemiopsis* phagocytes perform explicit roles in mediating innate immune responses? Does the range of phagocytic cell types associated with innate immunity in ctenophores reflect an early diversification of phagocytic immune cell types in the animal stem lineage? Do functional immune cells in ctenophores utilize conserved or divergent networks of gene expression? Are novel traits associated with *Mnemiopsis* immune cells a reflection of unique adaptations to pathogens present in estuarine/coastal environments? Informing these basic questions will improve our understanding of ctenophore developmental, cell and physiological systems providing additional insight into the diversification of cell types associated with metazoan innate immunity.

Cryptic Persistence of Abdominal Legs in Insects Enabled Diverse Outgrowths with Novel Functions

Heather Bruce, Nipam Patel

An iconic feature of insects is the apparent lack of legs on the abdomen, which is believed to be due to the repression of the leg-patterning gene *Distalless* (*Dll*) by abdominal Hox genes. However, in contrast to these molecular observations, it is not widely appreciated that the embryos of most insect groups do in fact form paired protrusions on most abdominal segments that appear to be homologous to the thoracic legs. However, these degenerate before hatching to form the abdominal body wall. To resolve this discordance between molecular and morphological observations, the expression patterns of pannier and araucan, genes known to distinguish proximal leg segments in all arthropods,

are examined in embryos of the flour beetle *Tribolium castaneum*. In *Tribolium* embryos, all pregenital abdominal segments develop leg-like paired protrusions, and the stripes of pannier and araucan expression that delineate the proximal leg segments of the thorax are also expressed in the same configuration around these abdominal protrusions. This suggests that insect abdominal legs are homologous to only the proximal portion of the thoracic legs, which in insect adults forms the body wall (lateral tergum and pleura). These cryptic, truncated abdominal legs – likely inherited from their crustacean ancestors – appear to be an important wellspring for new functions in insects, such as caterpillar prolegs, gills, and structures for camouflage, and aposematic warning.

Tadpoles cope similarly with organic and conventional agricultural environments

Amanda Bryant, Caitlin Gabor

Organic agriculture has gained popularity as a means to preserve biodiversity while maintaining agricultural production, with governments investing millions of dollars on incentives for farmers to make the switch. However, some approved organic chemicals have similar negative impacts on amphibians as conventional agricultural compounds including altered growth and development, altered behavior, immunosuppression, and endocrine disruption. Amphibians exposed to chronic stressors, including agriculture, can alter aspects of their glucocorticoid (GC) profile (baseline, agitation, recovery, stress responsiveness, and negative feedback) which aids in avoiding frequent or long-term GC elevations which can help with coping in polluted or stressful environments. We compared GC profiles of *Hyla versicolor* tadpoles from three treatments: natural ponds (no farming within 500m), ponds near organic farms, and ponds near conventional farms. We hypothesized that the GC profiles would differ between the three treatments. We found that tadpoles from conventional and organic agricultural water bodies had lower stress responsiveness and faster recovery to baseline corticosterone than those from natural ponds. It is unknown what tradeoffs may exist between observed GC alterations and other factors like immunity, metamorphosis, and breeding. Agricultural tadpoles maintained their capacity to cope with challenges in the same way regardless of agricultural method used indicating that current efforts to incentivize farmers to switch to organic farming methods may not be sufficient to address negative agricultural impacts on amphibians.

Genome-guided development of domestic dog probiotics from gut microbes isolated from wild wolves

Jessika Bryant, Evan Forsythe, Bruce Seal

The incidence rate of inflammatory bowel disease (IBD) and related gastrointestinal (GI) distress is increasing among domestic dogs. There is some evidence that this may be related to diet, which is drastically different than that of their wild progenitor, the grey wolf (*Canis lupus*). Here, we cultured three bacterial isolates from the wolf GI tract that show a high potential as probiotics for dogs. We performed whole genome sequencing and phenotypic assays to understand the taxonomic and functional properties of these isolates. Our isolates include two members of the *Enterococcus faecalis* species complex, which we designate as CIWae7 and CIWae12B, and a novel *Paenibacillus* sp., which we designate as CIWae2A. We performed phylogenetic analyses from whole genome alignments to determine phylogenetic relationships of our isolates and existing genomes on GenBank. Our functional assays showed that these isolates inhibited growth of *Staphylococcus aureus* and *Micrococcus luteus* bacteria, suggesting antibacterial properties likely valuable in probiotic candidates. Our ongoing genomic analyses of gene composition are revealing enzymes associated with probiotics and starch digestion, including alpha-amylase, cellulase, lipases, and pectin lyase, suggesting these isolate genomes encode metabolic repertoires well-suited for a probiotic candidate. Taken together, our genomic and functional analyses are important steps in developing these strong candidates as dietary supplements to treat IBD in domestic dogs.

Echinoderms rely on rapidly evolving repertoires of immune receptor genes

Katherine Buckley

Animal immune systems have evolved to recognize and appropriately respond to microbes. This includes not only protecting the host from harmful pathogens but also promoting the growth of a beneficial microbiota. From an evolutionary perspective, this raises the question: how do long-lived, multicellular organisms maintain the genetic diversity required to recognize the array of unique molecules generated by rapidly dividing microbes? Vertebrates generate a repertoire of immune receptors using adaptive immunity: sophisticated mechanisms of somatic diversification in which the genomic DNA that encodes immune receptors are rearranged in B and T lymphocytes. In contrast, invertebrates rely on germline genes that encode immune receptors. Initial analyses of genome sequence from the purple sea urchin (*Strongylocentrotus purpuratus*) revealed surprising expansions in the multi-gene families encoding immune receptors (10 to 20-fold more genes than vertebrate orthologs). Results will be presented from analyses of recently-available genome sequencing data from several additional echinoderm species, including representatives of other echinoderm classes (asteroids and ophiuroids). Findings suggest that expanded families of immune receptor genes are common throughout this phylum, although the sizes and natures of these families vary considerably among species. Notably, gene family size may correlate with specific life history traits such as life span and environment. This work will shed light on fundamental aspects of immunity encoded within animal genomes.

Predicted prophages of bacterial isolates collected from amphibian skin

Emma Bueren, Noah Wax, David Haak, Jenifer Walke, Lisa Belden

Host-associated communities of microorganisms (microbiomes) can influence the health of their hosts. For example, the bacterial skin microbiome of amphibians can mediate the outcome of amphibian exposure to the fungal pathogen, *Batrachochytrium dendrobatidis* (Bd). However, the influence bacterial viruses (bacteriophages) have on the amphibian skin community is currently unknown. For part of their replication cycles, some bacteriophages integrate within bacterial genomes as prophages. In this state, the prophage may carry genes that benefit their bacterial host, such as competitive toxins, resistance to other bacteriophages, or auxiliary metabolic genes. To better understand how prophages may influence the bacterial community within the amphibian skin microbiome, we completed a bioinformatic survey of 46 bacterial genomes, representing 14 different genera isolated from the skin of Virginia amphibians. A total of 72 unique prophages were predicted. The median number of prophages per genome varied between genera, with *Hafnia* spp. and *Stenotrophomonas* spp. containing the most prophages. Some prophages, predicted from various hosts, may encode potential metabolic genes, such as phosphoadenosine phosphosulfate reductases. Other prophages, primarily found in *Janthinobacterium lividium*, appear to encode putative chitinases, a previously proposed mechanism of Bd inhibition. While the relationship between prophages and Bd inhibition remains unclear, this study highlights several possible roles of prophages in the amphibian skin microbiome.

Science Outreach Partnerships 101: Making great outreach programs by partnering with local nonprofit

Carrie Buo

One of the challenges of grant applications is creating dynamic and effective outreach programs for the Broader Impacts section. Many times researchers fall back on training undergraduate students because they are unsure of where to start or how to bring their research to the community at large. This talk will explain the benefits of locating and partnering with existing non-profit organizations in your community to create outreach programs that are not only exciting, but can make a difference in the lives of participants and the larger community. We will also discuss what makes a program effective for different audiences and age groups, volunteer recruitment, supply costs and management, and give an example of a partnership between two non-profit

organizations and a research facility. This talk is geared toward the Inclusion, STEM Education, and Partnership societal outcomes of NSF grant applications, but discussion about other outcomes and impacts is welcome.

Science with a smirk

Carrie Buio

In Portland, OR there is a large population of LGBTQ+ youth ages 13-24, with up to 25% experiencing poverty and/or homeless (Tomlinson, 2022). Organizations in the area have dedicated themselves to providing various services and programming to these young people, including job readiness/career exploration. AKA Science, a non-profit after school program housed within Impact NW, has partnered with a Portland LGBTQ+ organization to create an evening of biology for teenage participants. This evening program consisted of three biology-based, hands-on activities created in partnership with LGBTQ+ scientists, followed by a video web chat with these scientists. This format allowed the participants to experience different aspects of biology and then connect with the scientists from all over the U.S. and discuss their science and their lives. It also encourages participants to see biology as a future career that is engaging and attainable.

The effect of male prostaglandin on courtship and female reproductive allocation in house crickets

Isabel Burger, Anna Parrott, Naomi Meurice, Kerianne Wilson

House cricket courtship and reproductive allocation are influenced by several factors such as male body size and acoustic signaling. Female reproductive allocation is also influenced by compounds in the seminal fluid of their mates. Specifically, prostaglandin has been shown to significantly increase egg laying activity in females. Females acquire prostaglandins from males during mating via spermatophores. Additionally, prostaglandin receptors have been observed along chemosensory pathways of both insects and other arthropods, suggesting house cricket females may be able to assess male prostaglandin levels during courtship. Given the importance of prostaglandin for female reproductive allocation, we sought to determine whether there was a relationship between male prostaglandin production and female courtship behavior and reproductive allocation. Naproxen was used to manipulate male prostaglandin production prior to each male being housed with a single female. Females were provided with substrate in which to lay eggs for 9 days following mating. Data on courtship behavior, egg laying rate, total eggs laid and fertility (based on the proportion of eggs that hatched) were measured. We hypothesized that females mated to males treated with naproxen would take longer to mount and have lower reproductive allocation.

Comparing thermal sensitivity and acclimation capacity between unisexual and sexual salamanders

Isabella Burger, Michael Itgen, Rob Denton, Eric Riddell

The increased genetic variation of polyploid, unisexual groups may result in high levels of tolerance for varying environmental conditions. This tolerance may provide an explanation for the persistence of certain unisexual species throughout millennia and may also influence vulnerability of unisexuals during environmental change. In this study, we compared 1) the thermal sensitivity of metabolism and resistance to water loss and 2) the acclimation potential of two sexual ambystomid species and their unisexual populations. We used flow-through respirometry to measure metabolic rate and resistance at 6°C, 14°C, and 22°C. Individuals were housed at 14°C during the first trials, then moved to 20°C for 3 weeks prior to the next round of experiments. Preliminary results indicate an increase in metabolic rate and resistance to water loss with temperature across both groups. While metabolic rate did not vary across populations, unisexuals exhibited a higher resistance to water loss. Furthermore, both sexuals and unisexuals acclimated, with no variation found between groups. This similarity in acclimation capacity exhibits the ability of the unisexual lineage to perform comparatively to their sexual counterpart. Furthermore, though we did not find any variation in metabolic rate between the two groups, the higher resistance to water loss in unisexuals may facilitate their persistence through future climatic shifts, as this lowered desiccation risk could allow for more activity time when compared to other sexual populations.

Functional feeding mode and diet underlie the exceptional oral jaw diversity of coral reef fishes

Michael Burns, Darien Satterfield, Nick Peoples, HoWan Chan, Anthony Barley, Michael Yuan, Alexus Roberts-Hughes, Khalil Russell, Marta Hess, Sarah Williamson, Katherine Corn, Michalis Mihalitsis, Dylan Wainwright, Peter Wainwright

Coral reef fishes exhibit extreme trophic diversity. We, therefore, explored how feeding mode and diet influenced the evolution of functional oral jaw diversity across the highly polyphyletic assemblage of fishes on coral reefs. We measured 13 linear traits from the jaws and craniofacial structures of 110 species from 40 families of coral reef fishes. We found that lineages of biters, which feed by removing attached prey from the benthos, evolved towards smaller and more anteriorly placed oral jaws with larger adductor muscle masses than lineages that rely on suction to capture free-swimming prey from the water column. Further, species in seven diet categories differed dramatically in the combinations of jaw traits that characterize each diet, indicating that evolution has capitalized on the complexity of the feeding mechanism during trophic diversification. Lastly, we reconstructed the evolutionary history of feeding mode and fit a model of multivariate character evolution. Biters have evolved the same amount of oral jaw disparity as suction feeders in less than a third of the time by evolving oral jaw elements ~2.9 times faster than suction feeders. Thus, benthic biting fishes are a relatively recent phenomenon and represent the most evolutionarily dynamic trophic category among coral reef fishes.

High Elevation is an ecological dead end for Appalachian salamanders

Edward Burress, Maya Stokes, Martha Munoz

The southern Appalachian Mountains are a global biodiversity hotspot for salamanders. Mountains may promote high species richness by acting as evolutionary catalysts and/or refugia. How mountains influence other dimensions of diversity – ecological and phenotypic – is uncertain. Here, we assess how abiotic factors that contribute to microhabitat availability vary with elevation gradient throughout the Appalachian Mountains, surrounding foothills, and lowlands. We compare this to elevational patterns in microhabitat use, phenotype, and macroevolutionary features (adaptive peaks), in a group encompassing 89 species of lungless salamander (Plethodontidae). We find that there are broad abiotic controls on microhabitat availability along the elevational gradient. As elevation increases, the spatial distribution of cave-forming rock-types decreases (determined by of large-scale geologic process), surface water availability also decreases (a function of climate and river network topology) as does soil depth (a function of geomorphic processes such as weathering). The presence of salamander microhabitat specialists is influenced by these abiotic factors, leading to a decline in microhabitat specialists, phenotypic disparity, and the number of evolutionary optima at higher elevations. While there is a multitude of microhabitat specialists at low and intermediate elevations, terrestrial species dominate salamander assemblages at high elevation. Eco-evolutionary processes operating within an abiotic framework of non-random spatial patterns in the availability of habitat leads to an ecological dead end for salamanders at high elevation.

Poor nutrition induces phenotypically plastic changes in size, but not shape, of rat lower molars

Robert Burroughs, Natasha Vitek, Devin Ward, Emma Pomeroy, Malgorzata Martin-Gronert, Sue Ozanne

Phenotypic plasticity may affect evolutionary responses to changing environments. The fossil record is a critical source of data on evolutionary responses, but most studies of plasticity are on traits which rarely fossilize. What is needed is to understand how environmental changes induce plasticity in fossilizable traits. We address this need by examining common lab rats (*Rattus norvegicus*) from a controlled feeding experiment where experimental animals were exposed to lower protein diet in utero and throughout weaning. This experiment allows us to link differences in early life nutrition to plasticity in molar

shape. We tested whether nutrition induces changes in relative sizes of cusps given previously documented decreases in tooth crown size among the animals exposed to the low protein diet in early life

We collected landmarks from μ CT scans of 21 (9 Control, 12 Low Protein), three-month-old individuals. Landmarks were placed to represent an outline to calculate a convex hull to estimate of tooth crown area and provide size correction and to estimate the size of major cusps on each molar. We recovered no significant difference in relative cusp size in low protein individuals vs. control individuals, consistent with isometric reduction in cusp size. These preliminary results suggest a specific pattern of plastic change that could be detected in the fossil record, pending future work confirming results with expanded sample sizes and phylogenetic scope.

How bee learning might influence the evolution of a common flower morphology

Jenny Burrow, Rachel Wilkins, Maggie Mayberry, Annaliese Novinger, Katherine Naumer, Haley Muse, Dalton McCart, Avery Russell

Pollinator learning often drives flower evolution, but how learning affects flower morphological evolution is poorly understood. In particular, how pollinators learn to manipulate flower morphology might alter pollination and foraging success. We studied how bee learning might mediate a common morphological change: transitions from free (anthers spread out and freely moving) to joined (anthers fused into a cone) architecture. First, we assessed how bees learned to manipulate free architecture and whether this affected bee pollen collection and/or pollen transfer among flowers. Experienced bees manipulated anthers into a partial cone, increasing pollen collection without affecting pollen transfer. These results suggest learning may drive shifts from free to joined anther architecture, given that partially-joined architecture benefits the bee without penalizing the plant. Thus, we next examined how architecture specifically affected pollination and pollen collection. We found that joined architecture increased pollen transfer yet decreased collection. Taken together, our results suggest bee learning facilitates evolution of a partially, but not completely-joined anther architecture.

Identifying novel markers of stress in sharks

Karson Burton-Reeder, Jane Khudyakov, Jayne Gardiner, Tonya Wiley

Sharks are crucial to oceanic food webs and ecosystems, but many species are threatened by stressors such as fishing, pollutants, and interactions with humans. Understanding how human stressors affect shark physiology is critical for predicting their impacts on animals, populations, and habitats. We are developing markers for assessing the health and stress state of four species of sharks that inhabit a highly urbanized ecosystem (Tamp Bay): Bonnethead Shark (*Sphyrna tiburo*), Blacknose Shark (*Carcharhinus acronotus*), Blacktip Shark (*Carcharhinus limbatus*), and Atlantic Sharpnose Shark (*Rhizoprionodon terraenovae*). Since corticosteroids, which are commonly used to detect stress in wildlife, are difficult to measure in elasmobranchs, there is a need for alternative biomarkers of stress in sharks. We measured metabolites (ketones, lactate) and oxidative stress markers in a cross sectional set of plasma samples from the four shark species. We determined baseline variability and identified species-, age-, and sex-specific differences in these markers, and are currently correlating them with contaminant and proteome profiles to identify novel metrics of health and stress in hammerhead and requiem sharks.

Freshwater habitats promote rapid rates of phenotypic evolution in sculpin fishes

Thaddaeus Buser, Olivier Larouche, Andres Aguilar, Michael Sandel, Brian Sidlauskas, Adam Summers, Kory Evans

The invasion of freshwater habitats by marine fishes is an exceptional case of habitat-driven biological diversification. Freshwater habitats make up less than 1% of aquatic habitats but contain ~50% of fish species. However, while the dominant group of freshwater fishes is older than that of marine fishes, it is less morphologically diverse. Unequal morphological diversification between two clades is classically explained by two phenomena: differences in the tempo and/or differences in mode of evolution between the two groups. We tested

for evidence of these two phenomena in the superfamily Cottoidea (sculpins), which contains a substantial radiations of both marine and freshwater fishes. We find that the morphology of freshwater sculpins evolves faster but under higher constraint than that of marine sculpins, causing widespread convergence in freshwater sculpins and higher incidence of morphological novelty in marine sculpins. The endemic freshwater sculpins of Lake Baikal, Siberia, are exceptions, and there we observed high levels of novelty more akin to that of the marine environment. We attribute the higher levels of morphological constraint generally present in freshwater taxa to niche conservatism. There are several tantalizing explanations for these findings, such as differences in habitat stability and/or habitat connectivity between marine and freshwater systems.

Molecular mechanisms behind Sexual Dimorphism in Pacific Salmon

Max Butensky

Pacific salmon have evolved sexually dimorphic mature phenotypes under the strict parameters established by reproductive competition followed by semelparity, a programmed senescence. Across the taxa oncorhynchus, emergence of secondary sex characteristics (ssc) is conserved strongly within sexes, with greater variation across species. Intersexual divergence, through emergence of specific ssc traits, serve purposes respective to reproductive strategies. Females conserve the mobilization of their energy store and prioritize allocation for oogenesis. Males expend their energy on the rapid development of ssc that aid prolifically in spawning behaviors fighting males and shielding mates. 70 years of literature have illuminated the ecological and physiological patterns integral to sexual maturation and provide a biological roadmap for an investigation into the mechanisms of variation in ssc across pacific salmon. Underlying these trends, the progressive maturation activated by the gonadotropins spurs the positive auto-regulating steroidogenesis within the gonads to produce mature gametes for expulsion. Systemic circulation of terminal androgens (testosterone, 11-ketotestosterone) positively correlated to ssc emergence is associated with transcriptomics from somatic and germ tissues in progressively maturing pink salmon (*O. gorbuscha*). From this comparison, we infer relevant gene pathways and identify targets for functional analysis ultimately providing key insights into androgen-activated phenotypes in teleost fish. Applications for this investigation include (1) conservation and managements of ESA listed species, (2) reproduction biology of fish with novel life histories, and (3) commercial aquaculture with increased quality and quantity yields.

Parallel Play: Convergent Evolution of Ecological Strategies By Separate Lineages of Coral Symbionts

Caleb Butler, Kira Turnham, Andy Hess, Todd LaJeunesse

Dinoflagellates of family Symbiodiniaceae form mutualisms with a diverse range of host organisms, including reef-building corals, soft corals, and other marine invertebrates. Soft corals (Class Octocorallia), unlike reef-building corals, appear to be more resilient to marine heatwaves, resulting in their abundances increasing globally. However, the identity, ecology, and evolution of their algal partners are often overlooked, despite being the functional unit of coral bleaching. We gathered samples across reefs in Australia, New Caledonia, Palau, Taiwan, Tanzania, and Thailand were collected to examine the breadth of Octocoral-associated Symbiodiniaceae diversity using genetic, ecological, and morphological data. We found that soft corals that obtain their endosymbionts directly from the environment tend to share the same Symbiodiniaceae in their respective sites, while maternally inherited endosymbionts lead to host-specific lineages. Similar patterns of endosymbiont evolution emerged between Octocorals and Hexacorals, with parallel patterns of host-symbiont associations from different radiations of genus *Cladocopium* across each site. Ultimately, this phylogenetic analysis enhances our understanding of the evolution of this symbiosis and allows us to formally describe species of Symbiodiniaceae that associate with soft corals, which may become more abundant as soft coral coverage increases in the future.

Dopamine neurons govern chemosensory-gated tadpole begging behaviors

Julie Butler, Lauren O'Connell

Our earliest interactions are with our caregivers, whom we rely on to satisfy our basic needs, such as food and safety. For Mimetic poison frog tadpoles (*Ranitomeya imitator*), recognizing and responding to a potential caregiver is crucial for survival. Tadpoles beg for food by vigorously vibrating their body back and forth to elicit feeding from mom. Begging is necessary to elicit feeding, but begging to a non-caregiver is energetically costly and increases predation risk. Here, we examined the sensory and neural basis of caregiver recognition and tadpole begging. We found that tadpoles rely on chemosensory stimuli for caregiver recognition, but multimodal stimuli are needed to elicit begging. We also found that dopamine signaling modulates begging behaviors. Stimulating dopamine signaling through D1 receptors increases begging, but blocking signaling through D1 receptors completely inhibits begging behavior. We also found that midbrain dopamine neurons in the caudal posterior tuberculum (cTP) regulate begging behavior through projections to a motor region in the hindbrain. Ablating cTP dopamine neurons inhibits begging towards a caregiver but does not affect other motor patterns. Those same midbrain dopamine neurons are activated when tadpoles are exposed to chemosensory stimuli from a caregiver, but not when presented with control water or conditioned water from a closely related species, indicating they are finely tuned for conspecific recognition. Together, this suggests that dopamine neurons in the cTP act as a command center for begging by incorporating sensory inputs and modulating motor output.

Evolution of toepads, lifestyle, and clinging ability in Papuan microhylid frogs

Marguerite Butler, Allison Fisher, Diana Gao, Sato Kaitlyn, Ethan Hill, Claire Fraser

Many species of frogs have enlarged toepads, which are particularly well-developed in species that are able to climb. Frogs remarkably can cling to wet or dry surfaces, which can be smooth or rough as they climb throughout the canopy or range from terrestrial to aquatic environments. Frogs of the subfamily Asterophryinae of the New Guinea region are remarkably ecologically diverse, having adaptively radiated into the five anuran lifestyles: terrestrial, fossorial (burrowing), scansorial (shrub), arboreal, and semi-aquatic, and presumably have different clinging abilities. We analyzed toepad morphology in 12 species of Asterophryinae and all 5 lifestyles from a community on Normanby Island, PNG. We analyzed toepad morphology in relation to clinging performance and microhabitat use data. We also compared toepad morphology across the two independently evolved arboreal genera. We discuss the evolution of toepad morphology in relation to ecomorphology in the diversification of Papuan microhylid frogs.

Nestling circulating glucose and triglyceride levels predict juvenile presence at a field site

Michael Butler, Zachary Cullen, Jordan Lam, Brooke Weiss, Jon Wallace

Using early-life information to predict later-life survival and behavior is a critical component of many studies. Working with younger vertebrates, and particularly nestling birds, offers many logistical advantages due to the relative ease of collecting morphology measurements and blood-based samples from individuals that are unable to escape investigator presence. These data become far more informative when they are associated with patterns that manifest days or weeks after sample collection. For example, many investigators collect information on nestlings related to body size (e.g., body mass, tarsus length) as a proxy for later-life survival. Here, we collected body size and circulating nutrient data from 149 nestling house sparrows (*Passer domesticus*) just prior to fledging, at which point we also affixed an RFID tag to each individual. Feeding stations within the field site were equipped to record the time and date of individual presence for the next 16 months. We found that while neither nestling body mass nor tarsus length predicted detection at a feeding station, nestlings with higher circulating levels of either glucose or triglycerides were more likely to be detected during the juvenile stage. Furthermore, these patterns became more pronounced for the subset of individuals that were

continually detected for at least a month at the field site. Future steps may provide context to these detection patterns, including differentiating between juvenile survival and natal dispersal.

What can frogs teach us about resilience?

Allie Byrne, Corinne Richards-Zawacki, Cheryl Briggs, Jamie Voyles, Roland Knapp, Erica Rosenblum

Examples of resilience in nature give us hope amid a growing biodiversity crisis. Naturally, as biologists we seek to uncover the underlying mechanisms that can help us explain the secrets of resilience across scales; from individuals, to species, to ecosystems, and beyond. The NSF-funded Resilience Institute Bridging Biological Training and Research (RIBBITR) was born of this shared interest, leveraging examples of amphibian resilience following a global disease outbreak to uncover shared patterns and processes linked to resilience across amphibian communities. In my talk, I will highlight insights gained through studies situated in two of these systems, namely the Mountain Yellow-Legged Frogs of California (*Rana muscosa/sierrae*) and the Golden Frogs of Panama (*Atelopus zeteki/varius*). Leveraging these two examples, each of which include long-term ecological datasets and whole exome investigations into molecular mechanisms linked to disease, I will present intriguing findings that serve to highlight shared and unique characteristics of resilience across scales and systems. Reflecting on this work, I will offer new insights and questions about resilience and consider what role biologists studying this phenomenon might occupy moving forward.

Leukocyte profiles and bloodborne parasites as a predictor of condition in a songbird

Andy Cabrera, Joel Slade, Daniel Baldassarre, Sonja Anderson

The correlation between high-quality plumage and fitness is mainly explained by the “good genes” model of sexual selection, whereby females select highly ornamented males so their offspring inherit beneficial alleles. Many studies have elucidated that quality in plumage coloration signals genetic quality through fitness, such as genetic reproductive success and nestling provisioning; however, only a handful of studies explored the relationship between leukocyte profiles, infection, and body condition. Our study explores the relationship between ornamentation, leukocyte profiles (e.g., H/L ratios), malaria infectivity, and body condition in male house finches (*Haemorrhous mexicanus*). Studies on house finch red carotenoid coloration and individual quality are well-established, whereby males exhibiting less red coloration tend to have lower reproductive success. In the spring of 2021 and 2022, we captured about 75 male house finches on the California State University, Fresno campus. We collected blood to identify bloodborne parasite lineages and to make thin blood smears for microscopy and breast feathers for spectrophotometry. We hypothesize that males with the highest quality plumage coloration will have decreased H/L ratios, no avian malaria, and have the highest body condition. Blood slides are currently being analyzed, and results will be discussed. Ultimately, our study will expand research on honest signals of immunocompetence and condition in free-living songbirds.

Macroinvertebrate community composition and diversity in wild rice beds

Abigail Cahill, Jessica Garcia-Lopez, Alyvia Martinez, Miles Newman, Adrian Sanchez

Wild rice (*Zizania palustris*) is an annual aquatic plant found in rivers and lakes in Michigan, forming emergent beds that are home to a diversity of macroinvertebrates. These animals are key components of aquatic food webs and indicators of river quality. We studied macroinvertebrate community composition and diversity in a short (ca. 200 m) stretch of the Kalamazoo River in Albion, Michigan, USA to see if there was a difference in these factors inside and outside of the rice beds. We used collectors deployed in the river for two weeks, which were colonized by invertebrates. The animals were identified using morphology, and we calculated taxon diversity and richness. This process was repeated in three seasons (fall, spring, summer). We found that seasonal patterns in diversity and richness were stronger than differences between habitat with and without wild rice: after the growing season, wild rice

sites had higher richness than sites without it. However, during the growing season, differences in diversity and composition did not clearly relate to the presence of wild rice. We also suspect that other, unmeasured factors such as nutrient availability are important in driving invertebrate diversity in this system.

Thermal stress on symbiosis onset and maintenance in developing *Exaiptasia diaphana* pedal lacerates

Jun Cai, Virginia Weis

The success of coral reefs depends on the cnidarian-dinoflagellate symbiosis which forms between the coral host and its endosymbiotic dinoflagellate symbionts. Under heat stress, homeostasis between host and algae is destabilized, leading the coral host to expel its symbionts (called coral bleaching). Corals eventually die if the symbiosis cannot be reestablished. Mass bleaching events, caused by elevated temperature from climate change are increasing in frequency and severity and threaten to cause the loss of 90% reefs by the next century. Given this threat, a better understanding of coral thermal tolerance and resilience is needed to help develop solutions for corals to survive. This includes characterizing the effect increasing temperature has on host development and symbiosis in juvenile polyps at the cellular level. We developed experiments to determine the effect of elevated temperature on asexually produced pedal lacerates, in *Exaiptasia diaphana* (commonly known as *Aiptasia*), a model system for coral-algal symbiosis studies, and a proxy for juvenile coral polyps. In our experiments, we examined the effects of elevated temperature on aposymbiotic, symbiotic and recolonized lacerates. Animal growth rate was measured using tentacle number and pedal disk size and EdU labeling to count the number of dividing host cells. Symbiont population health was measured by quantifying symbionts in hosts and by measuring photosynthetic efficiency using a PAM fluorometer.

Too Humid to Handle: The Effect of Ambient Humidity on Ant Adhesion, Locomotion, and Behavior

Mandy Cai, Stephen Yanoviak, Alyssa Stark

Ants are ecosystem engineers that rely on their adhesive tarsal pads to climb while foraging, escaping from predators or unfavorable conditions, and defending territory. The glue-like secretion on their tarsal pads allows *Camponotus pennsylvanicus* to forage in Pennsylvania where relative humidity (RH) can range from 20-100% and substrate wettability dictates how the moisture in the air will interact with various surfaces. The highly variable RH in temperate ants' environment and ability of thin water layers to disrupt the ant adhesive system raises the question of how temperate ant adhesion, locomotion, and behavior may vary with RH and substrate hydrophobicity. Therefore, experiments were conducted to test for the effect of RH and substrate wettability on ant performance by testing shear adhesion and running speed while also observing behavioral changes of *C. pennsylvanicus* in six RH setpoints (30, 40, 50, 60, 70, 80, 90% RH) on three substrates (hydrophilic glass, hydrophobic polypropylene, and intermediately wetting polycarbonate). We hypothesized that adhesion and running speed would reduce, and mitigating behaviors (stop, turn around) would increase on glass more than the other substrate due to elevated water layer deposition at high RH on this hydrophilic substrate. The results of this study will improve predictions about how variable climate conditions, particularly those related to climate change, will influence this key member of most global ecosystems.

Ride along Microplastics: microplastic dispersal across recreational, agricultural, and game trails

Sophi Cain, Jason Davis

Microplastics to be a pervasive global problem, exhibiting seemingly unlimited in a range of environments. From the tops of mountains to the tissue of various organisms, including humans, microplastics have proven to be all but omnipresent. This begs the question: how are microplastics distributed across environments, and how do natural and anthropogenic actions contribute to their dispersal. In the current study, soil samples were collected on and off hiking, agricultural and game trails at multiple sites in Far Southwestern Virginia. We explored various factors related to soil composition, use and

location map patterns in their distribution that may be related to trail type and location. Preliminary results suggest that microplastic distribution and type may be correlated with trail features.

Leg Jiggling - A Model for Understanding Biological Spring-Mass Systems

Ofubofu Cairns, Samantha Falcone, Rachel Fleming, Thomas Roberts

Cyclic motion is common across diverse biological movements. Some cyclic locomotor activities rely on elastic structures, such as tendons, and are governed by the physical rules of spring-mass systems. A simple, accessible biological model could be beneficial for exploring how physics and physiology interact in cyclic movements. We used nervous leg jiggling as our model, investigating its rhythmicity using force and displacement transducers. We tested the hypothesis that spring-mass dynamics would constrain preferred leg jiggling to a narrow range of frequencies. Preliminary data support this prediction; subjects were able to match a metronome at very low frequencies but not at frequencies just above or below their preferred. This result is consistent with the frequency-energy relationship of driven spring-mass systems. Adding mass to the leg resulted in increased jiggling frequency in most participants. This is not consistent with the physics of simple spring-mass systems, which would predict a decrease in frequency with increased mass. We speculate that the muscle volume recruited increased with added mass resulting in greater leg stiffness and thus higher frequencies. We find that leg jiggling is an effective model for understanding more complex physical and physiological interactions in biological cyclic motion. These observations may help explain patterns observed during other cyclic motions, including beak behavior in woodpeckers, which provided the original motivation for our study.

Navigating Turbulent Environments: Insights from Fish Schools

Michael Calicchia, Rui Ni

Despite decades of research into fish locomotion, the mechanisms that fish utilize to navigate through chaotic, turbulent environments remain elusive. Of particular interest is how fish cope with turbulent flows, where the largest eddy size (e.g., integral length scale) is larger than their body length. We hypothesize that fish utilize collective motion to filter out the largest eddies and generate a more coherent hydrodynamic signal within the school that is readily detectable by their lateral line. To investigate this hypothesis, experiments were performed in our cutting-edge FATE (Fish Aquarium with a Turbulent Environment) facility at Johns Hopkins University. By leveraging a unique jet array system, we control the turbulence independent of the mean flow. This enabled us to study how schools of Giant Danios (*Devario Aequipinnatus*) swim in flows with a fixed mean velocity but increasing energy dissipation rates. For each case, the dynamic behavior of the schools was recorded to analyze fish school size and shape using statistical approaches. This ongoing work aims to reveal the underlying principles that fish schools utilize to navigate through complex fluid environments.

The insatiable sweet tooth: molecular adaptations to increased sugar consumption in mammals

Jasmin Camacho, Andrea Bernal, Selene Swanson, Nicolas Rohner

Excessive sugar consumption is an unusual adaptation in mammals. Many mammals that consume too much sugar, including humans, will develop negative effects to blood glucose homeostasis. Blood glucose regulation is crucial to survival because glucose serves as a primary source of energy for the body's cells, especially the brain. Exceptionally among mammals, bats have independently evolved nectar-eating in multiple lineages. To identify metabolic adaptations unique to nectar-feeding bats, we tested glucose regulation across different bat species with a standardized stable isotope glucose tolerance test. Various species of nectar bats maintained high [U-13C] glucose levels at rest, likely through kidney reabsorption, whereas fruit bat species rapidly lowered [U-13C] glucose levels at rest, likely through insulin signaling. In order to reveal the genetic basis behind metabolic adaptations in nectar bats, we conducted a comparison of genome coding regions and found parallel amino

acid substitutions in genes functionally enriched for sugar and fat metabolism, DNA damage response, immunity, and lifespan. Based on initial examination of the [U-13C] enriched proteome, it appears that in insect bats, protein glycation, which can produce damaging advanced glycation end products, is correlated to increasing blood glucose levels. By integrating complementary -omics data, we will gain new perspectives on metabolic adaptations and the roles played by both convergent and divergent genetic changes.

Evolution of cranial morphology in replicated transitions to subterranean environments in Eurycea

Henry Camarillo, Ruben Tovar, Dana García, Tom Devitt, David Hillis, Bhart-Anjan Bhullar, Martha Munoz

Organisms can adapt to changes in their environment by either evolving novel structures that can appear at any stage of ontogeny (e.g., changing the shape of existing structures), or by changing the time and order that structures appear during development (i.e., heterochrony). Groundwater salamanders (genus *Eurycea*) can serve as a model system to understand how shifts in habitat influence morphological evolution due to their repeated independent transitions of subterranean habitats. One of the demands that has likely shifted with invasions to subterranean habitats is the difference in feeding ecology due to changes in abiotic (light availability) and biotic (prey availability) between surface and subterranean environments. Here, we compare differences in the musculoskeletal system among species of *Eurycea* salamanders to test how independent shifts to cave environments result in changes to feeding morphology through ontogeny. We compare divergent species through development to identify differences in the timing and patterns of muscular development. We utilize diceCT to reconstruct cranial elements in 3D and use VolumeGraphics Studio to isolate musculoskeletal structures.

Mechanical properties of snake skin vary longitudinally and following large prey ingestion

Lucy Campbell, Bruce Jayne, Thomas Roberts, Jarrod Petersen

The ability to ingest prey larger than their head (macrostomy) is a widespread, derived trait in snakes that involves distending the skin and metabolic upregulation. However, data remain sparse for how the material properties skin vary: after feeding, among longitudinal locations, and among species. We recorded uniaxial stresses and strains in circumferential loops of skin from four fasted and four recently fed boa constrictors. We also tested skin from different pre-loacal longitudinal locations in additional fasted snakes including two non-macrostomate genera (*Afrotyphlops*; *Anilius*) and a highly specialized macrostomate genus that eats only bird eggs (*Dasypeltis*). The neck skin of fed boa constrictors had a lower elastic modulus (26.7 ± 8.1 MPa $\pm$ s.e.m.) than the neck skin of fasted snakes (60.9 ± 4.2 MPa $\pm$ s.e.m.), and both were significantly lower than mid-body and caudal skin values of fasted snakes ($p < 0.05$). Compared to the other taxa, the longitudinal variation in the skin properties of the non-macrostomates was minimal, whereas the egg-eating snakes had neck skin with much lower stiffness that reached much greater strain before failure than skin from more posterior locations. Hence, the extent of longitudinal variation in skin properties is both species-dependent and affected by feeding. Whether the differences associated with feeding arise from active remodeling of the skin or from damage that needs subsequent repair is an interesting question for future studies.

Analysis of humeral robusticity in individuals with unilateral septal apertures

Timothy Campbell, Stephanie Baker

Septal apertures (SA) are defects in the bony septum that separates the olecranon and coronoid fossae on the distal humerus. Previous research has found that their presence is highly variable between populations, occur in higher frequencies in females and on left humeri, and are significantly associated with the non-dominant hand. Several hypotheses have been proposed for their etiology with both support and lack of reported in the literature. Here, we test the robusticity hypothesis which postulates that more gracile humeri are more susceptible to SA development using 44 individuals (31

females – 13 males) with unilateral SA housed at the Texas State University Donated Skeletal Collection (TXSTDSC). Seven standard liner measurements were taken including humeral maximum length, midshaft maximum diameter, and five additional measures on both proximal and distal ends. Robusticity estimates were generated using the first two measures to calculate humeral robusticity ratios (HRI), while all measures were used to calculate geometric means (GM). Bilateral robusticity estimates were analyzed using paired-t and Wilcoxon signed-rank tests. These analyses recovered significant differences ($P < 0.05$) in GM between humeri with SA and their contralateral, while HRI comparisons were not significant ($P 0.10-0.05$). These results may indicate that estimates which incorporate more information than bivariate ratios better recover size differences between paired elements. Future research on SA will include calculating cross-sectional geometry estimates of robusticity using mCT data.

Investigating Sediment Traits to Hindcast the Distribution of the Marine Angiosperm *Zostera marina*

Palmer Campbell-Kaswell, Sandy Wyllie-Echeverria, Bruce Finney

Eelgrass (*Zostera marina*) is an essential ecosystem engineer and keystone species throughout its distribution in the Northern Hemisphere. Consequently, these marine plants are valued and monitored. In the San Juan Archipelago region of the Salish Sea, ongoing monitoring since 2000 documents that several eelgrass populations disappeared, declined or became fragmented over this time period. One site where decline and fragmentation occurred is False Bay, San Juan Island, a marine preserve managed by the Friday Harbor Laboratories, University of Washington. In summer 2023, to understand the cause for eelgrass loss between observations made in 1994 and the present day, we extracted and examined sediment cores from the area where eelgrass existed in 1994. During daytime low tide events, we extracted five cores along a 365 m transect through the targeted area and analyzed these for grain size distribution, organic material content, and stable carbon isotope composition. Our objective was to correlate these characteristics with the historic distribution of eelgrass. We found that approximately the top 30 cm of each core was composed of homogenous fine to very fine sand with a comparatively low organic material content. Below this layer, the variance in both grain size and organic material increased dramatically. We suspect this shift in sediment characteristics may have influenced eelgrass loss in False Bay, but are waiting on radiocarbon dating results to determine when the shift occurred.

Walk The Line: The Energetic Cost of Modulating Walking Speed Via Stride Frequency or Length

Matthew Cannata, Noah Chernik, Reuben Jacobson, Stratos Kantounis, Melody Young, Michael Granatosky, Edwin Dickinson

Controlling speed in animals can be achieved by modulation of either stride frequency or stride length. While the relationship between these variables is well-documented, the central question of whether animals primarily modify stride frequency or stride length to increase their speed remains unresolved. Additionally, the underlying evolutionary, ecological, and anatomical reasons guiding the preference for one speed regulation strategy over the other remain unknown. In general, larger species tend to regulate speed by modifying stride length. This phenomenon is thought to decrease locomotor costs by reducing the total number of strides needed to cover a certain distance and by lowering swing phase expenses. However, the validity of this hypothesis lacks empirical validation. To address this gap, we conducted experiments with human subjects, manipulating stride length and frequency across various speeds to analyze their metabolic effects. Interestingly, at all speeds, deliberately controlling stride length led to significantly higher transport costs compared to unrestricted walking or maintaining a consistent stride frequency. This indicates that reducing the number of foot contacts with the ground is the most effective approach for minimizing locomotor expenses. Furthermore, for larger species, the costs associated with swinging the limbs during locomotion are likely substantial, and longer strides contribute to diminishing these swing phase costs. These findings provide experimental confirmation of the long-held assumption that longer strides result in more energy-efficient gaits.

Love in the heat: Avian courtship displays in response to a thermal challenge

Susan Cantonwine, Tara Empson, Melissa (Misty) Proffitt, Maëlle Lefeuve, Elizabeth Derryberry

A changing climate has increased the occurrence of temperatures outside of species' thermal comfort zones. As a result, many songbirds are experiencing sub-lethal and lethal effects of warming temperatures. For instance, on hotter days birds tend to produce fewer songs, a well-studied courtship display. Non-song courtship displays are less well-studied, yet dancing for the female can serve as an important male mating signal. We hypothesize that heat impacts male investment during courtship. To test this, we recorded several visual and auditory courtship behaviors in male zebra finches (*Taeniopygia castanotis*) at either a thermoneutral temperature (35°C) or during a heat challenge (43°C). Our song output results showed that most males sang less in the heat challenge, which is consistent with previous work. Although a few males in a heat challenge sang more than those in the thermoneutral temperature, this is likely attributed to individual variation. We predict that thermally-challenged males will also perform fewer non-song courtship displays due to heat stress. Such disruption of male courtship behaviors could potentially impact signaling of male quality and reduce reproductive success. Further, variation among individuals in courtship performance in heat could translate to differential fitness in the wild. This work allows us to understand more about how rising temperatures could affect sexually selected traits in songbirds.

Effects of short-term exposure to pesticide mixtures on free-swimming behaviors in goldfish

Esmira Cantu, MD Rahman

Aquatic organisms are noxious in toxic environments, on account of escalating pollutants defiling their natural habitats. The prevalence of pesticides in the aquatic environment continues to increase due to anthropogenic activities and poses a threat to aquatic organisms. These include compounds such as pesticides (more aptly called biocides) that contribute to a wide variety of stressors vitiating aquatic ecosystems and perniciously influence the life and behavior of aquatic organisms. The aim of this study was to determine the effects of short-term exposure (5-day) to an environmentally relevant pesticide mixture (low- and high-dose: metolachlor 2.4 and 12 µg/L; linuron 2.0 and 10 µg/L; isoproturon 1.2 and 6.0 µg/L; tebucazazole 1.2 and 6.0 µg/L; alconifen 0.8 and 4.0 µg/L; atrazine 0.4 and 2.0 µg/L; pendimethalin; 0.4 and 2.0 µg/L; azinphos-methyl 0.8 and 4.0 µg/L) on swimming behaviors of goldfish (*Carassius auratus*, a model teleost species). Behavioral analysis showed a dose-dependent, time-dependent, decrease in distance swam and the prolonged time they stayed in each region of the tanks. Collectively, these results indicate that pesticide mixture influences fish behavior and negatively impacts natural swimming patterns in teleost species.

Pesticides alter tissue morphology and induce oxidative stress and apoptosis in kidneys of goldfish

Esmira Cantu, MD Rahman

Aquatic organisms' life is damaged in toxic conditions due to escalating pollutants tarnishing their natural habitats. Anthropogenic activities, such as agriculture, introduce an ever-increasing variety and volume of chemical contaminants in aquatic environments. These include compounds such as pesticides (more precisely biocides) that contribute to a wide variety of stressors corrupting aquatic ecosystems and deleteriously influencing the life of aquatic organisms. In this study, we analyzed the dose-dependent effects of pesticide mixtures (low- and high-dose: metolachlor 2.4 and 12 µg/L; linuron 2.0 and 10 µg/L; isoproturon 1.2 and 6.0 µg/L; tebucazazole 1.2 and 6.0 µg/L; alconifen 0.8 and 4.0 µg/L; atrazine 0.4 and 2.0 µg/L; pendimethalin; 0.4 and 2.0 µg/L; azinphos-methyl 0.8 and 4.0 µg/L) (exposure at 22 °C for 1 week) on the morphological and cellular alterations in the kidneys of goldfish (*Carassius auratus*). Fish exposed to pesticides showed a significant increase in oxidative and nitrate stress biomarkers, apoptotic nuclei of cells, and antioxidant expression in kidney tissues. These results suggest that pesticide mixtures alter tissue morphology and impair cellular functions in teleost species.

Sticky feet do more than stick: adhesive pad use in level and vertical walking by Argentine ants

Yakun Cao, Andrew Chacon, Agasthya Valluri, Nick Gravish

Adhesive pads (arolium) of ants are well-known for their role in increasing adhesion force on smooth surfaces. Previous studies have shown that ants activate their adhesive pads when climbing or walking upside down to generate the adhesion forces which balance the torque (force) caused by gravity. However, it is unclear if these adhesive pads aid in executing other maneuvers which may require high traction forces such as body acceleration. To address this, we collected Argentine ant workers (*L. humile*) from 8 different locations around UC San Diego campus and recorded more than 4000 videos with them moving horizontally and vertically on a glass surface over a speed range of 0.9 – 27.5 body lengths/sec. In each video, we used a high-speed camera setup at 400 fps to capture the ant body and limb kinematics as well as the real-time adhesive contact area illuminated by a frustrated total internal reflection (FTIR) device. Climbing ants consistently used adhesive pads for resisting the overturning moment from gravity. We observed that adhesive engagement did not show up in most normal walking videos. However, strides characterized by higher acceleration had a greater tendency of showing adhesive pad contact. This implies that ants strategically employ adhesive pads to amplify traction force during such motions.

Marine heatwave influences life-history tradeoff of an intertidal gastropod

Mary Capossela, Kit Yu Karen Chan

Marine heatwaves (MHWs) pose a considerable threat to marine ecosystems. While mass mortality of marine invertebrates has been documented repeatedly, few studies to date have examined MHW impact on life history tradeoffs, specifically survivorship vs. reproduction. We examined how marine heatwaves of two different magnitudes (2 degree heat day (DHD) and 4DHD) impact the mortality, growth, and reproductive output of the gelatinous egg mass laying snail *Lacuna vincta*. These conditions had already been documented in the Salish Sea, where the organisms were collected. Exposure to MHW significantly reduced the probability of survival of the snails; however, the average shell growth amongst survivors and the total number of egg masses laid were comparable between MHW treatments and control. Yet, we observed that some egg masses laid towards the end of the 4DHD wave contained fewer eggs. With increasing frequency, duration, and intensity of MHW, the observed combined reduction in survival and number of offspring suggest *L. vincta* populations may decline in the future. More broadly, our work suggests that intertidal organisms which experience regular temperature fluctuations are not immune to such extreme events.

Exploring the Genetic Basis of the Tan Streak Mutation in Deer Mice

Isabel Carino-Bazan, Shuonan He, Hopi Hoekstra

Coloration varies vastly amongst organisms, even between individuals of the same species. One such example is *Peromyscus*, which are found across North America and display profound coat color changes as they adapt to distinct environments. A naturally-occurring color morph in *Peromyscus* is known as Tan Streak (TS), which gives mice a distinctive white coat with a tan-dorsal streak. However, the molecular nature of the TS mutation is yet to be fully explored. Melanocortin type-1 receptor (Mclr) is an important regulator of mammalian pigmentation synthesis, and deer mice carrying CRISPR-induced Mclr mutations display a strikingly similar coat coloration to TS mice. Through our experiments, we aim to test our hypothesis that the TS phenotype is due to loss of function of the Mclr gene. To test our hypothesis, the two methods we used were spectral analysis of the flat skin coat color, which allowed us to quantify and compare brightness, hue, and saturation along the dorsal-ventral axis, and genotyping of the Mclr locus. Spectral analysis showed us that brightness, hue, and saturation in TS and Mclr-mutants are comparable and that these measurements are significantly higher than those of the wild-type flat skins. From genotyping, we detected four nucleotide mutations,

three of which resulted in amino acid changes, in the Mc1r coding region of TS samples. These two results allow us to deduce that the phenotypic similarity between Mc1r-mutants and TS mice denotes a genotypic correlation.

How noise affects forage fish behavior

Nora Carlson

Anthropogenic noise is garnering more and more attention as our oceans and waterways continue to increase in volume every year. While we know that this noise negatively affects a number of species in a variety of ways from altering anti-predator response, schooling behavior, movement patterns, and overall stress and condition the majority of studies have taken single noise sources into account within experiments limiting our understanding of if and how different types of noise regimens result in different degrees of disturbance. Our experiment addressed this question by placing a common forage fish, Pacific sand lance (*Ammodytes personatus*) into four different acoustic environments. We tested how noise affects a range of behaviors including: schooling, time spent in refuges, and acute anti-predator responses during the experiment, as well as measures of overall condition upon completion to determine how responses to noise change across sources and how these changes could impact forage fish availability and quality to the predators that rely on them.

A comparison of the rates of morphological and physiological evolution in woodland salamanders.

Sarah Carnes, Nathalie Alomar, Martha Munoz

Woodland salamanders (Plethodon) are lungless, terrestrial salamanders found in the United States, a hotspot for salamander biodiversity. While the morphological evolution of these salamanders has been explored, less research has focused on the rate of physiological evolution. Specifically, skin resistance to water loss and metabolism could play an important role in the diversification of these salamanders due to their reliance on cutaneous respiration. Since the hydric and metabolic physiology is closely tied to their environment, I hypothesized that rates of physiological evolution will be faster than morphological rates in the genus *Plethodon*. Linear morphological measurements were taken from the literature and averaged across several *Plethodon* species. Skin resistance to water loss and metabolic rates were collected in the lab across the same *Plethodon* species. The Brownian motion rate parameter was estimated for each trait in RStudio and compared. The rate of physiological evolution was, on average, greater than the rate of morphological evolution by two orders of magnitude. These preliminary findings for the rates at which woodland salamander traits evolve hint that *Plethodon* physiology could be a crucial component in their diversification and is a potential underappreciated trait dimension. This also raises concerns that potential deviations caused by climate change will be detrimental to physiological maintenance.

The Metabolic and Temporal Effort of Ecdysis in Timber Rattlesnakes (*Crotalus horridus*)

Max Carnes-Mason, Steven Beaupre

The semi-frequent replacement of the epidermis is a requisite energetic expenditure in squamates. In snakes, ecdysis requires metabolic effort in three distinct quantities: biosynthesis of new tissue, physical removal of the old skin, and energy sequestered in the new tissue. Ecdysis also requires significant temporal investment as animals alter their behavior during ecdytic cycles. The metabolic and temporal efforts associated with ecdysis have been poorly studied. Herein, we present the first measurements of the cost of skin biosynthesis in ecdytic snakes. We used open-flow respirometry to measure the metabolic effort and duration of ecdysis at 25C in nine Timber Rattlesnakes (117g-1100g). We found that energetic effort of biosynthesis scaled with body mass, but effort of physical removal was variable and unrelated to body size. We estimated that total effort of a shed event accounts for 3% (~163 kJ) of the total annual energy budget of a 500g adult, the energetic equivalent of consumption of ~2 adult mice. Using measurements of metabolic effort, we were able to determine the duration of the process and found that a shed takes approximately 4 weeks at 25C, with energetic investment beginning up to two weeks prior to any visible evidence of impending shed. Our data

demonstrate that ecdysis is a significant energetic and temporal cost for snakes, and further emphasize the need for additional studies of frequency and timing of ecdytic cycles.

Uncovering the Senses: Interspecific variation in filoplume morphology and numbers

Anthony Carnevale, Vanya Rohwer

Feathers are marvels of engineering; they are strong, lightweight, provide excellent insulation, signal to mates or competitors, provide camouflage, and allow birds to fly across continents and oceans. However, feathers are also dead structures but birds are able to sense when they are damaged or displaced. The current hypothesis for how birds sense feather position and condition is through specialized hair-like feathers called filoplumes. Filoplumes are positioned immediately adjacent to larger flight and body feathers and are heavily innervated, suggesting they may function as sensory feathers. Filoplumes also appear to covary in length and number with their companion flight feather. However, if filoplumes are sensory feathers, then their morphology or number should be associated with the life span of individual flight feathers, the risks of breaking feathers, and a species' flight ecology. This study provides some of the first data exploring such predictions by looking at filoplumes in the outer wing feathers of over 100 bird species, ranging from flight-averse ground-dwelling rails to albatrosses. Preliminary data suggests intriguing patterns: filoplume number and length increase with the length of their companion flight feather and, to a lesser degree, with feather lifespan. How filoplumes vary with flight ecology appears more nuanced, however. Taken together, the data offers support for some predictions that link filoplume morphology and number to sensory feather function.

Totally tubular: exploring morphology in bioluminescent tubeshoulders (Platyroctidae)

Emily Carr, Rene Martin, Jack Degnan, John Sparks

Functional innovations play major roles in constraining or contributing to the evolutionary diversification of organismal morphology. Among fish key innovations, bioluminescence (light produced by a living organism) is critical to the success of many deep-sea fishes. Bioluminescence is useful for a myriad of behaviors and interactions in the deep sea, including the use of bioluminescent lures for predation and the presence of light organs on the ventral surface for a camouflage called counterillumination. Light organ location and size can be influenced by the constraints of an organism's natural history and physical structure, including its body shape. This sometimes results in fishes with unique body morphologies (e.g., hatchetfishes) or constraints on body shape (e.g., lanternfishes). In this study we investigate the deep-sea tubeshoulders (Platyroctidae), fishes that possess a postcleithral light organ associated with their shoulder girdle that excretes bioluminescent fluid. Many tubeshoulders also possess additional light organs on the lateral and ventral surface of their bodies. Tubeshoulders are an understudied group compared to many other deep-sea fishes, with most tubeshoulder species being rarely collected. In order to advance our understanding of various aspects of tubeshoulder morphology, including their unique bioluminescent structures and body shapes, this study uses histological methods and geometric morphometrics to investigate the morphological diversity in the tube-organ structure and body-shape variation across the Platyroctidae.

The combinatorial effects of temperature and salinity on the nervous system of the American lobster

Katrina Carrier, Patsy Dickinson, Yasemin Altug, Isabella Kane, Rania Janmohamed, Kaitlyn Elias, Daniel Powell

As a result of climate change, ocean temperatures are rising, and ocean water is becoming less salty in some regions while other regions become saltier. This has important implications for the nervous systems of marine osmoconformers and poikilotherms. Changes to the ionic concentrations in their extracellular environment can fundamentally affect neuron function, as can changes in external temperature. The nervous system of the American lobster (*H. americanus*) is a useful system to understand the effects of salinity and

temperature perturbation, as it contains circuits whose neural output is well characterized and that produce well understood functional output. The stomatogastric nervous system (STNS) and cardiac nervous system control the movements of the foregut and heart, respectively. We established the upper limit of temperature that these systems can withstand in normal physiological saline. We then determined whether combinatorial changes in temperature and saline concentrations alter the ability of the systems to withstand elevated temperature. Both systems continued to function at higher temperatures in lower saline concentrations (0.75x) compared to standard (1x) or higher saline concentrations (1.25x). Upon exposure to each saline concentration tested, the temperature tolerance of two neurons contained within the STNS differed, where the PY neuron's crash temperature was higher than that of LP.

Using diving performance to explain changes in foraging ecology over ontogeny in garter snakes

Elsie Cecilia Carrillo, Rita Mehta

Ontogenetic shifts, or changes from birth to adulthood, play an important role in the foraging ecology of many vertebrates. Some snakes experience ontogenetic shifts in diet, reflecting their ability to capture and consume prey, including garter snakes that switch from feeding on terrestrial prey as a juvenile to more aquatic prey as an adult. We question whether there are physiological or behavioral changes over ontogeny that facilitate dietary shifts in two closely related juvenile semi-aquatic garter snakes with different lifestyles. For this study, we selected the Lake Chapala garter snake (*Thamnophis eques obscurus*) which is more aquatic, and the checkered garter snake (*Thamnophis marcianus marcianus*) which is more terrestrial. As a proxy for foraging behavior, we quantitatively compared diving performance metrics over the first two years of life using three experimental assays: 1) voluntary submersion in a PVC tube to examine breath-hold ability, 2) head submersion in water to examine the dive response, and 3) free swimming and diving in a small wading pool to examine locomotor mode. We hypothesized that foraging behavior changes over ontogeny as snakes gain competency in prey acquisition due to changes in diving performance. The results of this study provide insight into whether dive metrics change over ontogeny, and ultimately help explain ontogenetic changes in diet in semi-aquatic garter snakes.

Arnold's Paradigm in the field: Morphology, performance, and behavior in *Lepidodactylus lugubris*

Rachel Carrock, Keegan Lutek, Alyssa Stark

Organismal performance is rooted in the relationship between morphology and behavior, and is crucial to understanding the way organisms interact with their environment. Extensive research has been conducted on how various abiotic factors in natural environments affect the adhesive and running performance of geckos. However, few studies have investigated how individual variation in morphology affects performance within a species of gecko. Moreover, no work has explored how differences in performance are related to key factors, such as foraging success, social interactions, and antipredator behaviors. To test for the relationship between morphology, performance, and behavior, we focused on one species of gecko (*Lepidodactylus lugubris*) living freely on Barro Colorado Island, Panama. We measured various morphological traits related to performance (i.e., body size, mass, toepad area, number of lamellae, hindlimb, and hindfoot length), adhesive and running performance, and documented behaviors in the lab and in the field while foraging. We hypothesized that morphological differences affect maximum performance, and that high performing individuals differ in their behavior, both in the lab and in the field, from low performers. The results of this study have ecological and evolutionary implications related to how morphology may have large impacts on organismal behavior, which can influence social interactions, invasiveness, and fitness.

An Electric Scent: the electrostatic enhancement of insect olfaction

Xavier Carroll, Marianne Alleyne, Jennifer Bernhard

Due to their small size, insects have a unique experience of electrostatic forces in their environment. Through flight, many insects accumulate surface charges through triboelectric interactions with the air. Even though the net surface charges are small, in the pico-coulombs range, they facilitate many environmental interactions. In bee systems, these include resource assessment, pollen transfer, and possibly enhanced olfactory ability. While the first two interactions are well documented, very little is known about the effects of surface charge on insect olfaction. This research project utilizes the proboscis extension responses and electro-antennograms to compare the olfactory ability of charged and uncharged honey bees, *Apis mellifera*.

Towards Dynamic Gaits in Paleozoic Tetrapods: Damping Forces in the Axial Column

Aja Carter, Diego Caporale, Daniel Koditschek

Many paleobiological and paleoecological sciences focus on modeling extinct taxa locomotor gaits. Generally, such studies focus exclusively on joint positions within appendages to generate hypothetical gaits. However, these studies cannot capture other necessary mechanical features, such as the function of joint capsules during dynamic locomotion, or include any role of the axial column in stable gaits. From a gait dynamics perspective, damping forces at the joint or across joints in response to perturbation comprise an essential element of stable locomotion in tetrapods. Currently, no methodologies are proposed to capture such mechanical information in extinct taxa, thus obscuring our understanding of past behaviors. In this study, we combine virtual kinematic analyses with multi-material 3D printed models and a custom-built bending machine to investigate pose space generated by multiple joints in series and dynamic stiffness and damping metrics in vertebral units. For this study, we selected *Diadectes sideropelicus*, a large-bodied Paleozoic herbivore for study. Our physical models produced a configuration subset within the osteological pose space, and the multijointed pose space was a simple scaled version of the single jointed space. Our virtual kinematic analyses suggest small-angle approximations are appropriate for this work, but the non-linear response to systematic mechanical forcing results suggests complex dynamics. Our study highlights virtual and physical models as a fossil-up line of evidence and captures essential measurements for subsequent robotic models.

Simulating bird-plant interactions in 3D to assess pollination loss in Hawaiian forests

Samuel Case, Alejandro Rico-Guevara

Animal pollination often depends on mutualistic interactions with nectarivores, which transfer pollen between plants while collecting the sugary reward. Nectarivores and plants may coevolve to exhibit matched functional traits, such as coupled bird bill and floral tube shapes, increasing the specificity and benefits of their interactions. When traits are mismatched, however, species may fail to interact, or interactions may negatively impact plants (e.g., nectar-robbing). The Hawaiian Islands have experienced high rates of extinction during the last ~800 years, and Hawaiian lobelioids (Campanulaceae), which have coevolved with nectarivorous Hawaiian birds, may be pollination-limited due to bird species loss. Nevertheless, historic plant-pollinator interactions are largely unknown, and it is uncertain whether extant nectarivores (native and introduced) may potentially compensate for bird extinctions by pollinating at-risk lobelioids. Thus, a mechanistic model is needed for identifying interaction outcomes for plant-nectarivore species pairings. We propose to simulate interactions from 3D digital models of birds and flowers to investigate how trait-matching metrics can be linked to pollen transfer in wild-filmed interactions. Using 3D scans of bird specimens and flowers from wild plants, we will predict outcomes for potential extinct and extant interactions across the archipelago to estimate mutualism loss. We will then validate these predictions with pollination experiments using 3D-printed models of extinct birds. Our results may benefit efforts to conserve remaining nectarivore and lobelioid species unique to the Hawaiian Islands.

Wingbeat frequency of bumblebees (*Bombus impatiens*) decreases after sublethal imidacloprid exposure

Julia Caserto, Matthew Huang, Corey Reese, Sunghwan Jung, Minglin Ma, Mary Salcedo

Pesticides negatively affect the ability of pollinator populations, such as bumblebees, to perform natural behaviors such as foraging and pollination. This study investigated the effects of the neonicotinoid pesticide imidacloprid on the wingbeat frequency (WBF) of bumblebees (*Bombus impatiens*) using a high-speed camera and controlled dosing of imidacloprid. Two sublethal doses of imidacloprid, 5 and 10 ng, were investigated, and flight recordings were captured from the front, left, and right positions of each bee at a framerate of 9000 fps. These video recordings were then analyzed using FIJI (ImageJ) to extract accurate WBF measurements. Body morphometrics including mass, wingspan, and body length were also measured. Bumblebees given imidacloprid exhibited reduced WBFs compared to those in the control group. The observed decreases in WBF suggest that exposure to imidacloprid may have a negative impact on flight performance. The implication of this finding is significant as reduced flight efficiency can impact foraging capabilities and overall survival. These results contribute to the understanding of the sublethal effects neonicotinoid have on bumblebees and emphasize the importance of sustainable pesticide management practices to ensure the health and stability of pollinator populations.

Experimental frequency response between thorax deformation and wing rotation in corn earworm moths

Caillin Casey, Braden Cote, Mark Jankauski

Insects have developed diverse flight actuation mechanisms, including indirect actuation. During indirect actuation, the indirect flight muscles deform the thorax exoskeleton which causes the wings to flap. Indirect actuation can be modeled as a two degree of freedom mechanical model where a parallel elastic element represents the thorax exoskeleton and flight muscles, and a series elastic element represents the wing hinge where the wings attach to the thorax exoskeleton. Previous studies suggest that wing inertia is much lower in insects with synchronous muscle than asynchronous which implies that the series elastic element deforms less during flight in species with synchronous muscle but the series elastic structure was not specifically assessed. Here, we determined a frequency response function (FRF) relating input thorax deformation to output wing angle in corn earworm moths. The dependence of FRF gain and phase on thorax deformation frequency can be used to identify the relative stretch of the series elastic element. Thorax compression was induced by an electromagnetic shaker and measured via a laser vibrometer, whereas wing angle was recorded via high speed videography and estimated using Deep Lab Cut. If wing rotation gain changes with thorax compression frequency, that is evidence that the series elastic element is significantly contributing to the wing actuation in corn earworm moths.

Take it or Leaf it: Can Bees Learn to Use Leaf Shape to Find Flower Rewards?

Moth Castagna, Jenny Burrow, Ciara Stewart, Avery Russell

A century of research demonstrates that pollinators use diverse floral cues to find food rewards. However, floral cues are not always reliable, as often occurs when unrewarding plant species mimic the floral cues of co-occurring rewarding plant species. When floral cues are unreliable, pollinators should learn to use other non-floral cues instead. Here we examined whether and when generalist bees (*Bombus impatiens*) would learn to associate differences in leaf shape with a pollen reward. We expected bees would rely more on leaf shape when learning petal color was more difficult, and vice versa. We therefore assigned bees to either of two treatments, differing in terms of how much artificial flowers differed in petal color; each treatment differed in leaf shape in the same way. As expected, bees learned much faster when petal color differed greatly. Yet when petal colors differed little, bees had a harder time learning petal color and did not show evidence of having learned leaf shape. Our results suggest that an inability to learn a non-floral cue (e.g., leaf shape) may reflect an unexplored and significant constraint on bee learning and memory that plants may exploit.

Life in a toxic world: poison frog microbiomes are shaped by alkaloid toxins

Stephanie Caty

Shifts in host-associated microbial community composition can have large effects on host health. In poison frogs (family Dendrobatidae), the skin microbiome is exposed to the alkaloid toxins that the frogs sequester from their diet and use for defense. Given the demonstrated antimicrobial effects of these poison frog alkaloids, these toxins may be structuring the microbial community, however this has never been studied. To test this, we first characterized microbial communities from toxic and closely related non-toxic frogs from Ecuador. Then we conducted a controlled lab experiment with a single species of toxic frog, *Oophaga sylvatica*, where the frogs were fed the alkaloid decahydroquinoline (DHQ) and their microbiomes were monitored over time as the frogs accumulated the toxin. To complement this sequencing work, we cultured microbial strains from *O. sylvatica* skin and characterized the impact of DHQ on growth. We identified three DHQ response phenotypes: susceptibility (reduced growth), resistance (no impact), and enhancement (increased growth). The majority of strains were either enhanced (16%) or resistant (37%). This complements our findings from the field and lab sequencing, where we found that toxin-exposed microbiomes are more species rich and phylogenetically diverse, and that taxa are differentially impacted by the presence of alkaloids. Taken together, these data suggest that poison frog microbiomes have specific adaptations, including the likely metabolism of alkaloids, that enable their survival in this toxic environment.

Examining the organismal responses of *Nematostella vectensis* to tire dust, a ubiquitous pollutant

Hannah Cavanaugh-Gouvea, Lloyd Haughton, Liz Burmester, Justin McAlister

Tire dust is a ubiquitous pollutant that forms from tread wear while driving vehicles. It is largely unregulated and understudied and contains microplastic particles which can leach chemicals and physically impede function in some organisms. Exposure studies have been conducted in Coho salmon, some freshwater fish, crustaceans, and larvae of keystone sea urchin species. Mechanisms and degree of toxicity vary by taxa, indicating that the chemical cocktail used to create modern tires may be detrimental in myriad and complex ways. Currently, our understanding of the effects of tire dust exposure in brackish marine invertebrates is untested, yet these organisms are vulnerable to high exposure due to excess runoff during storm events and the filtration services inherent to estuarine ecosystems. We examined the effects of exposure to tire dust and its chemical leachate components in the starlet sea anemone, *Nematostella vectensis*, which is native to saltmarshes of the Eastern coast of North America. *N. vectensis* is an established model system for developmental and ecotoxicological studies. We measured organismal responses in behavior, respiration, regeneration, and reproduction. Our findings will inform our understanding of the physiological consequences of this pervasive urban pollutant on a cnidarian system. We will discuss next steps in this line of research and consider further testable hypotheses regarding the impacts of tire dust in coastal urban ecosystems.

Thermal physiology in a 'nested' mutualism: CTmax in cleaner shrimp, host anemones, and client fish

Eleanor Caves, Kara Chatterton, Rebecca Varney

Mutualisms—mutually beneficial interspecies interactions—are widespread and provide important ecosystem services, but mutualisms require precise overlap between species in space and time, and thus may be particularly sensitive to climate impacts. The cleaner shrimp *Ancylomenes pedersoni* provides cleaning services to reef fish “clients” by removing ectoparasites, positively impacting reef fish health. *A. pedersoni* is an obligate anemone-dweller, living primarily on individuals of the host *Bartholomea annulata*, which serve as “cleaning stations,” or set locations from which cleaners advertise and provide cleaning services. Thus, successful cleaning interactions are characterized not only by a mutualism between cleaner shrimp and client fish, but also between cleaner shrimp and host anemone. Here, we examined the thermal limits of the cleaner shrimp *A. pedersoni* and its anemone host

B. annulata at various acclimation temperatures and compared those limits to published values for reef fish clients. We show that CTmax is higher in *A. pedersoni* than in *B. annulata*, and that CTmax increases with higher acclimation temperatures. Nearly all client reef fish, however, are able to tolerate higher temperatures than both cleaner shrimp and their host anemones. Thus, our data indicate that, as temperatures warm, cleaning interactions are at high risk from the death of host anemones, followed by the death of cleaner shrimps, with the highest risks being associated with fast, extreme heatwaves rather than slow, gradual warming.

Sensorimotor strategies for wind direction estimation in flying insects

Benjamin Cellini, Stanley Stupski, Jaleesa Houle, Floris van-Breugel

Localizing the source of odors is critical for animals to discover food sources and potential mates. As chemosensory information is displaced by the wind, it is critical that organisms can construct an accurate estimate of the direction of ambient wind. For flying insects, this is not a trivial task because much of their sensory measurements are not necessarily calibrated or represented in the brain in consistent measurement units. Recent work has revealed that the angular direction of certain sensory quantities—orientation, air speed, and motion—are encoded in the central complex, and potentially integrated to estimate wind direction. We applied a novel nonlinear observability tool to analyze trajectories from freely flying flies and investigate what motor actions and sensor combinations would best facilitate the estimation of wind direction in the context of insect flight. Our findings reveal that changes in course direction are critical for estimating wind direction in steady conditions, but unnecessary in the case of dynamic wind. In the case where insects do not have access to a reliable air speed direction measurement, an acceleration measurement or internal control signal can be used in its place. We present an observability-informed estimator demonstrating the viability of these strategies. Altogether, our results elucidate active sensing strategies in insect flight and our methodology provides a framework for analyzing sensorimotor decisions across a range of animal behaviors.

Investigation of avian avoidance response to wind turbine blade patterning

Henry Cerbone, Emily Scott, Marco Heerenbrink, Graham Taylor

In recent years, we have seen a substantial uptick in avian deaths caused by wind turbines. This is largely due to the visual challenge posed by the motion of wind turbine blades for birds in flight. Recent work has proposed various mitigation schemas involving patterning of wind turbine blades to increase visual contrast with some field studies being carried out. However, a quantitative understanding or framework of the underlying mechanism, how visual patterning effects a various features of a bird's response to wind turbines. These features include attempt to avoid, avoidance response latency, and probability of avoidance response among others. We seek to begin answering this question utilizing principles from optical flow and a mock wind turbine blade in a motion capture set-up. By having the birds fly towards the mock blade, we are able to quantify their avoidance response latency and response probability via motion capture data. This allows us to relate avoidance response metrics (probability and latency) to wind turbine blade patterning across several genera of birds. Based on previous work aimed on reducing visual smear, we stratify our experimental results with respect to background contrast. Unlike in previous work, we are able to measure individual bird reaction time relative to changes in patterning during flight.

Possible Mechanism for Energy Recovery from the Tendons of the Peduncle of Harbor Porpoise

Alexa Cesari, Jesse Placone, Nicole Ramo, Michael Rosario, Danielle Adams, Frank Fish

Harbor porpoises (*Phocoena phocoena*) swim by vertical movements of the tail. The tendons located in the caudal peduncle are attached to caudal vertebrae to generate propulsive oscillations. For the efficient swimming stroke, it would be beneficial to recycle energy by elastic mechanisms. Previous

research indicated that the tendons were incapable of elastic energy storage. The construction of the tendons, however, suggestion a potential mechanism to recycle elastic energy. The tendons of the caudal peduncle were mechanically tested including extensor caudae medialis (ECM), extensor caudae lateralis (ECL), and medial hypaxialis lumborum (MHL). Cyclic tests were performed on the tendon fascicles at 2 and 4 Hz. Stress relaxation tests were also made. Polarized light microscopy was used to visualize the fibril crimp as tensile forces were applied to fascicles. Uncrimping of isolated fascicles was visualized at strain mean values of 0.25-0.55%. Maximum elastic moduli of fascicles taken to failure were 1039.5-1185.8 MPa. Elastic hysteresis measurements indicated that the caudal peduncle tendons displayed elastic energy storage properties up to 99% during tail beat oscillations for routine swimming at 2 Hz. The irrecoverable work for the fascicles in MHL was higher than in ECM and ECL. The mechanical properties of porpoise tendons indicated that elastic energy could be stored and recovered, indicating cycling of elastic energy to reduce power expenditure during swimming by porpoises.

Pressure to Pointed Perfection: Habitat Influence on Stingray Serration Evolution

Jules CHABAIN, Philip Anderson

Stingray (Myliobatiformes), a sister group of sharks, is a diverse group of cartilaginous fishes with distinctive characteristics, like a flattened-body, viviparity, and a barb located on their tails. This barb has serrations covering the main shaft of the tool and is used exclusively for defense against predators through a whip-like movement. These serrations vary in morphology from a triangular knife tooth to an intricate harpoon head. Across biology, such animal weaponry is often also used to influence female selection and intraspecies combat, but the stingray barb is unique in that it is thought to be purely defensive against predators. Given the unifunctionality, it is surprising that there is such a diversity of barb morphologies both across and within species. In order to better understand the foundation of this serration diversity, we must understand how habitat, and thus predation, influenced their evolution. We used PGLS to explore the relationship of this morphological diversity to differing environmental pressures. The results show a strong phylogenetic signal independent of environment for the serrated shaft length. We investigate the evolutionary speed on traits in between marine and freshwater environments. These results show that the barbs of freshwater stingray diversify faster than marine. Understanding the influence of microhabitat on barb morphology evolution will help us to bridge the gap between evolution and mechanics of animal weaponry in differing environments.

Fluid ejections in Nature

Elio Challita, Pankaj Rohilla, Saad Bhamla

Fluid ejections are a universal phenomenon in biology, serving crucial functions across organisms of all sizes, from microscopic fungi to enormous whales. These ejections facilitate key biological processes like excretion, venom delivery, hunting, spore dispersal, and plant guttation. This presentation reviews the complex interplay between fluid mechanics and biological function in these ejections, analyzing them across length scales from microns to meters, and time scales from milliseconds to seconds. We examine both active nozzles powered by muscles, and passive nozzles utilizing gravity or osmosis. A framework based on dimensionless numbers like the Weber and Bond numbers categorizes the mechanisms, elucidating the transition from dripping to jetting. This exploration reveals underlying principles of biological fluid ejections, with potential applications in soft robotics, manufacturing, and drug delivery. By connecting organismal biomechanics, physics of living systems, and fluid dynamics, this presentation provides insights and directions to further understand this universal phenomenon across the spectrum of life.

Firefly immunity: factors influencing survival during bacterial infection.

Moria Chambers, Owais Gilani, Edith Simpson, Ryan Walker, Sarah Townsend, Aidan Sullivan, Madden Tuffy, Zhengkai Zhu, Deeshani Patel, Sarah Lower

The extent to which host, pathogen, and environmental characteristics affect fitness post-infection is a central question in ecoimmunology, however since infection experiments require many individuals and extensive controls, studies are often limited to lab-adapted organisms. *Photinus pyralis* is a charismatic firefly that is abundant during its annual summer emergence in the Eastern United States. Over the past few years, we have assessed the relative importance of pathogen identity and dose, pre- and post-infection environment, host condition and age on survival after bacterial infection in wild-caught adult male *P. pyralis*. We discovered that *Serratia marcescens* and *Providencia rettgeri* infections increased mortality, but that other pathogens that are highly lethal in fruit flies did not cause mortality in fireflies even at high doses. Initial body condition and the date of firefly capture also significantly impact mortality during infection, suggesting that age may be an important factor as well. While our initial study did not show an effect of collection locations, collections the following year, during a hot dry summer, revealed that fireflies collected from locations with more human disturbance were smaller and had poorer body condition as the season progressed. These results have important implications for firefly conservation as climate change may increase the likelihood of extreme weather and it's important to understand how this might impact firefly development and susceptibility to infection.

Cryptic diversity of the coral-associated bivalve *Pedum spondyloideum* in the Indo-Pacific

Benny Chan, Yao-Feng Tsao

The coral-associated scallop *Pedum spondyloideum* lives in fan-shaped burrows within massive corals in the Indo-Pacific. Little is known about the basic biology and ecology of *Pedum* because it lives in deep burrows, and collection would need to destroy the entire head of the host coral, making it infeasible to collect samples for studies. Currently, *P. spondyloideum* is considered a single species that is distributed in the world's coral reefs. In the present study, we developed a method that can collect *Pedum* without the need to break the coral heads, and therefore, a large number of samples can be collected for population genetic studies. Based on the markers COI, 12S, and 16S, *Pedum spondyloideum* is composed of two species in the Indo-Pacific, and these two species differ in the color of the gill flaps (white and purple). Species with white gills have a wider distribution, ranging from the Indian Ocean to the Pacific Ocean, and inhabit shallower depths (5-10 m). The purple gill species is distributed in the Pacific, ranging from Okinawa, Taiwan, and Papua New Guinea and is distributed in deeper depths (20 m). Further studies are needed to compare the host usage of these two species of *Pedum* in the Indo-Pacific region

Fertilization kinetics in a changing ocean

Kit Yu Karen Chan, Wing-ho Ko

Marine organisms have complex life histories. For broadcast spawners, successful continuation of the population requires the gametes to make contact in the water column for sufficiently long periods for fertilization to occur. Anthropogenic climate change has been shown to impact fertilization success in various marine invertebrates, including urchins which are key grazers in their habitats. Gamete performance of both sexes declined when exposed to elevated temperature and/or pCO₂ levels. Examples of reduced performance included slower sperm swimming speed and thinning egg jelly coat. However, such responses to climate change stress were not uniform between individuals. Such variations could serve as the basis for selection. Fertilization kinetics has long been modeled as a particle collision process without consideration of such within-population variations. Here, we present a modified fertilization kinetics model that incorporates individual variations in performance in a more environmentally-relevant regime. Numerical simulations highlight that the relative influence of gamete performance change is dependent on egg size and sperm-to-egg ratio. Incorporating variability

derived from experimental data improved prediction accuracy. This work demonstrates that to better understand the fate of organisms in the face of climate change, we need to both consider the mean and variance of the response to capture adaptive potential.

Acute social isolation changes the transcriptome of structures of the social decision-making network

Madeleine Chang, Patricia Lopes

The social environment can critically influence animal physiology and behavior. Yet, a lot of research aimed at testing physiological responses to various stimuli is carried out in animals temporarily separated from their group-mates for testing. Here, we examined the effect of acute social isolation on the transcriptome of three brain structures part of the social decision-making network: the hypothalamus, the nucleus taeniae, and the bed nucleus of the stria terminalis. We used zebra finches as a model since these are gregarious birds in the wild. We compared the transcriptome of males housed with a known female to that of males from whom the known female was removed for 2.5 h. All brain regions studied showed differences in gene expression, with the bed nucleus of the stria terminalis having the largest number of differentially expressed genes between treatments. We present important genes and pathways most drastically affected by social isolation and discuss how experiments involving acute changes to the social environment can influence experimental results.

Inequities in academia as evident in vertebrate collections

Nari Chang, Sharndee Kaur, Ulrike Muller

Biological collections are crucial in academia as they are a historical record of biodiversity over time. While collections are fundamental in understanding biodiversity, they also reveal inequalities in academic practices and institutions. Several coloniality studies have focused on species nomenclature, yet collection metadata, such as the demographic characteristics of collectors, also reveal inequities. In this study, we analyze the vertebrate collections at Fresno State, UC Berkeley, and Cal Poly Humboldt, focusing on a subset of taxonomic groups (mammals, birds, rodents, carnivora). We analyzed the collectors' names to determine likely geographic ancestry and gender in order to gain a better understanding about trends and possible causes of inequity. We found that collectors with female first names are severely under-represented and that collectors with last names from Northern European countries are severely overrepresented. We also found that collectors with female first names became more common after the 1990s and contributed more specimens from taxonomic groups with predominantly small-sized species (rodents vs carnivores). Our findings contribute to a wider understanding of the inequalities within biological academia. In the future, we will add more data from more collections to examine the validity of our findings.

Patterns in habitat use of the newly-described Talladega seal salamander (*Desmognathus cheaha*).

Nicholas Chang, Will Hutchinson, Carolyn Keogh

Changing land use has dramatically altered aquatic ecosystems across the globe and poses a major threat to the unique freshwater biodiversity of the Southeastern United States. Understanding the habitat use of aquatic biota is necessary to predict how species respond to changes in environmental conditions, as well as to identify suitable habitats and restore degraded ones. The newly-described Talladega seal salamander (*Desmognathus cheaha*) of Georgia, Alabama, and Florida, USA, is threatened by land use change in certain parts of its range. We evaluate the habitat use of *D. cheaha* in a series of urban streams in metro-Atlanta to identify the role of substrate composition, water depth, and cover object size and material in predicting within-stream occupancy. In addition, we compare temperature, water quality, and cover availability with presence and abundance data to examine salamander occupancy along an urbanizing gradient. Our analyses suggest cover object size to be a strong predictor of fine-scale occupancy, and that *D. cheaha* abundance is negatively correlated with large daily fluctuations in water temperature, but not necessarily with higher average temperatures. Data on substrate and

cover availability will be included in a full analysis. Our data reduces knowledge gaps about habitat suitability for this species, and improves our ability to appropriately address conservation needs.

Characterization of Slow Rhythmic Muscle Activity in the Arms of *Octopus bimaculoides*

Weipang Chang, Melina Hale

Neuronal network oscillations and rhythmic muscle activity are fundamental for coordinating many physiological processes in organisms. While octopuses do not appear to have rhythmic central pattern generators that function in walking, their individual arms have the potential for rhythmic movements. We recorded spontaneous electromyographic (EMG) activity in isolated octopus arms and found ultra-slow rhythmic bursting (0.05 Hz). Bursts, activity exceeding 2% of the maximum EMG amplitude and >0.5 seconds duration, with spikes-to-spikes intervals < 20 ms, were subjected to coefficient of variation (CV) analysis. We identified instances of regular (coefficient of variation of 14.2%) burst intervals in a subset of recordings (23 out of 59 arms). We also investigated how neurotransmitters regulate bursting activity. Low concentrations of acetylcholine enhance the amplitude of bursting while reducing their frequency. In contrast, high concentrations of acetylcholine completely block bursting, indicating a dose-dependent effect on octopus motor function. Glutamate's impact varies with concentration—modest levels enhance bursting amplitude while higher levels first enhance amplitude and then inhibit bursting. GABA consistently enhances the amplitude of bursting, suggesting its role in modulating rhythmic contractions. Serotonin and octopamine application minimally affect muscle activities, emphasizing acetylcholine, glutamate, and GABA's specific roles in octopus arm motor control. By revealing neurotransmitter effects on rhythmic muscle activities, this study enhances our understanding of local motor control mechanisms in octopus arms, and these animals' remarkable movements.

Initial immune state of rewilded lab mice as a driver of host susceptibility to intestinal whipworms

David Chang-van-Oordt, Daniel Metz, Oyebola Oyesola, Seokyoon Chang, P'ng Loke, Clayton Cressler, Andrea Graham

Linking natural immune variation to parasite susceptibility remains a challenge in ecoimmunology and disease ecology. Identifying the mechanisms that bridge this gap can inform our understanding of host susceptibility in natural systems and better connect immune variation with disease dynamics. Studies in laboratory mice (*Mus musculus*) kept in outdoor enclosures ('rewilding') show that outdoor exposure can increase susceptibility to the intestinal whipworm *Trichuris muris*. The immune phenotype of rewilded mice prior to infection may change the ability of the host to resist whipworm infections, but initial immune state is rarely studied. Here, we study how the initial immune phenotype may impact the ability of laboratory mice to resist a *Trichuris* infection. We characterized the immune phenotype, via complete blood cell counts and circulating IgG1 and IgG2a concentrations, before and after infection in rewilded and laboratory-control mice from resistant and susceptible strains. We then compared the resulting worm burden by treatment (rewilding vs. control) and strain (resistance vs. susceptible). In resistant mice, rewilding may increase susceptibility by biasing the immune response against new microbes present in the outdoor enclosure. Therefore, worm burdens should increase in rewilded resistant mice compared to controls. Meanwhile, susceptible mice, which already mount an incorrect immune response, may show similar worm burdens as controls. With this study, we explore how changing the immune state can impact host susceptibility and disease dynamics.

To Feed a Sea Monster, Functional Morphology of Lizard Jaws and its Application on Extinct Mosasaurs

Arion Chao, David Grossnickle, Sharlene Santana, David DeMar

Mosasaurs were a group of aquatic lizards that lived during the Late Cretaceous period, in which they greatly diversified and achieved a global distribution. Investigating their ecological traits, including diets, could help elucidate their roles in ancient marine ecosystems. Currently, our understanding of mosasaur

diets is based partly on preserved stomach contents and comparison of gross tooth morphologies. However, these methods only provide a limited view of the diets and associated morphological specializations across different species of mosasaurs. Diets can also be inferred from jaw morphology because animals feeding on foods with different mechanical properties have jaw shapes that are adapted to apply different bite forces. In this study, we inferred the diets of several species of mosasaurs by quantifying the relationship between diet and jaw morphology in extant lizards. We measured the jaws of 139 extant lizards representing all major clades except Serpentes and covering four major dietary groups: carnivores, insectivores, durophages and herbivores. We then used jaw measurements and beam theory to calculate maximum bite forces, which have been shown to be correlated with diet. Using phylogenetic comparative methods, we compared the inferred bite forces among extant diet groups and then applied our findings to mosasaur jaws to infer their diets. We find that different species of mosasaurs differed in bite performance, reflecting specialization to various diets.

Distributed visual-motor coordination in the bay scallop *Argopecten irradians*

Daniel Chappell, Martin (Ric) Wehling, Daniel Speiser

Complex visually-mediated behaviors are commonly thought to be the result of cephalized visual organs feeding information into central neural circuits to form sensory representations. These, in turn, inform motor centers to enact body movements. This co-evolutionary relationship between visual and motor circuits is found in numerous taxa, but animals with distributed visual systems often deviate from this trend by having numerous visual organs dispersed across their bodies. Additionally, distributed visual systems are associated with a diversity of neural architectures, and it is largely unknown if these nervous systems employ similar visual-motor circuits as those canonically found in cephalized animals or if they use novel strategies to efficiently form visual neural representations and enact behaviors. Bay scallops (*Argopecten irradians*) have distributed visual systems consisting of dozens of eyes radially dispersed across their mantle tissues. It was previously thought that scallops peripherally downsample visual information gathered by their high-resolution eyes to ease the processing burden on their simple nervous systems. We found that bay scallops demonstrate interactive visual behaviors requiring high-resolution neural maps of their surrounding environment which are likely embodied in the somatotopically organized lateral lobes of their visceral ganglia. Scallops serve as a unique model system in which to study the neural basis of distributed vision and to learn how animals effectively sense, process, and behave using a balance of centralized and distributed network topologies.

Craniomandibular integration patterns and evolutionary rates in cat-like carnivorans

Narimane Chatar, Margot Michaud, Davide Tamagnini, Valentin Fischer

The cat-like morphology is a textbook example of convergence, appearing in different vertebrate groups including two carnivorans families: felids and nimravids. Despite being grossly convergent, sabertoothed clades are now known to exhibit variable craniomandibular shapes. The gross shape and proportions of the craniomandibular complex of these groups have already been studied in detail; it is now time to untangle their disparity through the lenses of phenotypic integration and evolutionary rates. Indeed, the cranium and mandible have various purposes: from feeding to sensory functions. Those bones can be viewed as an arrangement of modules responding to different constraints that, if evolving independently, can lead to greater phenotypic diversity. We looked at the covariation between those two bones but also their different modules and computed specific rates of evolution using a database of 3D surface scans composed of 91 mandibles and 89 crania, spanning Oligocene to extant taxa. We quantified the shape using 3DGM with a total of 38 landmarks and semi landmarks on mandibles and 72 on crania. Our results show that the strength of integration is lowest in groups exhibiting longer upper canines; those same groups also show a burst of morphological evolution at the beginning of their evolutionary history. We therefore hypothesize that a low degree of integration coupled with rapid rates of evolution were two key components to develop a sabertoothed morphology.

Color and vision in hummingbirds - Insights from genes and behavior

Soumyadeep Chatterjee, Alan Brelsford, Chris Clark

Male hummingbirds have iridescent throat feathers, the gorget, which serves as a visual signal during courtship displays. Spectral tuning adapts vision to a particular color environment and occurs due to mutations in the opsin genes. Have these birds undergone spectral tuning to match their spectral sensitivities to the gorget color of the males which vary from short to long wavelength colors? The opsin gene, shortwave-sensitive 1 or SWS1 is being studied in 29 bee hummingbird species. Mutations that switch amino acids in the pigment protein could shift spectral sensitivity towards violet or red wavelengths. Amino acids in positions 86 and 90 could influence spectral sensitivity and have been shown to be variable in other species. However, this gene was not found in a BLAST search of existing hummingbird genomes indicating that they may exist in the unassembled portion. Hence, this specific region of the gene will be sequenced after targeted amplification by PCR to see if they vary among different species. The obtained DNA sequences will be translated into the corresponding amino acid sequences. Another objective will be to identify other amino acid sites that show large variation among species and may contribute to spectral tuning. Further ahead, operant conditioning experiments will be performed to determine whether hummingbirds differ in their ability to differentiate between shades of conspecific colors vs. allospecific colors.

Spatiotemporal structure of foraging and path integration errors by fiddler crabs, *Uca pugnator*

Ruma Chatterji, John Layne

Path integration is the navigational process by which animals construct a memory of a previously visited location by continuously measuring and summing their movements to form a single home vector pointing to the starting location. It is intrinsically error prone, subject to random errors and, potentially, to systematic errors in either measurement or the summing algorithm. Both types of errors lead to the formation of an incorrect vector memory and thus to an error in homing, and are theoretically distinguishable in the same manner as accuracy is distinguished from precision. Because the errors are incurred when animals move, they are also theoretically predictable from those movements. We analyzed the behavior of fiddler crabs (*Uca pugnator*) as they used path integration to perform foraging excursions. These excursions ended in varying degrees of homing error. We measured the location and orientation of both the body and eyes, frame-by-frame, from video recordings of natural foraging excursions. We computed different characteristics of the structure of the paths such as the time, length, tortuosity, body and eye rotations, orientation relative to the burrow direction, step sizes and directions, and bearing. These were analyzed in detail in an attempt to identify a path-related cause of the direction and size of the homing errors, and whether these errors were systematic or random.

Density-dependent impacts on physiology of North American elk (*Cervus elaphus*)

Eaqaan Chaudhry, Kelley Stewart, Leo Fletcher, Carolyn Miller, Brian Dick, Michael Wisdom, Cynthia Downs

Large mammals exhibit strong density-dependence of population dynamics ecologically mediated by interspecific resource competition. It follows that changes in population density likely alter the relationships among physiological traits because physiological functions are interconnected and underpinned by resource-based allocation. We experimentally manipulated population density of North American elk at Starkey Experimental Forest and Range in northeast Oregon during the spring through autumn and examined whether density-dependent changes in hair cortisol concentration and fat reserves mediate immune defense at the beginning of the winter. Elk residing in a high-density population exhibited greater cortisol concentrations in hair than those in a low-density population, indicating greater stress or metabolically demanding activities associated with more intense intraspecific resource competition. Unsurprisingly, elk in the high-density population had lower fat reserves entering the winter than those in the low-density

population. Constitutive, complement-based immune defense did not differ with population density. As such, changes in cortisol and fat reserves did not correspond with shifts in immune defenses among populations. Our findings regarding immune defense suggest that the relationship among hair cortisol, fat reserves, immune defenses, and population density is complex. Our overall results for this population corroborate existing work indicating that ungulate stress physiology is impacted by population density. Being cognizant of density-dependent effects on physiological stress across different taxa will be beneficial in monitoring wildlife health and improving population resilience.

The tail of myliobatid stingrays: understanding structure and function

Julia Chaumel-Cerda, George Lauder

Unlike other batoid rays, myliobatid stingrays –manta, eagle and cownose rays– are capable of actively swimming in the water column, migrating long distances, maneuvering in complex landscapes and forming large schools when swimming. This lifestyle is reflected in their distinctive body morphology and locomotor style. For example, one of the notable characteristics of myliobatids is a slender whip-tail that can be >3x the animal's body length. However, the biological significance of this long caudal appendage for locomotion and sensing has never been explored. In this project, we undertake the first biological characterization of the myliobatid whip-tail in a multidisciplinary analysis of its morphology, kinematics and hydrodynamics. The presence of muscles, nerves, and vertebral column along the tail indicates that myliobatids can actively move and sense the tail. The most striking finding is the presence of lateral line canals on both sides of the tail which, in contrast to other fishes, are highly ramified into numerous subcanals (which reach both the ventral and dorsal side). Neuromasts are found in the lateral canals as well as in pit organs on the tail surface. Tail morphology and length also influence hydrodynamics, likely increasing body stability while swimming. These results indicate that the tail is a complex organ, acting as a mechanosensory and tactile appendage, and plays an important role in the swimming ability of myliobatid rays

Shaggy regulates elongation, polarity, and segmentation in Tardigrada

Raul Chavarria, Frank Smith

Tardigrades have a highly simplified anteroposterior (AP) axis. Shaggy, an inhibitor of canonical Wnt signaling, is an important regulator of AP axis development in other animals. We used RNA interference (RNAi) in *Hypsibius exemplaris* to test whether shaggy regulates development of the highly derived tardigrade AP axis. In shaggy RNAi specimens, embryogenesis stalled at the elongation stage, a stage during which the AP axis normally forms. Some embryos that proceeded past the elongation stage showed duplicated anteroposterior body axes. Six3, a marker of anterior fate, was either lost or highly reduced in some shaggy RNAi specimens. Moreover, caudal, a marker of posterior identity, was expressed ectopically in some shaggy RNAi specimens. These results may reflect a role of shaggy in regulating AP axis development by promoting anterior fate, a function that is conserved in most other bilaterians that have been investigated. Additionally, some specimens exhibited excess hindgut tissue that failed to ingress, which could reflect posteriorization of the body axis. We also recovered specimens that exhibited segmentation defects that ranged from minor fusions between segments to the loss of several segments. Shaggy regulates segmentation in insects. Therefore, the segmentation defects we recovered in *H. exemplaris* could represent a conserved segmentation function of shaggy. In summary, shaggy regulates development of important features of the highly derived tardigrade body plan.

Elucidating Effects of Predation on Biofilms by Transcriptomics in the Squid-Vibrio symbiosis

Daravuth Cheam, Isabella Ma, Miranda Magdaleno, David Real, Michele Nishiguchi

Environmentally transmitted symbioses have a multitude of ecological factors that influence their overall fitness. One model system to study ecological

selection is the beneficial association between sepiolid squids and their *Vibrio* symbionts. A key symbiotic feature important for a successful symbiosis are the production of *Vibrio* biofilms found in and outside squid hosts. Prior to infection, *Vibrio* biofilms are susceptible to biotic factors such as grazing by protozoan predators. Here, we experimentally evolved *Vibrio* biofilms with various protozoan predators to examine the effects of predation on symbiotic *Vibrio* biofilms outside the host. We compared transcriptomes from a number of evolved strains at specific conditions to assess differences in gene expression between biofilms treated and untreated with predators. We observed a number of genes involved in biofilm formation that were differentially expressed between grazed and ungrazed biofilms. Differences in gene expression were also found between evolved and ancestral biofilms, as well as across strains from different host species. Specifically, genes involved in biofilm regulation, light production, and motility changed across generations as well as between strain type. These results demonstrate that experimentally evolving biofilms under predation pressure selects for genes used in defensive mechanisms that are specific for the maintenance of the association. Thus, ecological factors can contribute to the evolution of microbial symbionts, which in turn may affect their subsequent interactions with their hosts.

Comparative anatomy of feeding musculature between muscle-powered and spring-powered salamanders

Jane Chen, Bhart-Anjan Bhullar, Martha Munoz, Henry Camarillo

The majority of plethodontid (lungless) salamanders reside in the Appalachian mountains of the United States, a region consisting of a diverse array of microclimates and microhabitats. Although the majority of lungless salamanders rely on direct development, some have re-evolved a biphasic lifestyle, with associated changes in microhabitat use. Here, we compare two clades of salamanders that vary in their feeding performance (*Desmognathus* and *Eurycea*). Previous literature has established that salamanders in these two clades rely on different forms of tongue capture: whereas *Eurycea* relies on a ballistic, spring-powered feeding mechanism involving a protrusible tongue, *Desmognathus* salamanders rely on a muscle-powered feeding mechanism involving an attached tongue. We use diceCT to examine how differences in tongue prehension (muscle-powered vs spring-powered) as well as differences in microhabitat use relate to musculature associated with feeding. Specifically, we look at two *Desmognathus* species, *Desmognathus monticola* and *Desmognathus orestes*, and two *Eurycea* species, *Eurycea guttolineata* and *Eurycea cirrigera*, to compare the musculoskeletal feeding system. We use VGStudio to segment specific muscular regions and compare how differences in muscle volume have evolved between species. This project investigates and interrogates how these differences in morphological features and adaptations may be linked to differences in life cycle and microhabitat use.

Swept Away: Risk of dislodgement during barnacle cyprid surface exploration

Michelle Chen, Julia Stern, William Ballentine, Mimi Koehl, Kit Yu Karen Chan

Many sessile marine organisms disperse via planktonic larvae, which must settle and adhere to surfaces to recruit onto new habitats. The fusiform cyprid larvae of barnacles explore surfaces by bumping, walking, and turning while leaving adhesive footprints. During metamorphosis, cyprids raise their bodies off the substratum into an upright position. Using dynamically-scaled models of the cyprid of *Tetraclita japonica*, we measured the forces and torques experienced by cyprids on surfaces exposed to ambient flows. The Reynolds numbers of the cyprids and models ranged from 123 to 1230, calculated using water speeds measured at the height of cyprids in fouling communities and on intertidal rocks (0.13 to 1.3 ms⁻¹). Cyprids in different orientations (anterior, posterior, or broad-side towards flow) and tilt angles (0°, 48°, and 90°) were tested. Preliminary analysis showed that the drag pushing cyprids downstream was greatest when they were oriented broadside to the flow, and when they were oriented 90° to the substratum during metamorphosis. Depending on orientation and tilt angle, cyprids can be dislodged from surfaces by being spun around or pushed downstream by ambient flow, or by being peeled off the surface. Thus, resisting both torques and forces are important in adhering to

surfaces. Our work showed that the risk of dislodgement for small planktonic organisms not only depends on ambient flow, but also on their orientations and behaviors.

Shaping avian flight: Effects of morphology and flexural stiffness on feather deformation in wind

Wei-Lin Chen, Ching-Wei Wang, Wen-Tau Juan, Kai-Jung Chi

Feathers play multiple roles in flight: flight feathers generate force, while tail feathers act as brakes. Previous studies on bird flight have primarily focused on wings, but rarely discussed how their constituents, the feathers, contribute mechanically. To address this question, we used male Mallard ducks as a model to examine feather morphology and material properties at different wing positions, as well as their spatial variation within a feather. Morphological results revealed similar trends intra-specifically: The total area of primary flight feathers (PFF) increased distally and reached its maximum at PFF#9; the cross-sectional area of the feather rachis had a maximum at its proximal end and decreased distally. Such spatial variation in feather size and shape may have mechanical consequences for flight. Mechanical tests suggested that the flexural stiffness (EI) of flight feathers showed dorsal-ventral asymmetry but exhibited similarity in the tail feathers, which is consistent with the observed feather deformation during flight. We further compared different feathers of similar shape and found that their deformation in the wind was proportional to their area and inversely related to EI, supporting our hypothesis that both the feather morphology and material properties could influence their deformation, and consequently their function during flight. We will quantify rachis morphology using micro-CT to determine which characteristics are responsible to feather bending and twisting, and ultimately their mechanical contribution to bird flight.

Effect of food availability and conspecific competition on *Ae. aegypti* with wAlbB infection outcome

Chun (Jessica) Cheng, Gonzalo Vazquez-Prokopec

The *Aedes aegypti* mosquito is majorly responsible for the transmission of dengue (DENV), Zika (ZIKV), and chikungunya (CHIKV) viruses, particularly in Central and South America. The introduction of the intracellular bacteria *Wolbachia* into mosquitoes has been found to halve the adult lifespan successfully as well as reduce mosquito fitness by a process termed cytoplasmic incompatibility (CI), where only gametes from males and females carrying *Wolbachia* lead to viable eggs. In wild *Aedes* sp. mosquitoes, increased food levels are found to be positively related to the body size of emerging adults. A two-way ANOVA test reveals that the interaction between diet and conspecific density is significantly associated with time to pupation as well as time to emergence into adult mosquitoes (p-value = 3.48e-06). Evidence has been obtained that mosquitoes carrying the wAlbB strain of *Wolbachia* reared under increased food limitation may lose the infection in comparison with mosquitoes that are reared with plenty of food. Inspired by the study explained above, I extended the work on the impact of larval conditions on *Wolbachia* persistence in mosquitoes by conducting a study quantifying how they impact the wAlbB strain of *Wolbachia* in *Aedes aegypti* mosquitoes. Understanding the impacts of food availability and density-dependent competition on *Aedes* sp. Larval performance is a key step for future guidance on mosquito biological controls. Under the theme of global warming, the factor of climate change displayed as a variance in temperature in the lab setting will be taken into account in further study.

Exploring resilience and recovery of bats from White-nose Syndrome

Tina Cheng, Winifred Frick

Novel pathogens are often a powerful disrupting force in natural systems, invading naïve host populations and placing severe pressures that can lead to, in the worst of cases, species extinctions, community collapse, and global biodiversity loss. White-nose Syndrome (WNS), caused by the fungal pathogen *Pseudogymnoascus destructans* (Pd), is an emerging infectious disease in hibernating bats in North America, which has spread unabated

throughout North America over the past two decades and currently threatens several bat species with extinction. Despite this, some individuals have survived the epidemic and some populations continue to persist with the disease, begging the question, why do some perish while others persist? In this presentation, I will review potential mechanisms leading to persistence in WNS-affected bat populations, including mechanisms of resistance and tolerance, and behavioral adaptations. I will also discuss the challenges faced by recovering populations. Finally, I will present conservation strategies and current efforts focused on facilitating resilience and supporting the recovery of WNS-affected populations. Given the severe impact of WNS on certain bat populations, whether some species will continue to persist remains an open question. While managing diseases is still an area of needed development, understanding mechanisms of resilience and recovery in hosts is also critical and can help the research and conservation community to better anticipate the best way to support long-term persistence of species affected by emerging diseases.

Reducing locomotor costs in neuroatypical children through high-intensity training

Noah Chernik, Reuben Jacobson, Melody Young, Stratos Kantounis, Samantha Lynch, Edwin Dickinson, Michael Granatosky

Children with developmental disabilities such as autism spectrum disorder often face behavioral and physical challenges throughout their lives, which limit their ability to partake in physical activity. This can negatively affect both short- and long-term health outcomes within this population, especially relating to cardiovascular fitness. Here, we measure the true metabolic costs in children with autism spectrum disorder using respirometry and assess whether these metabolic costs change as a consequence of participation in a high intensity running and agility protocol. To analyze this, we conducted a longitudinal study measuring oxygen consumption and carbon dioxide production in neuroatypical children, ranging in age from 11-13. Trials were performed at a fixed speed, which was adjusted for participant's leg length. Measurements were taken prior to, during, and after a six-week exercise program. We observed that the energetic cost of locomotion decreased for each participant over the six-week running program, proving the efficacy of the program, and laying the groundwork for future training protocols in this population group. From a comparative perspective, neuroatypical participants reduced their cost of transport from 33% greater compared to neurotypical children of the same age to just 17.3% greater than neurotypical children. Our data show that participation within the program not only increases the fitness levels of these children but help narrow the energetic differences between neurotypical and neuroatypical children.

High intensity running improves locomotor performance in neurotypical and neuroatypical children.

Noah Chernik, Samantha Lynch, Melody Young, Edwin Dickinson, Stratos Kantounis, Matthew Cannata, Reuben Jacobson, Jon Gustafson, James Virga, Michael Granatosky

Children with neurodevelopmental deficits (e.g., Autism Spectrum Disorder, Cerebral Palsy) often present with physical and motor comorbidities. These physical deficits manifest in abnormal running mechanics and gait patterns characterized by long stride durations, short stride lengths, and minimal trunk and pelvic rotation. Here, we developed a twelve-week high-intensity training program involving eight neuroatypical children aged 9-15 and a control sample of eight neurotypical children of similar ages. Children met twice per week, with each session comprising of running trials at varying velocities, inclines, and durations. We used a four-camera system to record kinematic and spatiotemporal data including velocity, stride length, stride duration, stride frequency, duty factor, and joint angles. In both the neuroatypical and control group, there was a statistically significant increase in running velocity, increase in stride frequency, decrease in stride length, and decrease in duty factor between the start and end of the training program: demonstrating the efficacy of a high intensity running program for improving gait performance and mobility in both neurotypical and neuroatypical adolescents. More importantly,

we demonstrate a convergence in running kinematics between the two study groups, signaling that some of the physical and motor impediments typified by neuroatypical children can be overcome by appropriate training protocols.

The effect of multifunctionality on morphological diversity in the bat rostrum

Vaibhav Chhaya, Tim Smith, Abigail Curtis, Thomas Eiting, Sharlene Santana

The morphology of multifunctional structures is thought to be the result of selective pressures acting on performance across multiple functions. Under mechanical, phylogenetic and developmental constraints, these functional demands may trade-off with each other and consequently influence morphological diversification. Involved in feeding, olfaction, thermoregulation and echolocation, the bat rostrum is a diverse structure that is suitable for studying these functional tradeoffs. Bats exhibit striking variation in external rostral shape, largely associated with dietary requirements. However, the internal morphology of the rostrum remains particularly understudied, notably the turbinal bones that are functionally important components of the nasal cavity responsible for respiratory air conditioning and olfaction. We use micro-computed tomography and geometric morphometrics to study diversification patterns in the external and internal morphology of bat rostra. We compare the external rostral shape to turbinal surface area and complexity across bat families, and test whether external shape constrains the relative turbinal size. Additionally, we examine the link between turbinal morphology and ecological factors such as diet and echolocation mode. Our preliminary findings indicate that species that rely on olfaction to find food (e.g., nectarivores and frugivores) possess an extensive and complex network of turbinates. Conversely, nasally echolocating clades have reduced turbinates, presumably enhancing echolocation performance. Together, these results underscore potential tradeoffs and facilitation among rostral functions that may underlie the morphological diversification of this structure.

How to Find Prey: Exploring the Mechanosensory Lateral Line and Visual Systems of the Bumblebee Goby

Shrija Chhetri, Margot Schwalbe

The mechanosensory lateral line system of fishes is thought to form hydrodynamic images of flow disturbances immediately around the body and can mediate several behaviors, including prey detection. Reduced canals, one of four types of lateral line canal systems among teleosts, are found in gobies (~1360 species) and are characterized by a reduction of cranial canals, the absence of a trunk canal, and extensive proliferation of superficial neuromasts on the body. Taxa with proliferation of superficial neuromasts tend to occupy hydrodynamically quiet and/or light-limited environments, and more superficial neuromasts on the skin may increase the sensitivity to local flows and to prey. Few studies have linked this lateral line canal pattern to prey detection, and thus we asked the question – how does a goby use its reduced lateral line canal system to find prey? We tested the ability of bumblebee gobies (*Brachyogobius doriae*) to detect live, mobile prey under light and dark conditions with an intact or chemically ablated-lateral line system. We evaluated several parameters of search and prey detection behaviors from recorded feeding trials. We have shown that bumblebee gobies can localize and capture prey under both light and dark conditions, and they perform different search strategies depending on available sensory systems. This study provides important insights into the functional and ecological significance of lateral-line mediated behavior in species with reduced lateral line canals.

De novo genome assembly and annotation of a saline-tolerant coastal rock pool mosquito, *Aedes togoi*

Jonathan Chiang, Ben Matthews

The coastal rock pool mosquito (*Aedes togoi*) is found along coastal areas of east Asia and the Pacific Northwest. It has a rare capability to tolerate high salinity water, whereas most mosquito species require freshwater to breed. This extreme physiological adaptation makes *Ae. togoi* an ideal organism to study the sensory and ion regulatory systems of mosquitoes. Highlighting

significant adaptations within these systems in mosquitoes is crucial to understanding their feeding and oviposition behaviour, geographic range, and host preference—all key factors that inform potential vector control strategies. However, we are currently lacking the genomic resources needed to properly study *Ae. togoi*'s uniquely adapted sensory systems and ion regulatory mechanisms that allow for high salinity tolerance. Here, we present a high-quality *de novo* genome assembly from a single *Ae. togoi* mosquito, utilizing PacBio HiFi long-read sequencing and Hi-C data. The genome assembly has a total length of 800.58 Mb, a contig/scaffold N50 of 653.6 Kb, and a (BUSCO) completeness score of 96.7%. The pickpocket (PPK) gene family, which is important in salt-sensing pathways in mosquitoes, was identified as the first of many to be annotated in several broad families of sensory receptors. This work lays groundwork for *Ae. togoi* to be further studied and opens new avenues for comparative studies to investigate physiological and behavioural adaptations within the diverse Culicidae (mosquito) family.

Between a rock and a hot place- genotype and environment alters physiology of a montane insect

Harvey Chilcott, Elizabeth Dahlhoff, Nathan Rank

Life at high elevation poses unique challenges for animals living there, including reduced availability of environmental oxygen and variable air temperatures during the growing season. While many studies have shown temperature as a strong selective force in local adaptation, most have underestimated the role of hypoxia for small bodied insects; also, the role of mitonuclear interactions in metabolic adaptation of animals living at high elevation is not well understood. Here we examine the role of hypoxia and thermal stress on Sierra Nevada populations of the beetle *Chrysomela aeneicollis*. Prior studies showed that populations found along a latitudinal temperature gradient are genetically distinct. Adult beetles were collected from northern and southern populations and placed into mating pairs with partners from the same (NN, SS) or crossed (NS, SN) populations. Offspring clutches were split and reared at high and low elevation until third instar, after which running speed and metabolic rate were measured at high elevation, before and after heat treatment. Heat-treated larvae ran slower and had lower metabolic rates than control larvae. Larvae reared at high elevation ran faster with higher metabolic rates than siblings reared at low elevation. Offspring of southern females ran faster than those of northern females, and metabolic rates were slowest in larvae from 'mismatched' parents. These results suggest that environmental and genetic factors interact to affect physiology and performance.

Effects of Northern Snakehead (*Channa argus*) mucus and scales on terrestrial friction

Francis Chiles-Lopez, Noah Bressman

The mucus coat serves many important functions for aquatic fishes, but its functionality for terrestrial behaviors in fishes have not yet been investigated. The Northern Snakehead (*Channa argus*) is an invasive fish to the Chesapeake Bay watershed and amongst the largest amphibious fishes, so it is likely impacted more by terrestrial friction than most amphibious fishes. The goals of this project were to investigate how snakehead mucus affects friction during terrestrial locomotion, assess if their mucus evolved to facilitate terrestrial behaviors compared to that of fully-aquatic fish, and determine how snakehead scales influence friction anisotropy compared to scale-less fish. Using freshly euthanized fish on two substrates, we pulled fish forward and backward using a force meter until the fish began to move to determine the force needed to overcome static friction. We tested each fish with its mucus coat intact and then again with it wiped off, allowing for paired comparisons. Snakehead mucus significantly reduced friction, more so than fully-aquatic fish. Additionally, scales exhibited frictional anisotropy, with less friction in the forward direction and more backward, mirroring snake scale anisotropy. Amphibious fishes like snakeheads may have evolved particularly slippery mucus to aid in terrestrial locomotion by reducing friction and energy required to move overland. This study also determined an artificial lubricant with similar properties to snakehead slime, which could aid in amphibious fish-inspired robotics. The mucus coat serves many important functions for aquatic fishes, but its functionality for terrestrial behaviors in fishes have not yet been investigated. The Northern Snakehead (*Channa argus*) is an invasive fish to the

Chesapeake Bay watershed and amongst the largest amphibious fishes, so it is likely impacted more by terrestrial friction than most amphibious fishes. The goals

Dining with Sharks: Exploring Feeding Regimens via Stable Isotope Analysis

Amber Chiadini, Adilene Landa-Gaulrapp, Maria G Quiceno, Melanie Cardenas, Johnneil Ferguson, Harlyn Hosten, Ciera Jarrett, Jordan Penn, Tatiana Restrepo-Padilla, Karin Sandager, Carlee Bohannon, Gabriele Larocca-Conte, Jasmin Graham, Catherine Macdonald, Sora Kim

This study illuminated the complex interactions shaping community structure and the shared resource utilization dynamics among meso and apex predators by delving into the ecology of three shark species native to Biscayne Bay, Florida: blacktip *Carcharhinus limbatus*, bonnethead *Sphyrna tiburo*, and blacknose *Carcharhinus acronotus*. We used stable isotope analysis (SIA), a technique to track energy flow within ecosystems, to explore dietary preferences and compare trophic levels. Biopsy samples were taken from each shark at the base of the dorsal fin by Field School (34 specimens: 10 blacktip, 14 bonnethead, and 10 blacknose). These samples were extracted of lipids and urea before SIA of carbon and nitrogen. Our results revealed that bonnetheads had lower $\delta^{15}\text{N}$ values and the largest $\delta^{13}\text{C}$ range, indicating a lower trophic level and contribution from food webs based on seagrass, respectively. Meanwhile, the blacktip and blacknose sharks overlapped in $\delta^{15}\text{N}$ values, which suggests similar trophic levels for these two shark species. There were also correlations between total length and $\delta^{15}\text{N}$ values for bonnetheads and blacktip sharks, suggesting ontogenetic changes in diet as they grow. These results indicate overlap and possibly competition for resources amongst blacktip and blacknose sharks, while isotopic composition suggests bonnetheads are predominantly in their own niche. The disparity and overlap amongst the species studied provides further understanding of the ecology and interspecies competition in Biscayne Bay.

Drifting in from Hell: Uncovering the Genome of the Vampire Squid

Yekaterina Chmykh, Jason Podrabsky, Annie Lindgren

The Oxygen Minimum Zone (OMZ) in the northeastern Pacific is a deep pelagic region with extremely low oxygen and high hydrostatic pressure – two environmental parameters that are lethal to many animals. However, there is a high density of ecologically and economically important organisms present in the OMZ, including cephalopods and fishes. Organismal adaptations required to survive in the OMZ are not well understood - discovering their mechanisms for survival will help us better understand how life evolves under extreme stress. *Vampyroteuthis infernalis*, also known as the vampire squid, is a Pacific OMZ resident and has deep-sea adaptations thought to facilitate survival in the OMZ, including having the lowest recorded mass-specific metabolic among cephalopods. In addition, *V. infernalis* occupies a unique position as a monotypic order in the cephalopod phylogenetic tree. To begin to understand the underlying genomic mechanisms that *V. infernalis* may be using to survive in the OMZ, we present here initial genome assemblies using long read Oxford Nanopore sequencing. A draft genome for *V. infernalis* will not only fill missing gaps in understanding cephalopod genomics and evolution, but it will lay the foundation to test hypotheses related to RNA editing, proteomics, and provide a better overall understanding of how the vampire squid has evolved particularly in oxygen minimum zones.

Vortex mechanism in microvelia-Inspired water-walking for thrust optimization using robotic design

Daehyun Choi, Johnathan O'Neil, Pankaj Rohilla, Saad Bhamla

Microvelia (Veliidae) is a genus of aquatic insects capable of swift movement (60~100 BL/s, where BL denotes the body length) on both land and water surfaces. This is achieved through their alternating tripod gait and hydrophobicity of their leg surface (i.e., tarsi). Their locomotion on water creates vortices as each leg makes contact with the surface and these vortices interact with the leg movements and play a crucial role in determining the thrust produced

by microvelia. To delve into this mechanism, a microrobot has been developed (BL < 10mm), which is light enough (< 1 g) to float on water, made of smart composite material fabricated by the micro-cutter. The alternating tripod gait is actuated by the shape memory alloy wire and its amplitude-modifying linkage enables controlling various parameters, including the amplitude and frequency of leg movements, as well as the phase difference and time delay between legs. Through experimentation, it has been revealed that the strongest thrust occurs when the hind leg makes contact precisely at the center of the vortex that is previously generated from the middle leg. This optimized strategy allows the microrobot to achieve water-walking speeds comparable to its movement on land (approximately 20 BL/s).

Hypoxia-Induced Edema in Avian Embryos

Wonil Choi, Rachel Neto, Haruka Wada

The relationship between global warming and hypoxia is of intriguing interest, given that increasing temperatures elevate oxygen demands for many animals. However, the understanding of how elevated temperatures and/or hypoxia alter embryonic development remains limited. This is especially critical for avian embryos, as they rely on eggshell pores for gas exchange; however, pore density is fixed upon oviposition, making them incapable of adjusting to changing oxygen demands. Additionally, pore density variations (up to 5-fold) among clutches pose a threat to avian populations in such conditions. In a prior study, we induced hypoxia in zebra finch embryos by physically covering the eggshells (30% shell surface) with beeswax and noted that 1) wax covering decreased hatching success and 2) high incubation temperature amplified this negative effect on hatching success by wax treatment. Furthermore, within the hypoxia-exposed group, edema occurred near the hatching muscle. To decipher the physiological processes of edema formation, we employed the aforementioned treatments and histologically evaluated each embryo's edema region and vital organs. Our findings reveal that hypoxia-induced embryos had significantly enlarged regions of edema compared to controls. Additionally, severe edema formation was correlated with hepatic autolytic cells and renal interstitial edema, while the brain and eye remained unaffected. The organ damage may stem from ineffective lactic acid clearance in the liver and kidney, potentially causing fluid leakage and edema.

Epigenetic Regulation of Aposematic Coloration in *Oncopeltus fasciatus*

Elizabeth Chou, Madeline Hoesel, Marie Tan, Lyanna Toh, Laura Park, Yuichiro Suzuki

Evolution of aposematic coloration is facilitated by canalization to reduce variability that might prevent the establishment of learned associations. Our study examined the role of epigenetic regulators in maintaining this coloration robustness in *Oncopeltus fasciatus*, which features varied pigmentation plasticity across different developmental structures. Polycomb group (PcG) proteins, which constitute the Polycomb Repressive Complexes (PRCs), are epigenetic regulators that affect gene expression through histone methylation and ubiquitination that have been previously linked to phenotypic plasticity in insects. Using RNA interference, we knocked down Pc, E(z), and jing: components of PRC1, PRC2, and a PRC2 scaffolding protein respectively, in 4th instar *O. fasciatus*. Treated insects were then reared at different temperatures (20°, 26.5°, and 33°) to examine affected phenotypic plasticity in different environmental conditions. Resulting melanization of adult *O. fasciatus* was compared to that of controls. Pc knockdowns had significantly more melanization across all temperatures, while jing and E(z) knockdowns demonstrated greater plasticity in response to changes in temperature. These results imply that jing and E(z) could play a role in maintaining the robustness of aposematic coloration in the face of temperature fluctuations.

Deciphering the Genetic Basis of Cell Complexity Evolution using Butterfly Wing Scales

Jordan Chow, Lizzy Sullivan, Rachel Cotter, Alexandra Colombara, Brian Counterman

From the early days of microscopy, the differentiation of cell types and the evolution of their complexity has intrigued biologists. Insect scales vary wildly

in their complex structures and offer a rich opportunity to study how the cytostructural complexity of cells can evolve. Insect scales are homologous structures to setae, which include hairs and bristles, however, scales, such as those on butterfly wings are dramatically more complex in their cytoskeletal structures than setae. Here, we attempt to identify pathways and specific genes that may be involved in the evolutionary and developmental transition of hairs and scales. Specifically, we explore the role of the Planar Cell Polarity (PCP) pathway in the development of butterfly wing scales. The PCP pathway is responsible for coordinating the orientation of cells, which has been shown to be critical for the proper development of wing hairs in *Drosophila*. We ask whether this pathway also regulates the development of scale development on the wings of the butterfly *Vanessa cardui*. Using a CRISPR gene editing approach to study gene function we have identified several genes from the PCP pathway that appear to be critical for proper wing-scale development. We present our results of phenotypic mutants, which confirm the role of the PCP pathway in wing scale development and suggest the PCP pathway may be critical in the transition from wing hairs to scales.

Examining Species Classification of New Zealand's Mite Harvesters in the Genus *Rakaia*

Rachel Christensen, Unitas Vang, Elsa Viereg, Zade Alafanji, Haley Heine, Sarah Boyer

The mite harvester family Pettalidae is a group of small terrestrial arachnids that reside in leaf litter microhabitats and caves throughout numerous landmasses that were once linked by the supercontinent Gondwana. Three genera of these animals, *Rakaia*, *Aoraki*, and *Neopurcellia*, are endemic to New Zealand. Although these animals have been found throughout the North and South Island of New Zealand, they are incredibly dispersal-limited, blurring the lines between populations and species and therefore complicating the use of traditional identification methods. Along with the very limited differences in morphology across species, these factors present a unique challenge in terms of classification and require a more integrative approach when analyzing this system. By implementing additional analysis of geographic and genetic data, past researchers have been able to further clarify the evolutionary relationships of these animals. However, some species boundaries still remain unresolved; specifically, a clade of species in the genus *Rakaia* from the eastern half of the South Island has not received comprehensive taxonomic treatment. With the hope of clarifying species boundaries in this group, we analyzed morphology using scanning electron microscopy photos, conducted a phylogenetic analysis based on the mitochondrial locus COI, and mapped collection localities.

Protein metabolism limits mass gain in brown bears

Amelia Christian, Charles Robbins, Troy Tollefson, Jessie McCleary-Smith, Chelsea Davis, Anthony Carnahan, Ellery Vincent, Heather Havelock, Perry Barboza

Brown bears (*Ursus arctos*) prefer energy-rich foods that are moderate to low in protein. We fed 4 males (180–290 kg) and 7 females (145–200 kg) isocaloric diets (18.7 KJ/g) at 10%, 20%, and 40% crude protein (CP) to test the hypothesis that metabolic responses to daily protein load constrains mass gain. Bears were fed at two intake levels (maintenance and twice maintenance) in each of three periods over the active season (May–September). Dry matter (DM) intake ranged from 1000 - 5700 g/day, with 136–1780 g CP/day and 22350–124120 kJ/day. Apparent digestibilities of DM (75%), CP (74%) and energy (80%) were not affected by intake; that is, digestive efficiency was sustained over the range of intake. Daily mass gain increased over the summer in both males and females. Mass gain increased through the range of daily digestible intakes of DM (15–65 g/kg BW^{0.75}) and energy (357–1532 kJ/kg BW^{0.75}). Mass gain increased with daily digestible CP (DCP) intake to a threshold of 11 g DCP/kg BW^{0.75}. Increased protein intake above this threshold did not increase rate of mass gain, which suggests a limit to metabolizing additional loads of protein. Brown bears have a greater capacity to metabolize energy than protein and may therefore prefer diets with lower protein loads (< 2.0% DCP) as energy demands increase with activity and fattening.

Postembryonic Expansion and Innervation of the Zebrafish Anterior Lateral Line

Theresa Christiansen, Vishruth Venkataraman, Noel McGrory, Victoria Prince

The lateral line is a sensory system common to aquatic vertebrates which senses hydrodynamic information in the environment. During development, this placode-derived system differentiates into a migrating primordium, which deposits sensory neuromasts at stereotyped locations, and an innervating ganglion. At juvenile postembryonic stages, neuromasts become encased in bony canals, while superficial neuromasts are added to maintain sensory density with growth. The anterior lateral line (ALL) consists of three major lines surrounding the eye and jaw, which are innervated by the dorsal (gAD) and ventral (gAV) lobes of the ALL ganglion, while the posterior lateral line (PLL) lies along the trunk and is innervated by the PLL ganglion. While zebrafish PLL development is well-studied, key details of ALL organization and innervation remain unknown. To fill this gap we adapted a CUBIC clearing protocol to visualize immunolabeled zebrafish nerves and neuromasts in 3-10 mm Standard Length larval specimens. We find that superficial neuromast lines form parallel to existing ALL canal lines, with neuromasts likely added through proliferation and coalescence of interneuromast cells. Interestingly, the primary superficial lines appear to be exclusively innervated by the ventral gAV ganglion, although the dorsal ganglion innervates many canal lines. These findings suggest that the gAV system may maintain placode-like tissue used during superficial line development. Future experiments will explore this model and evaluate the role of innervation in generating neuromast expansion.

Aftermath of severe winter storms on a colonial tunicate invading the rocky intertidal of CA

Annie Chu, Tyler Soberanis, Emily Chapman, Erika Ono-Kerns, Patricia Cristales, Sarila Young, C. Sarah Cohen

San Francisco Bay is heavily invaded, but less than a handful of known invasive species have ranges extending to the relatively untouched outer coast of California. *Didemnum vexillum* (Dvex) is a yellowish, highly invasive colonial tunicate, an invertebrate that has spread to fouling communities worldwide. Dvex is abundant at Point Bonita, a National Park site at the mouth of the Golden Gate, demonstrating the spread of invasive species to the outer coast from more common bay and fouling community sites. After back-to-back atmospheric rivers depositing heavy rainfall across the span of five months during winter 2022-2023, Dvex at Point Bonita was significantly reduced; remaining colonies were thin, patchy, and heavily regressed. Similar Dvex population loss after heavy rainfall has been observed on the Irish east coast. These results suggest that salinity stress and mechanical removal of colonies on rolling boulders due to high wave energy had severe impacts on population abundance at Point Bonita. Data collected across survey trips before and after heavy winter storms revealed the extent to which they affected Dvex abundance at Point Bonita, documenting the gradual resurgence of Dvex populations during summer 2023. Several management strategies have been discussed, and spot treatments showed promise. As Dvex continues to spread along the outer coast from this crucial site, intertidal and subtidal ecosystems will be smothered by this highly invasive species.

Evaluating the use of Machine Learning and Bioacoustics to Quantify Avian Migration in a Major City

Madison Chudzik, Benjamin Van-Doren, Jorge Garcia, Sara Lipshutz

Billions of migratory birds travel across North America each fall and spring, and among cities in the U.S., Chicago poses the greatest risk to migrants due to its skyscrapers along Lake Michigan. Recording nocturnal flight calls, vocalizations emitted during migration, provides a species-specific view of this phenomenon. This acoustic monitoring can provide insight into taxonomic composition and migration intensity at a local scale. However, the large amount of data recorded in a migration season requires a substantial time investment to annotate and identify calls at the species level. Nighthawk, a deep-learning model, detects and identifies nocturnal flight calls, providing an efficient form of analysis. We deployed a network of eight acoustic recording units across Chicago during the spring 2023 migration season. We manually annotated

50 hours of recordings to train and evaluate Nighthawk's performance and compared total flight call counts to migration intensity from a nearby Doppler radar to assess its accuracy in measuring migration. We find that once customized, Nighthawk provides accurate nocturnal call analysis and measure of migration. Total flight call counts closely reflected the recorded radar migration intensities. Furthermore, most flight calls were identified at the species level, revealing variations in taxonomic density and composition between each site. Quantifying migration on a local scale can help identify areas of importance in ecological traps, such as big cities, and inform effective mitigation decisions.

No discernible difference in thyroid concentrations between African savanna and hybrid elephants

Daniella Chusyd, Claire Goodfellow, Janine Brown, Steve Paris, Nicole Boisseau, Stephanie Dickinson, Tessa Steiniche, Colin Chapman, Richard Mutegeki, Patrick Omega, Nelson Ting, David Allison, Michael Wasserman

The Albertine Rift is the largest known hybridization zone for African elephants (i.e., offspring of African savanna, *Loxodonta africana*, and forest, *L. cyclotis*, elephants). However, there are no published data assessing how different elephant species respond to shared habitats. In Kibale National Park, Uganda, single fecal samples were collected from November 2020 through August 2021 (n=124). Dung was genotyped at 14 microsatellite loci to determine sex and species (i.e., African savanna, forest, or hybrid). We quantified fecal thyroid hormone (T3) concentrations. Linear Mixed Models accounting for repeated measures were performed and adjusted for sex and season. There was no discernible difference in T3 between savanna (mean 211.25, SD 125.25 ng/g, n=33) and hybrid elephants (mean 232.90, SD 142.06 ng/g; n=88) (p=0.430). There was only one forest elephant (345.41 ng/g) and thus it was not included in the analyses. Female elephants (mean 269.17, SD 141.80 ng/g, n=44) had higher T3 concentrations compared to males (mean 204.72, SD 130.01 ng/g, n=78) (p=0.005). Thyroid hormone plays an important role in overall energy expenditure and resting metabolic rate. Based on this study population, it appears that hybrid elephants demonstrate similar T3 concentrations compared to savanna elephants living in a shared environment. With knowledge of hybrid elephant biology is in its infancy, this study was an important first step.

Effect of Antibiotics on Brain Development in Wild Birds: Cognition in the Age of Climate Change

Avia Ciaccia, Jennifer Houtz, Nora Prior, Maren Vitousek

The gut microbiome regulates early development among diverse groups of vertebrates. In particular, the gut microbiome is influential in early brain development and adult brain function. Evidence indicates that climate change induces gut dysbiosis through varied mechanisms, suggesting that the brain function of wild animals may be altered in the age of climate change. Recent research supports the existence of a gut-microbiota-brain axis in birds. Understanding the effect of a dysbiotic microbiome on brain development will allow us to predict cognitive variation and better conserve wild birds in the face of climate change induced microbial dysbiosis. Here, we administered an antibiotic to wild-caught juvenile Tree Swallows (*Tachycineta bicolor*) to induce gut dysbiosis. Intestinal microbiome samples were analyzed for bacterial community composition. Expression of glutamate receptors, dopamine receptors, and an inflammatory marker were measured in brain tissue from the Nidopallium caudolaterale, the avian seat of complex cognition and correlate of the mammalian Prefrontal Cortex. Glutamate and dopamine are implicated in neuroplasticity and executive functioning, respectively. Differences in receptor expression may suggest variation in cognition induced by antibiotic treatment, implicating the ability of avian species to behaviorally adapt in the face of climate change induced microbial dysbiosis.

Geometric morphometrics analysis reveals that long bone morphology depends on habitat and body size

Robert Cieri, Joshua Jevremov, John Capano, Christofer Clemente

Biomechanical scaling predicts positive allometry in skeletal robustness and changes in bone shape with increasing body mass. This is because bone strength depends on cross-sectional area which increases as M^{2/3}, much lower than mass, and thus without these changes bones would become

proportionally weaker. Previous work in mammals has shown that habitat, locomotor mode, and relatedness substantially influence long bone shape, such that allometric aspects of bone shape may be confounded by differences in life history, posture, and phylogenetic inertia. Varanoid lizards offer an ideal opportunity to investigate the influence of body size on long bone shape because this group maintains similar body proportions and posture over a body size range from 1g to 70 kg. Here we apply geometric morphometrics to the femur, tibia, fibula, humerus, ulna, and radius digitized from computed tomography scans of 34 varanoid species ranging in snout-vent length from 93-812 cm. Our results show that body mass and habitat have significant effects on the long bones of varanoid lizards, while phylogeny has a minimal influence. Size-related allometry of most bones involves increasing shaft robusticity and changes in the shape of the distal and proximal articular surfaces. Interestingly, we found that body size has relatively more impact on the shape of the upper limb bones (stylopodium), while habitat is a bigger driver of bone shape in the lower limb bones (zeugopodium). Similarly, the influence of body size on the lower limb bones was greater in the forelimb than the hindlimb. These results show how long bones scale in sprawling animals, providing insight into evolutionary pressures on bone geometry in stem tetrapods. Biomechanical scaling predicts positive allometry in skeletal robustness and changes in bone shape with increasing body

Structure and function in the Cetacean pulmonary airway tree

Robert Cieri, Robert Shadwick, Marina Piscitelli-Doshkov, Merryn Tawhai

The pulmonary systems of Cetaceans are the largest in evolutionary history and tell a fascinating story about secondary adaptation to marine life. Many species of Cetacean use explosive ventilation, where high volumes of air are exhaled and inhaled rapidly during a brief surface interval. Cetaceans also expose their respiratory systems to frequent high external pressures during diving. Cetacean lungs usually contain only one lobe, have a high degree of smooth muscle and cartilage reinforcement, and have subjectively relatively larger airways than those in terrestrial mammals. These adaptations are thought to enable high relative flow rates, prevent barotrauma, and facilitate the movement of air from the gas-exchanging to the conducting airways during lung collapse at depth, but how these traits and airway topology vary within Cetacea, and the influence of these adaptations to the flow of air through the lung is unknown. We are studying the structure and function of the pulmonary airway tree in whales and dolphins using computed tomography (CT) scans and computational fluid dynamics. Airway geometry, such as branching angle, the pattern of branching, relative airway diameter, airway cross-sectional shape, and relative parenchymal to airway volume vary between terrestrial mammals and Cetaceans. Although we lack CT data for the largest Cetacean species, an allometric approach may give us insight into how breathing works in the largest animals of all time.

Symmetry/asymmetry in the stalked eyes of larval *Idiacanthus* (Stomiiformes: Idiacanthidae)

Todd Clardy, Allison Smith, Clay Pollock, William Lucht

One of the most extreme morphological transitions in larval fishes, and indeed all vertebrates, involves the development and reduction of stalked eyes of *Idiacanthus*. The eyes of larval *Idiacanthus* are elliptical and positioned at the tips of cartilaginous rods that extend outward from the neurocranium, extending up to 27% of the body length. During development, the eye stalks shorten into the neurocranium, and eye shape normalizes to a round shape. While various aspects of eye morphology in *Idiacanthus* have been studied, the symmetry in eye stalk growth and reduction has not been examined. The symmetry/asymmetry of *Idiacanthus* eyes may provide important insight in the evolution of symmetry and asymmetry in the vertebrate bauplan. In this study, we measure various aspects of the *Idiacanthus* eye on both left and right sides, including eye stalk length and eye area for larval stages and eye area for adult stages. Initial findings show that eye stalk length varies between 2-15% between left and right sides within individual larval *Idiacanthus*. In adults, the variation in eye morphology between left and right sides is much less. Our findings suggest that eye morphology of larval *Idiacanthus* is more variable than those of the adults.

The Tour de Fins: Bluegill brake like bikes

Andrew Clark, Eric Tytell

In nature, fish often swim unsteadily, speeding up and slowing down regularly, but we know relatively little about how they modulate speed. In particular, we know very little about routine deceleration. Several studies have examined rapid deceleration, but, to our knowledge, no one has studied slower decelerations. Therefore, we studied routine braking behavior in bluegill sunfish, *Lepomis macrochirus*. We used a programmable “car” with an attached chamber that is mounted in the working section of a flow tank, allowing us to control both flow speed and car speed to compel fish to slow down at varying rates. We highlight the roles of the pectoral and pelvic fins during gradual braking maneuvers. Here we present evidence suggesting that bluegill brake somewhat like a person on a bike. At the lowest speed, they cease caudal fin undulation (like gliding to a stop on a bike); at intermediate speeds, they flare the pelvic fins (like using the back brake); and at the highest speeds, they flare the pelvic and pectoral fins (like using the back and front brakes together).

How Nightjars produce loud wing-snaps during courtship

Chris Clark, Juan Areta

Many species of nightjar reportedly produce ‘clapping’ sounds with their wings during courtship. To record these displays, we illuminated N = 6 male Scissor-tailed Nightjars (*Hydropsalis torquata*) with infrared light on a road near Salta, Argentina, in spring and early summer (Sept-Dec 2022) on pre-dawn nights immediately following a full moon. Amongst the moonlight, the nightjars produced 4 different types of nonvocal sound, the most common of which were short, sharp, loud, atonal ‘tk’ sounds. These sounds were produced in multiple contexts: during a jump display, in a flight display, and immediately after copulation. High-speed video revealed these ‘tk’ sounds were produced when a bird rapidly elevated and pronated his wings to slam opposing wrists together. The videos falsify the hypothesis that sound is produced by clapping, i.e. a pulse of air accelerating to escape a constricted space (as in human hand-clapping), as there was no contact by the surface of opposing wing-feathers. Instead, we conclude the physical acoustic mechanism is likely snapping, i.e. impulsive collisions between stiff elements (wing bones) which then vibrate, similar to wing-snapping of *Manacus manacus*. We finish by speculating about whether other birds produce snapping or clapping. There is acoustic evidence that hints many species of nightjar in the genus *Caprimulgus* do produce wing clapping, as do certain owls and possibly a hummingbird.

Experimental epidemics reveal that host sex and sociality impact transmission within host groups

David Clark, Jason Walsman, Faith Rovenolt, Isabelle Weiler, Vineet Nayak, Paige Person, Joshua Tamsen, Jessica Stephenson

Understanding parasite spread and persistence within host populations is critical to predicting how emerging infectious diseases will impact host populations. Both heterogeneity among hosts and social interactions between hosts can impact the transmission of parasites within populations. We used a Trinidadian guppy and monogenean worm system to assess the impact of host sex, social contact, and worm intensity on the transmission of parasites within groups. We recorded the behavior of 17 groups of 6 female and 3 male guppies for 2 days before and 7 days after the introduction of an infected or sham-infected female. We tracked the movement of individuals to assess contacts with conspecifics and screened the fish for parasites to quantify transmission over the course of each day. We found that male guppies were more likely to stay uninfected longer and had lower established infection loads than female guppies within these groups. Additionally, groups with higher contact with infected hosts had higher infection prevalence a day after the release of the infected female. These results are a promising start to disentangling the importance of host sex, sociality, and worm intensity in the establishment and transmission of parasite infections across groups. Further elucidating these factors and the relationships between them is critical to understanding and predicting the spread of emerging pathogens within host populations.

Extreme insect sap feeding illuminated with 3D imaging and computational fluid dynamics simulations

Elizabeth Clark, Rodrigo Almeida

Insects that feed on the xylem sap of plants can inadvertently spread bacterial pathogens, leading to lethal infections in grapes, almonds, blueberries, and other important crops. As such, a thorough understanding of how these insects feed on xylem sap is critical to pinpointing how pathogen transmission occurs. However, the mechanics of ingestion in these insects remain poorly understood due to an important technical challenge: in contrast to most insects, the mouthparts of xylem sap-feeders are internal, as is the plant material on which they feed. We have developed a novel approach for visualizing behavior in small insects through integrating synchrotron-based 3D imaging, 2D x-ray video imaging and computational fluid dynamics modeling to illuminate how *Graphocephala atropunctata*, an important vector of xylem-limited bacterial pathogens in California agriculture, performs xylem sap-ingestion. Through the application of this methodology, we were able to calculate important feeding parameters such as the rate of ingestion, the force application required to begin ingestion and to meet observed excretion rates, and maximum force application capabilities based on muscle anatomy. As this is the first study that integrates this set of innovative digital approaches to illuminate how piercing and sucking insects ingest food, this study establishes a new methodological workflow for using computational tools to understand and combat insect-borne agriculturally significant diseases in plants.

Trouble in Sunshine Creek: Exploring virtual reality as an effective science communication tool

Elizabeth Clark, Laura Lynn Gonzalez

3D imaging data is becoming widely used in many fields of biology. However, the presentation of this type of data continues to take place almost exclusively on systems designed for 2D media (e.g., a computer screen, a powerpoint, a publication figure). The reliance on 2D outlets for sharing 3D data grossly underutilizes the power of this type of data and its utility for conveying information. Virtual reality represents a promising avenue for easily sharing 3D data with a range of audiences. However, the cost of creating VR experiences and the technical barriers to do so limit the generation of high-quality scientific content with emerging research in VR. 10k Science is an app for scientists to easily create bespoke VR experiences using their own 3D data. Here, we used 10k Science to construct a VR experience in which users can learn about plant and insect anatomy as well as insect-vectored plant diseases through free exploration in a virtual vineyard. Feedback from diverse groups of participants including farmers, high school students, educators and scientists indicate that our VR experience is an effective learning module for conveying important information about vital plants, insects, and plant diseases in California. These results strongly suggest that this approach represents a promising tool for scientists in a wide range of fields to effectively share their 3D imaging data with a broad audience.

Painted turtle personality: *C. picta* exhibit behavioral syndromes across contexts

Morgan Clark

Behavioral syndromes, correlated suites of behavior, or animal personalities, are underexplored in herpetology, and how behavioral syndromes yield evolutionary and ecological insight into freshwater turtle species is a mystery. In particular, members of the globally imperiled reptile order Testudines have long been considered automatons, lacking intertwined sets of behaviors and the cognitive abilities to make complex decisions. Some studies have described correlations between behavioral traits such as aggression, sociability, and boldness, but how the relationships between these traits are correlated with other behavioral outcomes is poorly understood. In this study, I examined personality traits in a wild population of painted turtles (*Chrysemys picta*). In the field, I performed behavioral assays under simulated predation and behavioral assays in a novel environment to test for a correlation in behavioral traits across contexts. I found that painted turtles exhibit a correlation in behavioral traits between and within contexts. Specifically, when placed in a novel environment, turtles that raised their heads quickly to explore were

also quicker to move their bodies to assess an unfamiliar setting. Most notably, painted turtles that displayed bolder behaviors under simulated predation were faster to explore a novel environment. These results demonstrate the presence of behavioral syndromes in a wild population of freshwater turtles and add insight into how this and similar species may respond to future predation pressures and environmental changes.

Does selection drive antenna morphology between reproductive signaling strategies in fireflies?

Noelle Clark, Ricardo Hernandez-Espinoza, Lindsay Waldrop

The Lampyridae family (fireflies and glow worms) is unique due to their diverse reproductive signaling methods. Some species use a visual signal (flashing or glowing), others use pheromones (chemical signals captured by chemosensory hairs on the antennae), and a handful use both. The use of pheromone signals for reproduction is suspected to be ancestral to lampyrids, with visual signaling evolving multiple times independently. A few species have secondarily reverted to pheromone signaling and have lost the trait of visual signaling. Previous work has found morphological differences in the eyes of visual versus pheromone signaling species. Here, we ask, are there morphological differences in the antennae between species who use pheromone or visual reproductive signaling? To explore this question, the flagella of antennae from 30 species of fireflies and a soldier beetle (outgroup) were imaged with scanning electron microscopy. A variety of morphometric measurements were made on the lengths, widths, and arrangements of chemosensory sensilla. We found that there are no significant differences in the lengths, widths, or arrangements of chemosensory sensilla between pheromone and visual signalers, despite having species representing five independent reversals to pheromone signaling. These results suggest that there is no strong selective pressure on antenna morphology from the switch to visual signaling.

Locomotor joint moments in Varanid lizards and the scaling of locomotion in sprawling tetrapods

Christofer Clemente, Robert Cieri, Taylor Dick, Peter Bishop, John Hutchinson

Geometric scaling predicts a major challenge to legged, terrestrial locomotion with increasing body size. Locomotor support requirements at dynamically equivalent speeds scale isometrically with body mass (M_1), while force generation capacity should scale $M_2/3$ as it depends on tissue cross-sectional area. Mammals compensate with more upright postures at larger sizes, but it remains unknown how sprawling tetrapods deal with this constraint. Varanid lizards are an ideal group to address this question because they span an large body size range with similar posture and body proportions. This study reports the scaling of joint moments from the hindlimb and forelimb from varanid species ranging from 7-37,000 g. Joint moments were calculated via inverse kinematics and inverse dynamics in forelimb and hindlimb musculoskeletal models with 23 and 26 muscles, respectively, and 12 degrees of freedom in OpenSim. Peak joint moments scaled generally with isometry ($M_1.30$ isometry; $M_1.31$ hindlimb mean; $M_1.26$ forelimb mean) with the exception of hip adduction was higher ($M_1.45$). The impulses of joint moments, however, scaled with positive allometry ($M_1.167$ isometry; $M_1.55$ hindlimb mean; $M_1.45$ forelimb mean).

Previous work found that muscle parameters (fascicle length, muscle mass, physiological cross-sectional area, and fibre cross-sectional area) as well as duty factor scale with positive allometry, suggesting that varanid lizards use both anatomical and kinematic adjustments to produce sufficient joint impulses for locomotion without suffering high peak joint moments. These results also suggest, however, that hip adduction may provide a biomechanical limit to locomotion at large body size in sprawling tetrapods.

Unlocking Kangaroo Hopping: Posture, Tendon Stress, & the Metabolic Mystery

Christofer Clemente, Lauren Thornton, John Hutchinson, Glen Lichtwark, Craig McGowan, Alexis Wiktorowicz, Jonas Rubenson, Taylor Dick

Compared to other mammals, kangaroos truly have different body form, locomotor biomechanics, and energetics. For example, in most mammals, the

mass-specific metabolic rate increases as an individual moves faster – the extra energy used to propel the body forward. However, hopping kangaroos defy this trend, with the energy use being independent of their speed. This unique ability is a complex function of limb proportions, body mass, and muscle-tendon structure which is yet to be deeply interpreted. To explore this topic, we created an OpenSim kangaroo musculoskeletal model, integrating 3D motion capture and force plate data in inverse simulations to analyze the kinematics and kinetics of hopping in red and grey kangaroos. We evaluated how body mass and speed influence (i) hindlimb posture, (ii) effective mechanical advantage (EMA), and (iii) ankle extensor tendon stress during hopping. Our findings revealed that alterations in ankle and metatarsophalangeal joint angles played an important role in decreasing ankle EMA. Surprisingly, these changes in kangaroo posture appeared to contribute to heightened tendon stress rather than mitigate it to minimize the risk of tendon rupture, thereby indicating a potential mechanism behind the speed-independence of locomotor cost, via elastic energy savings. This study enhances our understanding of the interplay between posture, tendon stress, and metabolic rate in kangaroos, offering valuable insights into their extraordinary locomotor capabilities.

Biorobotics reveals mechanisms underlying bird swimming and invertebrate walking

Glenna Clifton

Animal movement is particularly complex in naturalistic environments, and the mechanisms underlying these movements are difficult to study when the animals are not lab-suitable, the behavior cannot be induced, or the motion occurs at extreme spatial or time scales. This talk will highlight three examples of how biorobotics helps connect the anatomy and behavior of freely moving animals to the mechanisms underlying performance. First, foot-propelled swimming birds have historically been considered to generate drag-based, and not lift-based, propulsive forces. To test this assumption, I attached cadaveric feet to an industrial robot programmed to replicate tracked loon swimming motions. Directly measuring the forces acting on the cadaveric feet revealed a larger contribution of lift. Second, observations of ants walking over rough ground revealed foot collisions during swing that flexed the distal limb. Incorporating this limb compliance into the virtual controller for a five-linkage planar robotic leg enabled collisions to progress towards successful stepping and improved walking on rocky terrain. Lastly, a scaling study of two intertidal crab species revealed a conserved 63% of the limb distal to the “knee”. Simulations of a crab-inspired robotic limb (planar, two-segments) identified the exact same limb ratio as best for stepping over hemispherical obstacles, which was confirmed in robotic experiments. Together, these examples highlight the power of biorobotics to inform our understanding of animal anatomy, behavior, and movement.

Can power amplification improve thermal robustness of muscle in the Western fence lizard?

Anthony Cobos, Natalie Holt

Actomyosin interactions in skeletal muscles power animal movement. However, the rate-limits of enzymatic processes associated with actomyosin interactions limit the force-velocity relationship and maximum muscle power output. Many muscles operate in series with elastic elements (SEEs). When these SEE are sufficiently compliant, muscles can store and release strain energy in them to amplify power output, resulting in joint powers that exceed muscle power. Given the enzymatic nature of actomyosin interactions, muscle performance is temperature sensitive. However, the temperature-insensitivity of elastic tissues means that ectotherms can utilize power amplification to increase thermal robustness. During running, *S. occidentalis* joint powers were estimated to range from 180.15-880.94 W/kg (15-35°C), exceeding theoretical limits of muscle power. Here we determine the thermal sensitivity of the gastrocnemius, and the potential of its tendon to function as a SEE and amplify power. We used temperature-controlled in-vitro muscle physiology experiments to determine gastrocnemius power output at 15, 20, 25, and 30 °C, and highspeed videography to track muscle fiber shortening against SEEs. The average muscle power was 80.01-137.25 W/kg (15-30°C), far below measured joint powers during locomotion. The Achilles tendon stored ~13 J/Kg of strain energy during a maximal fixed-end contraction. The ability of

S. occidentalis to store energy in SEEs, coupled with the discrepancy between measured muscle and joint powers, provides evidence for power amplification increasing thermal robustness in this system.

How does oxygen availability affect ion transport in aquatic insects?

Jamie Cochran, David Buchwalter

Oxygen availability is central to the energetic budgets of aquatic animals and is highly variable in freshwater systems globally. This variation is further exacerbated by anthropogenic activities (e.g., eutrophication). To investigate how oxygen availability is linked to ion transport in aquatic insects, we used radioisotopes (^{22}Na and $^{35}\text{SO}_4$) to measure ion uptake rates across a gradient of oxygen partial pressures in the caddisfly, *Hydropsyche betteni*. We hypothesized that suppression of ion transport is imposed due to limited ATP production at partial pressures below the P_{crit} (the oxygen level below which they no longer oxyregulate). Our findings show Na and SO_4 uptake decreased by 1.5 and 3-fold respectively at the P_{crit} (7kPa) and 7 and 9-fold respectively below the P_{crit} (2kPa). Interestingly, we also observed a 1.4 and 2-fold decrease in Na and SO_4 uptake respectively at partial pressures right above the P_{crit} (13 and 11kPa). This raises the possibility that *Hydropsyche betteni* may sense oxygen reduce transport of Na and SO_4 to conserve energy (even at partial pressures above the P_{crit}). We plan to present these data in addition to experiments in progress in the mayfly *Neocloeon triangulifer* evaluating how oxygen availability affects ion (^{22}Na and $^{35}\text{SO}_4$) transport, body fluid osmolality, and expression of several ion transport (e.g., SO_4 transporter, Ca ATPase and Na/K ATPase) and hypoxia-associated genes (e.g., LDH and EGL-9).

Flow field surrounding bluegill sunfish (*Lepomis macrochirus*) during suction-feeding

Jensine Coggin, Duvall Dickerson-Evans, Rory Miller, Erin Hackett, Roi Gurka

Suction-feeding is a common technique for capturing prey by aquatic organisms. This feeding method allows for the adaption of predators by enabling them to consume a wide range of prey. Suction-feeding is a complex fish-fluid interaction governed by the balance of various hydrodynamic forces: inertia, unsteadiness, viscous and pressure gradient that are described by the momentum equations of the fluid. This study measures the flow field surrounding a bluegill sunfish (*Lepomis macrochirus*) while it suction feeds. Particle image velocimetry was utilized to measure all three velocity components of the flow surrounding the fish during suction-feeding along with high-speed imaging of the suction-feeding for characterization of the kinematics. Quantification of all three velocity components allows for insights on the flow dynamics of suction-feeding and potential quantification of some of the hydrodynamic forces (e.g., viscous and pressure gradient).

Growing defenses: insights into the cellular development of lumpsucker armor

Karly Cohen, Gareth Fraser

Dermal odontodes are specialized structures encompassing scales, spines, and tubercles while teeth are exclusive to the oral cavities of vertebrates, primarily used for food processing. The Pacific spiny lumpsucker (*Eumicrotremus orbis*) has evolved large, tooth-like armor to survive the harsh environment of the subtidal waters of the North Pacific Ocean. Their armor is composed of enamel cones that spiral and coalesce into large scales. Damaged odontodes cannot heal and so lumpsuckers must continuously build new armor, either through replacement or constant growth. Here, we investigate the initiation, growth, and cellular development of lumpsucker armor to better understand how individual cones recruit additional cusps to form the adult morphology. We found that initial placodes are temporally restricted and odontode development begins as early as 2-weeks post hatching. We also found that odontodes develop in waves of rapid growth followed by periods of stagnated development. Lumpsuckers invest early in armor development illustrating the importance of these scales for survival in harsh intertidal environments. We hypothesize that adult dermal odontodes form through a

signaling cascade where the initial placode serves as a base for growth that initiates the development of the next sequential cone, ultimately forming the signature spiral. These pathways likely persist into adulthood, allowing individuals to continuously grow and potentially replace damage scales. Additionally, dermal odontodes may be more present in teleosts than currently defined.

Circadian rhythms and fitness of free-living house sparrows across a light pollution gradient

DiDiAlice Coker, Elisset Poveda, Jenny Ouyang

Human activities change habitats faster than species can adapt, threatening many with extinction. Light pollution is a stressor that interferes with natural light-dark cycles and disturbs sleep and wake patterns, perhaps most noticeably in city-dwelling birds. Abnormal light exposure from artificial light at night may perturb behavioral rhythms important for survival and reproductive success. We quantified artificial light at night levels at three locations varying in degrees of urbanization, caught 10 pairs of house sparrows breeding at each location and outfitted them with radio tags. We monitored their behavioral rhythms continuously for ~10 days and their parental feeding rates. We show how behavioral rhythms are related to reproductive effort and success across varying light at night exposures.

Identification of brain regions involved in formation of zebra finch song preference

Melissa Coleman, Ella Thunen, Divya Ahuja, Melina Soberg

Social monogamy is a complex behavior in which animals form a pair-bond for at least a single breeding season. We are interested in how the nervous system controls the formation and maintenance of pair bonds in the monogamous zebra finch. During pair bond formation in finches, males sing a unique song to attract females and females select a male based, in part, on the quality of his song - an honest signal of fitness. As a result, females form and maintain a preference for their partner's song. Therefore, we use female song preference to understand the neural mechanisms of partner preference and pair bond formation. To identify areas of the female finch brain involved in song preference, we looked for areas of the brain activated by playback of partner song in paired finches different than areas of the brain activated by non-partner song in unpaired finches. We identified these areas using immunohistochemistry of the immediate early gene (IEG), *egr1*. As a first step, we are quantifying differences in *egr1* expression in an area of the brain important for song discrimination (called the caudomedial nidopallium, NCM) and an area important for partner preference in other species (the striatum). Preliminary analysis suggests an increase in neural activity in both areas.

Linking fitness to transcriptional and epigenetic plasticity in a gall-inducing insect

Nate Collison

Grape phylloxera (*Daktulosphaira vitifoliae*) is a gall-inducing hemipteran insect and major pest to grape cultivation worldwide. The molecular mechanisms by which phylloxera feed, induce galls, and overcome host defenses are still unknown, although secretory effectors likely play a key role. The phylloxera genome retains an extraordinary expansion of over 2,000 predicted effector genes. Some of these effectors have been functionally characterized recently and have predicted roles in modulating broad aspects of host biology, including cell division, defense response, and cell death.

The mechanisms that drive host plant specificity and biotype evolution in phylloxera remain largely unexplored, although are of critical importance for pest management. In related aphids, rapid transcriptional plasticity at effector and chemosensory genes contribute to host specificity. Epigenetic regulation is thought to play a role in this plasticity, but empirical evidence is lacking. In Chapter 1 of my dissertation, I look at transcriptional and epigenetic plasticity in grape phylloxera as it moves to new host plant genotypes and determine the effect of this plasticity on phylloxera fitness. I hypothesize host change will cause (1) differential expression of predicted virulence effector genes, (2) positive correlation between fitness and virulence effector expression, and

(3) DNA methylation changes linked to transcriptional activity. These data will provide novel insight to the molecular nature of virulence and host adaptation by insect herbivores, and potentially inform pest management strategies.

Behavioral and mechanical responses of flying pollinators to clutter and wind

Stacey Combes, Nicholas Burnett, Katherine Jordan

Bees and other flying pollinators forage widely for floral resources and play a critical role in natural and agricultural habitats. They often encounter challenging flight conditions such as wind and environmental clutter, and how flying animals contend with these challenges is of increasing interest to biologists and engineers. These aspects of the environment are also changing, due to urbanization, agriculture, and large-scale shifts in precipitation and wind patterns; understanding organismal responses to these changes is critical for predicting future impacts on pollinator populations, natural ecosystems, and global food supply. We synthesized published findings to identify strategies that pollinators adopt to contend with wind and clutter, and we present findings of a study examining how wind and clutter affect bees' route preferences and flight performance. We find that insects display three strategies in challenging environments: fly slowly and carefully (avoiding collisions), get through challenges quickly (less time near obstacles, so fewer flapping-wing collisions), or avoid flying through challenging environments entirely. These strategies have different implications for energetics, resource intake rates, and pollinator longevity. Additional studies are needed that combine wind, clutter, and other challenges, test pollinators' choices between different environments, and track pollinators' microhabitat use while quantifying environmental features in the field. These studies will inform predictions of how pollinator responses to changing flight environments may affect colony growth, pollination services, and ecosystem structure.

The role of the spinal cord in the zebra finch (*Taeniopygia guttata*) reproductive stress response

Devon Comito, Robin Hinks, Allegra Estrada, George Bentley

Many think of the hypothalamic-pituitary-gonadal (HPG) axis as the main regulator of reproductive activities in vertebrates. Various external stimuli can stimulate the hypothalamus to synthesize and release gonadotropin releasing hormone (GnRH) to upregulate reproductive activities and gonadotropin inhibitory hormone (GnIH) to inhibit reproductive activities. These neuropeptides can then regulate gonadotropin release from the pituitary and steroid release from the gonads. This is a relatively time- and energy-consuming process with systemwide repercussions that may not be efficient or effective in response to unpredictable environmental stressors. We propose the existence of another axis, the hypothalamic-spinal-gonadal (HSG) axis, a transient fine-tuning regulator of the reproductive system. We have immunohistochemical evidence of GnIH and GnRH in the avian spinal cord and propose that the hypothalamus may send direct neural signals to the gonads via the spinal cord. Here we use behavioral assays and immunohistochemical analysis to examine the effects of food restriction and immune challenges, two stressors known to affect reproductive behavior and physiology, on zebra finch (*Taeniopygia guttata*) behavior and the HSG axis.

Towards the Integration of Somatic Mutations into Models of Coral Development and Evolution

Trinity Conn, Jessie Renton, Zoe Dellaert, Valérie Chamberland, Benjamin Werner, Thorsten Reusch, Ilana Baums

Adaptation to changing conditions is fueled by selection on standing genetic diversity and the introduction of new mutations in a population. Somatic mutations, while traditionally considered detrimental due to their association with cancer and senescence, accumulate during an organism's lifetime and can contribute to genetic diversity in clonal, colonial organisms such as corals. Unlike non-modular organisms like humans, in colonial organisms, deleterious mutations can be purged at the cellular or polyp level without colony-wide death. Conversely, adaptive mutations can rise in frequency within a colony as some polyps with novel mutations create genetically distinct branches. Here

we catalogued somatic genetic variation across eight colonies of *Acropora palmata* in Curaçao. Forty-eight genomes were sequenced to a depth of 70-100x and used to document shifts in mutation variant allele frequencies within colonies. Mutation load was high even in colonies aged six to ten years. While mutations were not fixed at the polyp or branch level, their allele frequencies followed a power-law distribution as is observed in aging human tissues. Mutation frequency distributions can thus be correlated with age in coral colonies; however, the relationship was not linear. Non-linearity in mutation accumulation suggests mutation-carrying stem cells may travel through the colony. Data presented here provide a framework for quantifying module-level selection of genetic variation in coral populations and the contribution of somatic mutations to adaption in colonial organisms.

Genome skimming resolves east Pacific *Pocillopora* species diversity and population differentiation

Michael Connelly, Victoria Glynn, Anabell Cornejo, Matthieu Leray, Sean Connolly, Andrea Quattrini

Biodiversity conservation relies upon accurate species taxonomy to support decision-making. Stony corals in the genus *Pocillopora* are critical ecosystem engineers in the tropical eastern Pacific (TEP), where seasonal cold-water upwelling and periodic El-Niño heatwaves limit the diversity of other coral taxa. However, the *Pocillopora* species taxonomy in the region is still unresolved due to high phenotypic plasticity, lack of diagnostic morphological characters, and low-resolution genetic markers used in previous studies, making it difficult for managers to identify conservation priorities. To address this gap, low-coverage whole genome sequencing, or genome skimming, was completed for 123 *Pocillopora* coral samples collected from Panamá, Costa Rica, and Clipperton Atoll. Sequencing data was used to recover mitochondrial and nuclear barcode loci and ultraconserved elements (UCEs) for species delimitation, and reads were aligned to the *P. damicornis* reference genome to calculate genotype likelihoods for population genomics analyses. Barcoding loci and species delimitation analyses revealed the existence of five distinct *Pocillopora* species in the TEP, and the presence of inshore-offshore population differentiation in *P. grandis*, the most widespread species in the region. Lastly, non-coral reads were aligned to algal symbiont (*Cladocodium* and *Durusdinium*) reference genomes to identify differential associations between *Pocillopora* host species and algal symbionts. Together, this study highlights the power of genome skimming as an affordable, high-resolution approach to assess stony coral species diversity, population genomics, and microbial symbiosis ecology.

Effect of prostaglandin inhibition on sperm quality and reproductive behavior in *Acheta domesticus*

Chloe Connolly, Kerianne Wilson

Prostaglandins (PGE) contained in semen play an important role in animal reproduction and can affect male sperm quality and female reproductive allocation. In some mammals, greater amounts of PGE are correlated with lower total sperm counts and sperm concentrations, although effects may be species-specific. Greater levels of PGE can also be associated with greater sperm velocity and frequency of sperm head rotations. The effect of PGE on insect sperm quality is poorly understood, though PGE has been shown to play a role in female reproductive allocation. In female house crickets, PGE2 increases lifetime fecundity by increasing the number of eggs laid per day and decreasing egg length. Our study explored the effect of PGE inhibition by naproxen on sperm quality in the house cricket. Short courtship trials were used to stimulate male spermatophore production. Spermatophores were collected and semen was evacuated into saline. Sperm quality was assessed by quantifying motility, viability and sperm count. We predicted that in accordance with the traits found in mammalian sperm, when treated with naproxen, the motility of *Acheta domesticus* sperm will decrease, the total sperm count will increase, and the percent viability will decrease. These results will inform the effect of prostaglandins on the male reproductive function of invertebrates and may provide insights into reproductive trade-offs.

The Effects of Diet and Acute Heat-Stress on Digestive Enzyme Activity in Mussels

Kwasi Connor, Beck Wehrle, Daniel Rankins, Jonathan Lopez, Diana Nieves

The mussel *Mytilus californianus* aggregates to form reefs within intertidal zones along the western shores of North America. Intertidal mussels throughout the world are under physiological stress due to elevated air and water temperatures resulting from Global Climate Change. Therefore, identifying physiological functions that are modulated by heat is critical for predicting how they cope with environmental change. A recent lab study suggests that the digestive system in mussels is negatively affected by cycles of low-tide heat. In the current study we asked whether an acute heat shock modulates the activity of the carbohydrases in mussels acclimated across a spectrum of diets. Results showed differences in specific activity of amylase and cellulase across diets in line with the nutrient balancing hypothesis. Acute aerial heat-shock did not have an effect on digestive enzyme activity but there was a slight effect of tide. Remarkably, starved individuals retained large digestive glands suggestive of higher potential for digestion. These results allow for a more critical assessment of the effects of marine heatwaves on nearshore fauna of the north Pacific coasts.

T3 hormone does not upregulate beta-1 adrenergic receptors or increase heart rate in chick embryos.

Molly Connor, Zoe Butler, Thomas Pirtle

Triiodothyronine (T3) is a lipid-soluble hormone that binds to nuclear receptors to promote transcription and therefore upregulates proteins in target cells. We hypothesized that increasing T3 levels (by administering T3) or decreasing T3 levels (by administering methimazole, an inhibitor of T3 synthesis) will, respectively, increase/decrease heart rate by respectively upregulating/down-regulating beta-1 adrenergic receptor. We administered control saline (chick Ringer's + 1% DMSO), T3 saline (T3 in chick Ringer's + 1% DMSO), and methimazole (methimazole chick Ringer's + 1% DMSO) to the air sac. Previously, our lab had tested this hypothesis pharmacologically, in isolated superfused chick embryonic hearts exposed to isoproterenol (10⁻⁹ to 10⁻⁴mM), which showed no significant change in heart rate at any of the test concentrations. Here we repeated these experiments with norepinephrine. Additionally, replication of electrocardiogram recordings, heart morphometrics, and superfused heart experiments performed on control chick embryos, the positive treatment T3 chick embryos, and the negative treatment methimazole chick embryos do not support the hypothesis that T3 upregulates beta-1 adrenergic receptors during embryonic heart development.

Scaling of Metabolic Rate and Tongue Projection Performance in Chameleons

Etti Cooper, Madison McIntyre, Krystal Tolley, Christopher Anderson

Among the best-known features of chameleons (Squamata: Chamaeleonidae) is their tongue projection feeding mechanism. Across their significant variation in body size, the maximal distance at which chameleons can project their tongue has a size-dependent component: smaller chameleons can project their tongues relatively farther than can large chameleons, up to 2.5 body lengths in small species. This scaling relationship has been hypothesized to be explained by mass-specific metabolic rates decreasing as body size increases. If smaller chameleons have a relatively higher caloric requirement due to this high mass-specific metabolic rate, an increased tongue projection distance would increase the relative area over which they are able to capture prey. We used stop-flow respirometry to measure metabolic rate and high-speed video to quantify tongue projection distance in five chameleon taxa to test whether mass-specific metabolic rate and projection distance decrease at a similar rate as body size increases. As expected, we found that relative tongue projection distance decreased as body size increased. Further, we found that absolute metabolic rate increases with a scaling factor of 0.87, which is consistent with other reports of metabolic scaling in squamates. Conversely, no significant relationship between mass-specific metabolic rate

and body size was observed. These data suggest that observed scaling patterns in tongue projection distance in chameleons may be explained by factors other than scaling patterns of mass-specific metabolic rate.

Compositional differences in the cerebellum of beluga and bowhead whales

Lisa Cooper, Matthew Smith, J.G.M. Thewissen

Within mammals, echolocating whales (e.g., belugas and dolphins) are noted for having an exceptional brain size relative to body size, including the cerebellum. It is unknown how the cellular composition of the brain differs in taxa with exceptional brain size, specifically in the total abundance of cells and the proportion of neurons to glial cells. This study utilizes the preserved brains of an echolocating beluga (*Delphinapterus leucas*) and a non-echolocating bowhead (*Balaena mysticetus*). Unlike belugas, the brains of bowheads are smaller, with a brain-to-body size ratio like that of terrestrial artiodactyls. We tested the hypothesis that the cerebellum of belugas contains a greater density of neurons and supportive glial cells than the bowhead. Subsamples of the cerebellum in both taxa were homogenized, stained to differentiate between nuclei of support cells (DAPI) and neurons (DAPI and anti-NeuN), and stained nuclei were counted. Results show the cerebellum of beluga whales has a greater cellular density, with greater counts of total cells and neurons. Combining these results with fossil data, we propose that the beluga lineage underwent several evolutionary events that increased brain and cranial cavity size and densities of glial and neuronal cells. Our ongoing work utilizes histology to test for differences in the composition of the inferior and superior colliculus in both taxa to test for critical differences in their sensory systems.

A Field Study on the Relationship Between Temperature and Behavior Patterns in *Oligocottus maculosus*

Sophia Cooper, Amy Cook

Organisms living in the intertidal zone face highly variable environmental conditions, of which many prove limiting factors to habitat suitability. One such organism, *Oligocottus maculosus* spends almost its entire life cycle occupying high and mid-range intertidal pools. The juveniles in particular are subject to large variations in temperature, spending much of their time in shallow pools exposed to full sunlight. To test the relationship between temperature and behavior, both focal animal samples and scan samples were utilized with an emphasis on behavior patterns associated with movement, feeding, and distribution within pools. Results indicate that although there may be a slight correlation between activity of juvenile fish and temperature, there is a greater likelihood that the behavior patterns observed were a result of an amalgamation of factors with one or more distinct characteristics responsible for alterations in activity levels at different temperatures. Of perhaps greater interest is the finding that behavior patterns may be more dependent on individual pool environments. Further investigation is required for more specific conclusions to be drawn and lab studies with a greater ability to manipulate environmental factors would be helpful in determining the individual biophysical characteristics contributing to the behavior patterns observed in this study.

Leveraging kinematic performance landscapes to model the macroevolution of feeding in reef fishes

Katherine Corn, Roi Holzman, Christopher Martin, T. Linscott, Josef Uyeda

A central goal of the evolutionary biology is understanding the processes that lead to the accumulation of phenotypic diversity. Biomechanists have an advantage for understanding this puzzle: the unavoidable physical and mechanical principles that govern organisms' movements. Yet how these physical principles directly affect the evolution of lineages through time remains unclear. A major innovation in the recent years is to use these biomechanical principles to estimate the topography of the performance landscape for a given task, such as building the performance landscape from hydrodynamic models of suction feeding strike. Here, we develop a model-fitting framework to estimate the evolutionary trajectory of lineages across performance landscapes, with reef fishes as a model system. We discretize the landscape to fit biogeography-style models estimating the history of lineages'

paths across the performance landscape, where transition rates between regions of the landscape are weighted by the performance gradient between them. This model-fitting framework provides a toolkit for asking a range of questions about the history of kinematic evolution and has wide applications for bridging macroevolutionary processes with the biomechanical principles that underly animals' every move.

Beyond thermogenesis: seasonal variation in response to reduced food in captive crossbills

Jamie Cornelius, Jessica Karr, Jalyn Devereaux, Ben Vernasco, Heather Watts

Temperate winters impose challenging conditions on resident songbirds, including shorter days and often lower temperature and food availability. Many studies investigating seasonal acclimatization have focused on thermal metabolic traits and these provide strong evidence for both preparative and responsive changes in thermogenic capacity to low winter temperature. However, a bird's ability to cope with seasonal extremes or unpredictable events is likely dependent on a combination of behavioral and physiological traits that attempt to restore allostatic balance. In addition to thermogenesis, such traits include the ability to gather information, seek shelter, find and assimilate nutrients and cope with reduced food intake. Here we compare results from a suite of integrative studies conducted in captive red crossbills (*Loxia curvirostra*) to investigate how response to reduced food availability varies seasonally. We find that crossbills cope better with time-restricted feedings in winter compared to summer and that this difference is only partially explained by differences in food intake. Social information about declining food improves the coping response to food restriction in both seasons and may be linked to differences in energy assimilation. Intestinal mass appears to be sensitive to both social cues and temperature. Our results highlight the importance of seasonal variability and a more integrative approach across physiological systems to investigate organismal responses to environmental challenges.

Protein and Histone PTM Alterations during Early Development in *L. tenuis* Due to Osmotic Stress

Meranda Corona, Dietmar Kueltz

Leuresthes tenuis experience a wide range of salinities in their early life stages. Due to the unique subterranean incubation of their eggs, it is not unlikely for this marine fish to hatch in non-seawater conditions, whereafter the larvae may transition to relatively diluted estuaries or concentrated harbors to develop as juveniles for several months. Consequently, they are an ideal system to study the impacts of ecologically relevant salinity stress on the alteration of proteins and histone post-translational modifications (PTMs) during early development. The study of the relationship between the critical window of development (CWD), in which sexual bipotential is lost, and the longevity of protein landscape and histone PTM changes has not been widely explored. Thus, hypo-and-hyperosmotic exposures of *L. tenuis* hatchlings during this window, and past it, attempt to identify if the CWD is relevant to the presence and persistence of protein and histone PTM changes under salinity stress. To elucidate this relationship, California grunion larvae were exposed in replicate to one of three salinity treatments (16ppt, 32ppt, 40ppt) for the duration of their CWD (68dph). Chronic post-CWD exposures and recovery times until 98dph were used to determine whether the CWD or prolonged salinity exposures were key in such alterations. After each timepoint, *L. tenuis* were culled and processed for histone PTM enrichment and proteomic analysis. Funding provided by NSF IOS-2209383 and NSF GRFP.

Cumulative Effects Of Multiple Stressors On Marine Mammals— Elephant Seals As A Model System

Dan Costa, Rachel Holser, Birgitte McDonald, Sarah Peterson, Joshua Ackerman, Dan Crocker

We examined the effects of multiple stressors on vital rates (survival and reproductive output) and health metrics (e.g., energy stores, allostatic load, stress hormones, immune status, contaminant burden) in female elephant

seals after exposure to combinations of acoustic disturbance, mercury load, increased cortisol (stress hormone), and body condition. We found a significant relationship between energy gain over a foraging trip (foraging success) and circulating blood cortisol level (GLMM $p < 0.001$). Females with very poor energy gain had cortisol levels as high as 20 $\mu\text{g dl}^{-1}$, a value 20 times normal levels. These females had a lower probability of pupping the following season. There was an interaction between muscle mercury and cortisol levels that affected circulating T3 levels additively ($p < 0.02$). This contrasts with the synergistic interaction between cortisol and muscle mercury on T4 ($p=0.006$) and immune function (IgE). Cortisol and mercury affect T4 and IgE. We induced endogenous cortisol and aldosterone secretion in 24 adult females by administering an intramuscular injection of ACTH., which lasted more than 24 hr. There was a strong negative relationship between the magnitude of the stress response and total adipose mass - animals with greater adipose stores showed a reduced stress response ($p = 0.006$ R^2 0.3). The lactation duration of females with elevated cortisol levels was longer (28.9 ± 1.95 days vs. 26.0 ± 2.11 $p = 0.0009$).

The targeted role of the proboscis and abdomen in mid-air oviposition by *Sa. chloropterus* mosquitoes

Nicola Costello-Zaragoza, Robert Hancock, Jonathan Dyhr

The diurnal mosquito *Sabethes chloropterus* disperses eggs via a mid-air oviposition strategy by catapulting individual eggs from the abdomen into small, water-containing holes. Gravid *Sa. chloropterus* female can precisely target the launch trajectory of eggs to oviposit in both vertically and horizontally oriented openings as small as 0.6 cm and from distances of up to 2 cm. This study aimed to identify sensorimotor targeting strategies allowing for this precision by tracking body and limb kinematics during oviposition. We video recorded *Sa. chloropterus* ovipositing in simulated tree holes at 1000 frames/second and tracked the proboscis, thorax, abdomen, and egg. Holes varied in size and were vertical, horizontal, or inverted facing. Our data suggests that *Sa. chloropterus* use the proboscis to target the launch vector of the egg. Prior to oviposition, the mosquitoes hover in place with the proboscis pointed at the hole. After ejection of the egg, the proboscis and abdomen are aligned parallel to each other. The angle of the egg launch vector relative to the final abdomen/proboscis vector was precise, with an average angle of $27^\circ \pm 6^\circ$. The angle of the launch vector relative to the pre-launch proboscis vector was more variable, with an average angle of $13^\circ \pm 13^\circ$. The consistent relationship between abdomen, proboscis and launch angle suggests egg targeting is dependent on the initial vector of the proboscis.

Empirical Evaluation of Wing Hinge Mechanics in Bumble Bees

Braden Cote, Mark Jankauski

In most flying insects, the flight muscles attach to the interior walls of their thorax rather than the wing base. Contraction of these muscles deform the thorax, and that deformation is transformed into wing rotation via a transmission mechanism called the wing hinge. However, the influence of the wing hinge on system dynamics is not well understood. Anatomically, the wing hinge is a compact region containing multiple steering muscles and tendons making it challenging to perform direct experimental measurements. We designed an experiment to simultaneously measure thorax deformation and wing rotation of bumblebees in tethered flight, allowing us to empirically evaluate the influence of the wing hinge on the thorax-wing system. Two trials were conducted, a control and a treatment where the mass moment of inertia of the wings was changed by trimming or weighing them. Our results show that clipping the wings has no effect on thorax deformation or wing rotation amplitude. Weighing the wings does not affect thorax deformation but leads to an increase in wing rotation amplitude by 7 degrees. Clipping the wings causes them to lead the thorax in phase by 15-17 degrees and weighing them causes the wings to lag the thorax by 6-7 degrees. These results suggest that the wing hinge is a compliant mechanism which can mitigate external influences propagating back to the thorax through the wing.

Many functionally connected loci underlie a hybrid origin of novel butterfly warning colors

Brian Counterman, James Ogilvie, Riccardo Papa

The role of hybridization in generating evolutionary novelty has become well-established, yet the genetic processes involved remain largely unresolved. For example, in *Heliconius* butterflies that are renowned for their diversity of brightly colored warning colorations on their wings, hybridization is rampant and often hypothesized as the source for much of the color pattern diversity. Despite a well-resolved genetic architecture for *Heliconius* warning color variation, there is no clear example of hybridization generating a novel genotype and accompanying novel wing coloration. Here, we use a combination of CRISPR gene-editing, RNA-seq, ATAC-seq, ChIP-seq, Hi-C, and population genomic datasets to test the prediction that hybridization followed by strong natural selection has generated a co-adapted complex of loci, referred to as “adaptive hubs”, that are responsible for the origin of a novel warning coloration in *Heliconius erato amalfreda*. We identified a handful of these adaptive hubs in *H. e. amalfreda*, which include *optix*, a gene known to regulate butterfly wing color pattern development, as well as several downstream targets that appear to be regulated by *optix*. Our findings suggest that allelic changes at the adaptive hubs are the result of hybridization and selection and that they are collectively responsible for generating the novel warning color pattern of *H. e. amalfreda*.

Developmental food restriction has long-term consequences on adult reproductive success

Victoria Coutts, Kayci Messerly, Haruka Wada

The developmental environment is crucial for shaping adult phenotype through modifying the morphology and physiology of an individual. It is hypothesized that this developmental plasticity is adaptive, yet few developmental stress studies have investigated consequences on fitness metrics, especially under nutritional stress. In this study, we exposed zebra finches (*Taeniopygia castanotis*) to either a control (ad libitum) diet or 40% restricted diet and measured development of sexually selected traits and reproductive success. Pictures of birds were taken at different points throughout development and early adulthood to analyze the development of beak color in both males and females and cheek patches in males. Females were also allowed to breed with nonexperimental males and we analyzed their clutch size, hatching success, fledging success, and brood mass. Food-restricted birds had significantly less red beak hue, but only on one side. Food restriction significantly delayed development of male cheek patches, and cheek patch color was less saturated compared to controls. Further, females previously exposed to a food-restricted diet had significantly lower fledging success and brood mass, but similar clutch size and hatching success compared to control females. These data imply that developmental stress poses long-term consequences and highlight the importance of measuring fitness-related metrics later in life to show either detriment or benefit to a developmental stressor.

Food restriction during breeding has implications for telomere dynamics in zebra finches

Victoria Coutts, Hannah Butterfield, Tonia Schwartz, Haruka Wada

Dietary restriction has many health implications including increasing longevity in some species. Telomeres, repeated regions at the end of chromosomes that shorten over time, are a common biomarker of senescence. Even though both diet restriction and breeding can alter energy demand, there is a lack of knowledge regarding whether diet restriction during breeding alters telomere lengths. We exposed adult zebra finches (*Taeniopygia castanotis*) to either an ad libitum diet or 40% food-restricted diet treatments during breeding. Blood samples were collected from parents at three timepoints, the time of pairing (before diet treatment began), when their offspring fledged, and when their offspring were nutritionally independent. DNA from red blood cells were used in a qPCR assay to determine telomere length and telomere rate of shortening across timepoints. The ad lib diet treatment group did not significantly alter telomere length between the pairing and fledging periods, but the food-restricted group significantly increased telomere length between the same time points. Despite these results, there was no significant difference between

treatment groups in rate of telomere shortening. Although typical breeding does not appear to alter telomere length during the most stressful part of the breeding period, food restriction during breeding appears to have a protective effect, highlighting the need to understand the complex role of diet during periods of high energetic demand.

The shared structure and biochemistry of extreme dormancy in divergent crustacean zooplankton

Joseph Covi

Both coastal and inland zooplankton produce dormant embryos that survive decades to centuries in anoxic environments. Until recently, it was unclear if divergent species used similar mechanisms to achieve this incredible physiological feat. Almost all research on the physiology of embryonic dormancy in the crustacea was conducted using the brine shrimp, *Artemia franciscana*, because it was the only commercially available species. Recent data on the ultrastructure and biochemistry of dormancy in the Antarctic freshwater copepod, *Boeckella poppei* provide a unique opportunity for the comparative examination of embryonic dormancy. Embryonic development in *B. poppei* appears to continue under very low oxygen tension, but arrests under anoxia while the embryos are still partially syncytial. This partially syncytial embryonic state is similar to that of brine shrimp embryos. Both *A. franciscana* and *B. poppei* experience intracellular acidification under anoxia. Levels of nucleoside triphosphates (NTPs), which includes adenosine triphosphate (ATP), also decrease dramatically under anoxia in both species. When oxygen is returned, intracellular pH and NTP levels are restored. This large reversible acidification and depletion of ATP would cause mortality in most eukaryotic species, and may be unique to crustacean zooplankton. These data provide the first comparative evidence that anoxia-induced dormancy in crustacean zooplankton is associated with intracellular acidification and an ability to recover from the depletion of NTPs when cell membranes are incomplete.

Temperature-dependence of regional heterothermy in a diminutive snake

Christian Cox, Albert Chung, Aaron Bindrim, Georgia Davidson, Sarah Dean, Katherine Haines, Alexander Heise, Elana Mauer, Katrina Pfennig, Ethan Sorrell, David Tepper, Charlie van-den-Oord, Michael Logan

Regional heterothermy, which occurs when there is variation in temperature among body regions, is relatively poorly understood in ectotherms. In particular, the drivers of regional heterothermy in small ectotherms remains mysterious as these animals should quickly equilibrate to environmental temperature. We sought to understand the effects of environmental temperature on regional heterothermy in the ringneck snake (*Diadophis punctatus*). Despite the small size of ringneck snakes, our previous research has documented large differences between head (measured in the mouth) and posterior (measured in the cloaca) temperatures in this species. Yet, how and why regional heterothermy might vary with environmental temperature is unknown. We measured both head and cloacal temperatures at three different environmental temperatures that spanned what ringneck snakes typically experience in the field (20, 25, and 30 °C). We also measured regional heterothermy at environmental temperatures close to the critical thermal minimum (15 °C) and voluntary thermal maximum (32 °C) for this species. We found that the difference between head and cloacal temperatures decreased with increasing environmental temperature and that cloacal temperature changed more with increasing environmental temperature than did head temperature. Our findings are consistent with regional heterothermy being driven by different materials properties between the cloaca and head or with active regulation of head temperature compared to cloacal temperature.

Biogeographic and evolutionary trends of feeding specializations in butterflyfishes (Chaetodontidae)

Adera Craig, Chloe Nash, Mark Westneat

The fish family Chaetodontidae, comprising 136 coral reef-dwelling butterflyfish species, is widely distributed across the Indo-Pacific, Western Pacific, Atlantic, and Indian Ocean. These fishes exhibit remarkable dietary diversity,

encompassing obligate and facultative corallivory, non-coral invertebrates, and zooplankton consumption. Advances in molecular phylogenetics and dietary analysis have significantly enhanced our understanding of their evolutionary ecology and feeding behaviors. In this study, we sought to analyze the evolutionary and biogeographic relationship across species regarding the feeding habits among the Chaetodontidae. Dietary data were sourced from previous literature, primarily relying on gut content analysis and supplemented by observational data. The acquired data facilitated the classification of distinct ecotypes. Here we present a new time-calibrated phylogenetic hypothesis for the family, using a multi-locus dataset of 132 species and a rich outgroup framework. Species distribution data were collected and subsequently integrated into geographic information system software to model the present-day range of Chaetodontidae species. Leveraging the identified ecotypes, the constructed phylogeny, and biogeographic insights, the research unveiled discernible patterns of evolutionary diversification and feeding ecology across the family's diverse species, revealing the intricate interplay between evolutionary history, biogeography, and dietary preferences in the butterflyfishes. With mounting threats to reefs, the Chaetodontidae, and particularly its coral-ivorous members, emerge as a set of potential indicator species, highlighting the urgency of conservation amidst escalating environmental pressures.

Immune response of *Pycnopodia helianthoides* to sea star wasting disease

Grace Crandall, Alyssa Gehman, Catherine Harvell, Steven Roberts

Sea star wasting disease has been impacting sea stars along the North American West Coast for nearly a decade, and the causative agent for this disease has not been identified. One of the hardest-hit species is the Sunflower Sea Star, *Pycnopodia helianthoides*, though it is unknown why this species is particularly vulnerable. A means to assess vulnerability and potential candidates for causative agents is to fully characterize their immune response. Using transcriptomics, healthy and disease-exposed *Pycnopodia helianthoides* were compared to identify differentially expressed genes. These genes were then characterized, and many associated with immune response were identified as being more highly expressed in stars that were exposed to disease and exhibited disease signs. Additionally, a multi-species experiment was performed using *Pycnopodia helianthoides* - the most vulnerable species, *Pisaster ochraceus* - an intermediately vulnerable species, and *Dermasterias imbricata* - a supposed resistant species, to compare disease sign progression when exposed to the same source of disease and to compare immune response using transcriptomics across species and time. This work will identify genes associated with resistance, will aid in identifying populations for conservation efforts, and may help point towards possible causative agents.

Different ways for the same result: heat dissipation areas role in maintaining bird body temperature

Kristen Crandell, Bret Tobalske, Donald Powers

A primary role of plumage is thought to be regulation of body temperature, traditionally viewed as an insulator. In most climates, avian heat dissipation occurs passively via radiation, conduction, and convection due to the thermal gradient between the environment and the animal. The muscles that power flight also produce significant heat that must be dissipated. How plumage interacts with these mechanisms is unclear. We examined the role of plumage as an insulator, or dissipator, of heat in Eurasian collared doves (*Streptopelia risoria*, $n = 8$). We measured internal body temperature and skin temperature (under the plumage) using thermal PIT tags and external temperature using a thermal imaging camera. Birds experienced four thermal regimes: resting, post-flight, heating via radiative lamps, and cooling via wind. Internal body temperatures remained consistent across resting, flight, and heated treatments, with a slightly lower (< 0.5 °C) temperature under convective cooling. Skin temperature (sub-plumage) varied with similar but more pronounced pattern within ± 1 °C. Heat dissipation occurred in local 'heat dissipation areas' (HDAs) which did not differ in maximum temperature changed in overall area. Under warm conditions, birds increased HDA surface area and added additional HDAs - most notably the shoulder and muscular wing, for heat shedding. Under cool

conditions, fewer HDAs were employed (most pronounced being the loss of the face and beak). We conclude that heat dissipation, or heat retention, is actively modulated by skin surface temperature in localized areas.

Stop, Chomp, and Roll: Rotational feeding behavior in marine sculpins

Callie Crawford, Shubham Vijay Kumar Yadav, Jonathan Huie, Emily Kane

In summer 2022 at the University of Washington Friday Harbor Laboratories on San Juan Island, Washington, we recorded 4 species of marine sculpin, *Oligocottus maculosus*, *Leptocottus armatus*, *Clinocottus globiceps*, and *Clinocottus embryum*, performing rotational feeding behavior in which the fish rapidly rotates about the body axis in order to break off a manageable piece of a prey item. These recordings were made while filming prey capture trials as part of another study. Although these behaviors have been observed in distantly related groups of fishes, these are the first filmed recordings of stout-bodied sculpin species performing rotational feeding behaviors consistently in a laboratory setting. The species observed in the study primarily prey upon small marine invertebrates and fish larvae which they bite or scrape off the substrate, or suction feed from the water column near the substrate. When offered large prey items, they are unable to obtain the food in their quotidian manner and instead bite off small pieces or perform the rotational behavior to assist in tearing off a manageable piece. We find that rotational feeding behaviors are readily employed in the lab by multiple sculpin species when offered prey items larger than their typical prey types. This finding suggests that rotational feeding may be an important but overlooked feeding mode in sculpins.

Using fecal glucocorticoid metabolites to assess the impacts of mining contamination on songbirds

Bridger Creel, Megan Fylling, Benjamin Colman, Creagh Breuner

Mining contamination (MC) is a global ecological disturbance with a substantial impact on riparian ecosystems that harbor diverse breeding songbird populations. This study investigates MC as a potential driver of riparian songbird decline by investigating the impacts of MC on health and survival in the nestling stage. Nestlings are particularly vulnerable to disturbance because their growth is rapid and energetically demanding. In riparian habitats, growth is fueled by insects that act as vectors for contaminant transfer. We expect that nestling songbirds are affected both by metal exposure and by habitat degradation (including prey depletion) from MC, but few studies have disentangled the mechanisms that may decrease health and survival. Glucocorticoids (GCs) can mediate organismal responses to environmental and internal stressors and may provide a tool to assess the effects of MC stressors on nestling songbirds. We are measuring fecal GC metabolites (FGMs) from nestlings of 6 riparian species across 4 habitats with varying contamination/restoration levels. We will examine whether nestling FGMs 1) reflect MC presence, and 2) predict growth and fledging success. Evaluating nestling FGMs, metal accumulation, and growth/fledging success within and across sites and species should provide robust insights into FGMs as indicators of MC's impact on songbirds. Initial findings reveal significant variation in nestling blood concentrations of lead, arsenic, and selenium across sites, exceeding negative-effect thresholds identified in prior studies.

Are vertebrates constrained to two sets of paired appendages? The evolution of prepelvic claspers

Karen Crow, Kayla Hall, Riley Jones

Holocephalans exhibit auxiliary appendages called pre-pelvic claspers (PPCs) that are located anterior to the pelvic fins, while pelvic claspers are pelvic fin modifications located posteriorly as modified metapterygia. Articulation points of the PPCs have not previously been imaged or evaluated in a comparative context, therefore, they may represent modified pelvic fin structures if they articulate with the propterygium. Alternatively, they could represent the only example of an independent third set of paired appendages in an extant taxon, if they articulate independently from the pelvic fin basal cartilages, challenging the current paradigm that extant jawed vertebrates are constrained to two sets of paired appendages. We evaluated PPC developmental growth

rates, morphology, and articulation points in spotted ratfish (*Hydrolagus Colliciei*, Holocephali), and compared variation in among representatives of the three extant holocephalan families. Both, the pre-pelvic and pelvic claspers exhibit a dramatic surge in growth at sexual maturity, suggesting synchronous development via shared hormonal regulation and puberty. MicroCT scans revealed that PPCs are not modified propterygia, nor do they articulate with the propterygium. They articulate with the anterior pre-pelvic process on the anterior puboischiadic bar (or pelvic girdle), suggesting that while they are associated with the pelvic girdle, they may indeed represent a third, independent set of paired appendages in extant holocephalans.

Geographic Variation in Optimal Egg Size in *Chrysemys picta*

Sara Crow, Beth Reinke

Optimal egg size theory predicts that females should produce offspring of a size and number that maximize fitness. In many species, this means having a few, large offspring that will likely survive. However, for a species with no parental care, we should expect to see a tradeoff between the amount of eggs and the size of the eggs within a clutch. In some environments, it may be better to have many offspring so that some might survive, while in others it may be better to have fewer offspring that are larger and can better survive unfavorable conditions. Environmental conditions, such as the length of the growing season, vary by latitude and may impact the optimal egg size for each population. To better understand the tradeoffs and geographic variation in optimal egg size we use two populations of *Chrysemys picta*, the painted turtle, at two different latitudes. We attempted to measure 30 clutches from each population, then compared the relationship between weight and number of eggs, standardized by female size. We expect to find that the further north site with a shorter growing season has fewer eggs that are larger than the site that's warmer with a longer growing season. Understanding how geographic variation affects optimal egg size will help us understand and predict changes in egg size in response to climate change. size theory predicts that females should produce offspring of a size and number that maximize fitness. In many species, this means having a few, large offspring that will likely survive. However, for a species with no parental care, we should expect to see a tradeoff between the amount of eggs and the size of the eggs within a clutch.

Modelling plasticity and the evolution of environmental sex determination in a changing world

Claudia Crowther, Lisa Schwanz

Species adaptation to environmental change involves co-occurring responses from many traits. Trait adaptation is influenced by changes in coevolving traits and phenotypic plasticity induced by the new environment. In species with temperature-dependent sex determination (TSD), the pivotal temperature (T_{piv}) and maternal nesting behaviour (N_b) have been identified as traits with the potential to evolve in response to rising temperatures that destabilise sex ratios. However, the impact of plasticity on the coevolution of these traits has not been explored. We used individual-based simulation modelling to ascertain the relative adaptive capacity of T_{piv} and N_b and determine how temperature-dependent embryonic survival and plasticity in maternal nesting behaviour influence the coevolution of these two traits. We found that T_{piv} evolved to ameliorate sex ratio bias more readily than N_b , though N_b played an important role in adaptation to extreme environments. Selection favoured increased evolution of N_b when embryonic survival depended on nest temperature. In contrast, the presence of behavioural plasticity to annual climate fluctuation reduced the relative adaptive capacity of N_b . Our research demonstrates the complexity of interactions that occur when species adapt to new environments and highlights the importance of plastic responses in shaping the course of evolution.

Effect of synthetic estrogen exposure on algal symbiosis and reproduction in the aggregating anemone

Lisa Crummett, Sebastian Castillo

Synthetic estrogen (17 α -ethinylestradiol) or "EE2" is a highly estrogenic endocrine disrupting chemical, and it is widely used in birth control pills. Globally, 47.5% to 71.5% of EE2 is removed from wastewater influent, on average,

depending on the treatment process. Incomplete EE2 removal from wastewater influent can result in effluent concentrations, and sometimes surface water concentrations, that are above the lowest observed effect concentration (LOEC) for EE2. Most studies that have examined the effect of endocrine disrupting chemicals on wildlife have focused on vertebrate species while far fewer have studied invertebrate species. We examined the effect of synthetic estrogen exposure on algal symbiosis and reproduction in the aggregating anemone, *Anthopleura elegantissima*. Anemones (64 individuals, 8 clonal groups) were collected from Point Fermin (San Pedro, CA) and placed into one of four EE2 treatment groups: control (0 ng/L), low (1.5 ng/L), medium (15 ng/L), and high (150 ng/L) EE2 concentration. Individuals experienced intermittent EE2 exposure over 4 weeks. Anemones were induced to spawn at the end of the EE2 exposure period. We present the effect of EE2 exposure on various dependent variables that we measured, including whether anemones spawned, gamete concentration after spawning, proportion that underwent longitudinal fission (asexual reproduction), symbiont density per mg of host tentacle protein, concentration of chlorophyll per symbiont cell, and photosynthetic efficiency as measured with a fluorometer.

Feeding efficiency of sunbirds and comparisons with other nectar-feeding birds

David Cuban, Yohanna Dalimunthe, Rauri Bowie, Steve Johnson, Alejandro Rico-Guevara

Sunbirds have evolved to feed on floral nectar and are distributed across a large geographical area from South Africa to Southeast Asia. Our recent work has revealed that the feeding mechanism used by sunbirds is consistent amongst members of the family, however, the feeding efficiency (caloric intake rate) has not been investigated. For 7 species, we collected feeding performance and morphological data across 2 global regions to investigate their feeding efficiency relative to species identity, morphology, and location. We offered individual birds multiple nectar concentrations (10, 20, 30, 40, and 50 percent sucrose solutions) in randomized order and recorded them feeding from artificial corollas with high speed cameras. We compare the results with predictions from mathematical feeding models and those of a distantly related, but ecologically convergent group of nectar-feeding birds: the hummingbirds. This study reveals how convergently evolved groups have adapted to their local environments and differential selective pressures and sets the groundwork for further investigation into the bird-flower interaction at the caloric level.

Evaluation Of Snow Cover Differences In Eastern Sierra Nevada 2018-2023

Giselle Cuevas, Nathan Rank, Elizabeth Dahlhoff

Montane environments experience high variability in temperature and precipitation within and among years. In California, this leads to high variation in snow pack, which affects organisms living at high altitudes, including the willow leaf beetle (*Chrysomela aeneicollis*), which occurs from 2800-3400 m in the Sierra Nevada range. Prior studies reveal that snowy winters impose different selective pressures than dry winters. During dry winters, overwintering beetles may be exposed to lethally cold temperatures, and during snowy winters, beetles must survive prolonged intervals without food under snow. I studied length of snow cover for sites where beetle populations have been monitored for 24 years, focusing on the exceptionally snowy winter of 2022-2023. I used three snow depth loggers from the California Data Exchange to estimate snow depth and 27 data loggers placed at the soil surface to estimate length of snow cover. There was a strong correlation ($r^2 = 0.79$, $n = 14$) between snow depth and snow cover length. During the 2023 water year, average duration under snow (210 days) was 33% greater than the mean for the past five years. Previous research has demonstrated that beetle populations grow one year after a snowy winter. We expect that in 2024 populations will grow after years of decline during the recent California drought.

The silk road to skeleton shrimp and whale lice

Brittany Cummings

Corophioid amphipods are poorly known crustacean taxa in marine epifaunal and biofouling communities. Unlike many mobile amphipods, corophioids

possess clinging body forms which enable bizarre anti-predator adaptations. For example, neon sea fleas use their colorful exoskeleton to purportedly mimic nudibranchs that are distasteful to fish; certain mast-building amphipods assemble their fecal rod nests off threatening sea urchin spines; skeleton shrimp adopt hyper-elongated body forms to masterfully blend into their branching habitat; and whale lice ride out their lives on giant whale hosts. Morphology suggests that silk glands ignited a cascade of key innovations that first led to tube-dwelling, then to diverse free-living lifestyles. However, there is no robust phylogenetic framework to reconstruct the history of trait acquisition across the corophioid clade *sensu lato*. I am using phylogenomics and broad sampling of numerous gene characters to develop a robust phylogeny of corophioid amphipods and assess the evolutionary dynamics of corophioid radiation. Fresh and preserved specimens from families across the breadth of corophioid phylogenetic tree space were collected from Pacific, Atlantic, Caribbean and Antarctic coastal regions. Nine transcriptomes were used to design baits for targeted DNA sequencing and a species phylogeny estimated using the concatenated maximum-likelihood method. The resulting phylogeny is used to reconstruct the evolution of key morphological traits. Implications of these results to amphipod systematics and ecology will be discussed.

Sheepshead Minnow do not adjust swimming activity in response to varying salinity

Rachel Cuomo, Nicolas Walker, Isaac Ligocki

Varying salinity in estuarine environments presents organisms with a physiological challenge. Particular taxa use diverse mechanisms to deal with changing salinity, we investigated whether an estuarine fish modifies its activity levels as a short-term response to acute changes in salinity. The Sheepshead Minnow, *Cyprinodon variegatus*, is a euryhaline fish that is abundant in the mid-Atlantic region. Fish were collected from Assateague Island, Virginia and transported to Millersville University where they were habituated to three different salinities within the range these fish regularly experience in the wild – 25 ppt, 30 ppt, or 35 ppt. Once habituated for 14 days, fish underwent a series of swim performance tests in which they could actively swim against the current or rest on the bottom of the swim tunnel. We predicted that fish would spend less time actively swimming when exposed to salinities different than that which they were habituated to. Fish were first tested in the salinity they were habituated to, then again in each of the other two salinities. Over the course of each one hour trial, the time each fish spent actively swimming was recorded. Contrary to our predictions, experimental fish did not differ in the time spent swimming depending on 1) what salinity they were habituated to or 2) whether the trial took place in a different salinity than that they were habituated to.

Global change in the sensory landscape: Color signal evolution across changing light environments

John David Curlis, Karla Alujevic, Leah Bakewell, Sasha Bishop, Brian Bock, Albert Chung, Elissa Connolly-Randazzo, Guillermo Garcia-Costoya, Hayley Crowell, Akhila Gopal, Noah Gripshover, Molly Hirst, Jillian Myers, Daniel Nicholson, Renata Pirani, Noa Ratia, Daniel Romero, Brett Seymoure, Jessica Stapley, Claire Williams, Perry Wood, Kelly Wuthrich, Christian Cox, Michael Logan, W. Owen McMillan, Alison Davis Rabosky

Studies of the effects of changing environments on phenotypic evolution are often limited to testing responses to climate. However, changes in habitat structure and their associated light environments can also have substantial impacts on phenotypic evolution, especially for organisms that use coloration for signaling. According to the sensory drive hypothesis, selection should favor signaling traits with colors that are most easily perceived in a given environment, yet such environments are changing rapidly due to anthropogenic activities. We combined a study of the historical and current spatial distribution of polymorphic lizards along a natural light gradient with a field translocation experiment to test the effects of varying light environments on the evolution of a colorful signal. Using the slender anole (*Anolis apletophallus*), which has two distinct dewlap color morphs, we tested the relationship between morph frequencies and the light environment over forty years in a mainland population. We also introduced hundreds of individuals to islands in the Panama

Canal that varied in canopy structure and light levels and tracked changes in dewlap coloration over multiple generations. Both studies strongly supported the sensory drive hypothesis; dewlap morph frequencies were associated with light variables on the mainland, and dewlap color changed predictably across generations on islands. These results have broad implications for how color may evolve under changing light environments, especially as humans continue to change habitat structure at unprecedented scales.

The ups and downs of an arboreal lifestyle: the impact of variable inclines on the kinematics of cha

Michael Curran, Christopher Anderson

An arboreal lifestyle presents numerous challenges to the variety of organisms living in these habitats, including navigating narrow perches, crossing open gaps and variable substrate inclines. Chameleons have evolved a plethora of specializations to cope with challenges faced by arboreal organisms, including pincer-like feet, and prehensile tails. Locomotion in chameleon has been well studied, with most research examining locomotor patterns at inclines of 0°, 45°, and 90° in the veiled chameleon (*Chamaeleo calytratus*). The impact of declines on their kinematics has received less focus in the literature. We performed locomotor trials with adult *C. calytratus* and *Trioceros j. jacksonii* walking on a meter long, 0.5 inch diameter wooden dowel positioned at a 45° incline, level (0°) orientation, and 45° decline. We quantified kinematic and performance variables, including velocity, joint angles of forelimb and hindlimbs, stride length, stride frequency, and limb extension, from biplanar high-speed video of locomotor trials for each species. Our results provide insight into how locomotor patterns shift during inclined, declined and level movement in an arboreal habitat. Further, these results can help illustrate the generality of locomotor patterns of navigating narrow perches across inclines in tetrapods.

E-PG neurons are needed to balance spontaneous saccades in *Drosophila*

John Currea, Sarah Fatkin, Mark Frye, Giovanni Frighetto

Many animals have an internal compass that integrates multisensory information to detect their orientation in space. In insects, the ellipsoid body of the central complex forms a ring attractor network with activation propagating around the ring according to the azimuthal rotation of a visual scene. In *Drosophila*, E-PG neurons needed to update this network can be inhibited genetically, eliminating idiosyncratic celestial cue orientation. In addition to distance navigation, flies explore their local environment with body saccades to orient toward vertical objects like plant stalks. Celestial navigation and object detecting algorithms must interact if a fly is to maneuver through its local environment while maintaining bearing toward a distant goal. Here we explore the behavioral consequences of silencing E-PGs on this balance using a virtual reality flight simulator. We find that in the presence of a natural scene, E-PG-silenced flies execute spontaneous saccades like controls, but they saccade left more often than right, reverse saccade direction less often, and therefore encircle a territory rather than migrate over distance. We find that visually-evoked saccades are less affected by E-PG silencing, suggesting that the compass does not participate in object-evoked saccades. Overall, the heading compass appears to balance the direction of object saccades, thereby compensating for rapid short-range maneuvers in order to maintain a distant goal.

Inflation: Anatomy and function of expansive paranasal chambers and nasal fossa in sac-winged bats

Abigail Curtis, Tim Smith, Sharlene Santana, Thomas Eiting, Nancy Simmons

Several groups of bats have prominent, bony inflations on the dorsal surfaces of their rostrum that contain paranasal chambers. Rostral inflations are commonly found in bats that emit echolocation calls nasally, and their size correlates with call frequency. Turbinal bones that function in olfaction and prevent respiratory heat and water loss are greatly reduced or modified in these bats. Similar rostral inflations are found in sac-winged bats (Family Emballonuridae), which

echolocate orally, but their function and relationship with surrounding structures within the nasal chamber are poorly understood. We used microCT, diceCT, and histology within a phylogenetic context to describe and quantify paranasal chamber, nasal chamber, and turbinal morphology in 43 emballonurid species. Results show emballonurid paranasal chambers are air-filled spaces lined with thin mucosae, with up to three paranasal chambers in an individual. Nasal chamber size and turbinal surface area are correlated with skull size. Total paranasal chamber volume is not correlated with skull size, nasal chamber size, or turbinal surface area, suggesting paranasal chambers are not significantly impacting nasal chamber or turbinal size. However, a posterior chamber formed by a nasal septal recess of Neotropical emballonurids likely impacts airflow. Additionally, we observed similarities in turbinal morphology between emballonurids and nasal echolocating species that have rostral inflations. Thus, rostral inflations and internal rostral morphology of emballonurid bats may also function in echolocation or other vocalizations.

Pleiotropic Roles of Leptin Signaling in *Xenopus* Tail Tip Regeneration

Grace Curtis, Robyn Reeve, Erica Crespi

Leptin, a hormone known for modulating appetite and metabolism, increases immune function and the rate of wound closure in adult mammals and amphibians. Recently, our lab showed that leptin administration increases regeneration rate in *Xenopus laevis* larval limbs and tail tips. We also showed that food restriction reduces expression of leptin protein in the tail and regeneration rate, and leptin administration via both intraperitoneal injection and implantation of a leptin-secreting bead at the amputation plane rescue regenerative ability in food-restricted larvae. To explore which molecular pathways are stimulated by leptin signaling during regeneration, we conducted RNASeq analysis of regenerating tail tips implanted with a saline- or leptin-soaked bead at 6- and 24-hours post-amputation (hpa). At 6 hpa, local leptin up-regulates genes associated with inflammation, cell proliferation, and cell migration, while down-regulating genes associated with pathogen response, apoptosis, and tumor suppression. These profiles are consistent with the increased closure of wound epithelia and reduced necrosis tail fin tissue after amputation. At 24 hpa, leptin up-regulates the angiogenic genes *klf2* and *egr1*. When we tested whether leptin increases angiogenesis, we found that leptin injection accelerates blood vessel growth into the blastema and regenerated ventral tail fin. Together, this work shows that leptin signaling has pleiotropic roles throughout tail tip regeneration, and supports a role for leptin as a nutritional modulator of regeneration.

Methane-Fueled Bacteria Power Invertebrate Life in the Deep Sea

Bianca Dal-Bó, Shana Goffredi

The list of animals known to harness methane as an energy source is limited, but growing. At deep-sea methane seeps off of southern California, we discovered 2 species of limpet (genus *Pyropelta*), a new species of sponge (genus aff. *Hymedesmia*), and a new species of sea spider (genus *Sericosura*) forming relationships with bacteria that utilize methane emerging from the seafloor. Based on molecular analysis, methane-oxidizing (MOX) bacteria comprised a substantial part of the hosts' associated microbiomes (23 to 45%). A nutritional influence by these bacteria was supported by the negative tissue ¹³C-carbon isotope values for all three genera (-43 to -48‰) and the uptake of ¹³C-labeled methane in shipboard experiments. Despite living in the same location, the animals hosted unique MOX bacteria, suggesting specialization of host-symbiont pairs. Separate analyses of male sea spiders and their egg clutches revealed a consistent microbial community, supporting the possibility of vertical transmission of bacteria. Of these invertebrate hosts, only sponges are so far known to host MOX bacteria, thus the discovery of methane-based nutrition in two additional animal groups contributes to our understanding of the sphere of influence of methane in these habitats, which are important methane sinks and biodiversity hotspots in the deep ocean.

Acute stress effects on Igf1 and metabolic pathways in olive rockfish

Zoey Dale, Henry Marden, Janae Shew, Hayley Mapes, Sean Lema

Physiological responses to stressors commonly include elevations in glucocorticoid hormones. Those increased glucocorticoids alter energy allocation and utilization through effects on glycolysis and gluconeogenesis. As part of that change, energy is typically shifted away from somatic growth. However, the specific mechanisms whereby elevations in glucocorticoids affect growth are not fully understood. Here, we examined effects of handling stress on glycemic status and endocrine growth pathways in juvenile olive rockfish (*Sebastes serranoides*). Olive rockfish were netted to create an acute 'handling stressor,' and a blood sample was rapidly taken for a 'baseline' (0 hour) time sample. Handled fish were then returned to their holding tanks for 2 hours, after which time fish were recaptured and liver tissue and blood collected. Fish exposed to the 'handling stressor' showed increases in plasma cortisol and glucose from the 0 hour baseline to the 2 hour 'post-stressor' sampling period. Accompanying those changes, liver mRNAs for insulin-like growth factor binding proteins (Igfbp)-1a and -1b were elevated at the 2 hour sampling time in handled fish. In fishes, type 1 Igfbps inhibit Igf1 action, and the upregulation of liver igfbp1a and igfbp1b gene expression suggests that increased type 1 Igfbp levels may act to inhibit growth following handling stress. These findings suggest some of the effects of glucocorticoids on growth may occur via Igfbp1 modulation of Igf1 availability.

The evolution of salp colony architectures and its consequences for multi-jet locomotion

Alejandro Damian-Serrano, Kaiden Walton, Anneliese Bishop-Perdue, Kelly Sutherland

Salps are urochordates that filter-feed on microbial production in the plankton in marine pelagic ecosystems. Salps form colonies of asexually-budded individuals that swim by multi-jet propulsion. Colonies develop into species-specific architectures with distinct zooid orientations. These architectures vary in frontal drag, thrust ratio, and locomotory efficiency. We (1) define the salp colony morphospace, (2) characterize the developmental pathways that build the different architectures, (3) assess their hydrodynamic consequences for locomotion, and (4) reconstruct their evolutionary history. First, we defined a universal comparative set of axes and planes based on the transversal double chain arrangement found in the early-developing stages of all colonies and defined adult zooid architectures as developmental transitions from this shared stage. Development shows that the morphospace is constrained to three transformation pathways, where all architectures are either final or intermediate stages towards bipinnate, cluster, or helical forms. To measure these architectures and their hydrodynamic properties, we collected and photographed specimens of adult and developing colonies via SCUBA diving, and measured the swimming speed of different species using in situ stereo-videography. To study the evolutionary history of these architectures, we inferred a new 18S gene phylogeny, reconstructed the ancestral states using models informed by developmental constraints, and identified categorical shifts in the evolutionary change of zooid orientations. We find that the ancestral salp architecture is most likely oblique or linear, with every other state being derived. Linear architectures are the fastest, most hydrodynamically efficient, and have evolved independently more often than any other architecture. Each of the three slowest-swimming architectures was derived at least once, suggesting that swimming speed is not strongly selected for across salps, and might be driven by ecological trade-offs with other traits.

Does the range of behavioral syndromes depend to the selection pressure present in an environment?

Mark Daniel, Jason Davis, Colby Quinn

Does the range of behavioral syndromes present in an environment correspond to the selection pressure experienced by the organisms living there? Do organisms living in an environment undergoing increased change exhibit a restricted range of syndromes relative to organisms living in more steady state or less highly pressured environments? To address these questions, we have developed a new integrated computer-based system for ecobehavioral

monitoring, interactive stimulus presentation, and data collection. To accomplish this, we custom-built a raspberry Pi integrated bird feeder/monitoring device around a Lego brick framework, capable of providing stimulus (in the form of an audio file) when birds landed to eat and of recording their behavioral response. Early testing demonstrated that general feeder microhabitat and location was strongly correlated to use; birds in remote areas did not visit feeders, however birds with experience feeding from anthropogenic sources adopted these feeders rapidly. Ongoing research focuses on developing a wider system of stimuli and recording on a larger scale from multiple environments and locales.

Muscle-tendon properties alter walking and running kinematics of pregnant and lactating rats

Nicole Danos, Adrien Arias

During pregnancy and lactation there is extensive remodeling of the muscle-tendon units that are responsible for walking and running. The Achilles tendon, which stores elastic potential energy during walking and running, becomes more compliant. The gastrocnemius, a muscle that contributes significantly to walking power, becomes 25% smaller during lactation. In addition to the 23% increase in body mass during pregnancy, these changes in muscle-tendon properties likely lead to significant changes to the walking and running gait of female mammals. In this study, we use high-speed videography on virgin, primiparous pregnant, and lactating rats (*Rattus norvegicus*) to quantify walking and running kinematics. We found that for all animals, higher velocity movements are powered by lower duty factors, as expected. However, for similar velocities pregnant animals use higher duty factors than virgin animals, while lactating animals use lower duty factors. Pregnant animals also employ asymmetrical gaits across a larger range of velocities. Additionally, we quantified leg compression, measured as the length from toe to base of the tail and found evidence of greater elastic potential energy usage in lactating animals. Together these data indicate that a) animal gaits differ depending on their pregnancy status, b) lactating and virgin animals have significantly different gaits, despite similar body masses, and c) these differences support the hypothesis that gaits will be more efficient during these metabolically demanding stages.

Interindividual Variation and Developmental Plasticity in Insect Flight Energetics

Charles-Antoine Darveau

Intraspecific variation in morphology and metabolic physiology associated with flight energetics is substantial in flying insects and represents the raw material for microevolutionary mechanisms to act on and give rise to macroevolutionary diversity among species. We conducted a series of studies on bee species to assess how variation in body mass and wing proportions relates to variation in metabolic rate during flight, but also how the metabolic phenotypes of the flight muscles vary with differences in metabolic rate. Flight metabolic rate variation can be explained by differences in flight kinematics, mainly wingbeat frequency, which in turn is a function of differences in wing size and proportions. These associations explain variations among individuals but also differences among morphologically distinct castes of bumblebees. We further investigated the role of developmental plasticity in coordinating the associations among these diverse phenotypes. Although we find sharp transitions in metabolic enzymes activity of the flight muscles in the first few days following adult emergence, experimental manipulating of wing size, body mass, or flight efforts has no impact on muscle metabolic phenotypes, indicating little plasticity at the adult stage. We are further exploring the role of environmental conditions in modulating flight properties during development. The coordinated development of phenotypes is likely an important window that sets adult phenotypes and ultimately leading to species differences in form and function.

Three-dimensional analysis of odor-guided orientation in freely flying hawkmoth, *Manduca sexta*

Shivansh Dave, Mark Willis

Moths, like many other animals, rely on olfaction to locate important resources like food, mates, and egg-laying sites. Nocturnal hawkmoths perform odor-tracking flight under quite low light conditions like moon-light and star-light to find the odor source, a robust behavior that can be reproduced reliably in the laboratory using a wind-tunnel with an attractive odor-source positioned upwind. Studying the flight trajectories, we can find the strategies flying moths use to track-down the source. Traditionally, this behavior in moths has been studied either using two-dimensional analysis (usually in horizontal plane) or just tracking their position. However, hawkmoths like *Manduca sexta* show a significant displacement both in horizontal and vertical plane while tracking odors. Also, studying their body orientation in three-dimensions along with the position, as done in this study, can help explain the complete behavior. We filmed the behavior and measured moths' body orientation in three-dimensions by tracking the moths' head and tail in two camera views. Since the odor-plume is invisible to us, we compared their trajectories to a three-dimensional plume profile that was measured using electroantennography (EAG) of an excised hawkmoth antenna. In this study, we have quantified how the three-dimensional track parameters, such as body orientation (yaw and pitch), flight velocities (ground and air) and angles (slip and track), change with respect to the plume profile to explain the overall behavior.

The evolution of reincarnation? - Acquisition of polymorphism in the chordate doliolids.

Bradley Davidson, C. J. Pickett, Joseph Ryan, Ipeknaz Icten

Although sequence variants associated with novel traits have been identified, it is difficult trace their impact on intervening scales including gene networks and cell lineages. We aim to address this question by studying doliolids, a highly divergent tunicate taxa. Doliolids are the only polymorphic chordate, transitioning through four distinct morphs specialized for locomotion, feeding, asexual or sexual reproduction. We have initiated genomic and developmental comparisons between *Doliioletta gegenbaui* and the primary tunicate model *Ciona robusta* to explore how alterations in doliolid signaling genes drove reallocation of embryonic cell lineages to generate novel organs. We are currently exploring this process by examining the emergence of additional doliolid muscle bands. Tunicate muscle band induction relies on FGF signaling. We have detected substantial alterations in *Doliioletta* FGF signaling genes including loss of the primary FGF-dependent transcription factor *Ets1/2* and loss of *Ets1/2* binding sites in *Doliioletta* regulatory elements. Loss of FGF-dependent induction and subsequent lineage reallocation serves as a working, testable model for acquisition of additional doliolid muscle bands. We have also begun to investigate the origins of novel structures associated with polymorphism. In particular, these efforts will focus on a derived appendage extending from the dorsal side of the primary locomotive morph. This appendage plays a central role in polymorphism as it contains three spatially distinct stem-cell lineages that gradually differentiate into feeding, asexual and sexual morphs.

Long-term population trends of the Piedras Blancas northern elephant seal breeding colony

Mackenzie Davidson, Heather Liwanag, Kathleen Curtis, Kate Riordan, Katie Saenger, Molly Murphy, Erin Schneider, Tess McIntyre, Paul Kessler, Elise Fiskum, Jenna Camargo, Avery Ancell, Brian Hatfield

Northern elephant seals (*Mirounga angustirostris*, NES) are an example of conservation success, having bounced back from near extinction to a total population of more than 200,000 individuals. The recolonization of rookeries (breeding sites) at different times presents a unique opportunity to analyze marine mammal population dynamics across their range and within individual rookeries. The Piedras Blancas (PB) rookery in San Simeon, CA was recolonized by NES in the early 1990s. The objectives of our study were to (1) determine whether the PB population is approaching carrying capacity (*k*), which is an indicator of the maximum population a habitat can support, and (2) explore how abundance of NES varies at each individual beach site of

the PB rookery across breeding seasons. Using published data from Lowry et al. (2014) and ground census data, we analyzed the total estimated NES population at PB and its seven established beaches during the breeding seasons from 1990-2023. We expected that the population numbers would begin to level off as the population approaches *k*, and we observed this in the years 2020-2022. This leveling out could be indicative of the PB NES colony reaching carrying capacity, or it could be a result of intense storms in the winter breeding season. Understanding the growth of this population and its movements will provide a better understanding of NES recovery and will help inform conservation and management efforts for this ecologically and culturally important species.

A TALE of how a segmented worm makes its nerve cord: *Capitella teleta* and cell fate specification

Johnny Davila-Sandoval, Javier Tabima, Neva Meyer

The origin of centralized nervous systems in animals has been debated as the amount of evidence in non-classical research organisms has increased. In vertebrates (Deuterostomia) and insects (Ecdysozoa), neuroectoderm is specified by a gradient of bone morphogenetic protein (BMP) signaling and activation of the MAPK cascade during dorsal-ventral axis formation. In the third major bilaterian clade, Spiralia, there is little evidence that antagonism of BMP signaling or the MAPK cascade are involved in neural specification. Our project aims to unveil mechanisms of neural specification in the spiralian annelid *Capitella teleta*. In this study and previously, we isolated blastomeres in *C. teleta* and found that micromere 2d, fated to form the ventral nerve cord (VNC), continues to form neural tissue in isolation. However, animal cap micromeres including 2d isolated at the 16-cell stage fail to form a VNC. This suggests that autonomous neural determinants and external signaling affect neural specification. To identify these factors, we are looking for differentially-expressed genes between whole and partial larvae in *C. teleta* via blastomere isolation and RNA-seq. In a pilot experiment, we detected several up- and down-regulated homeobox genes and other transcription factors, including Spiralia-specific TALE genes. For future directions, we will sequence additional blastomere isolations to understand neural specification in *C. teleta*, which should enable a better understanding of how neural mechanisms have evolved.

3D analysis of muscle microanatomy within the feeding apparatus of musteloids

Cassidy Davis, Edwin Dickinson, Madison Manzo, Aleksandra Ratkiewicz, Ashley Deutsch, Adam Hartstone-Rose

The superfamily Musteloidea boasts a significant amount of dietary, ecological, and body size diversity, spanning three orders of magnitude in body mass and encompassing dedicated hypercarnivores, frugivores, insectivores, and folivores. This dietary diversity has been linked to differences in masticatory anatomy: for example, musteloid species that frequently consume larger food items exhibit significantly longer muscle fascicles than other closely-related taxa. As recent advances in 3D anatomical visualization (e.g., diffusible iodine-based contrast-enhanced computed tomography; DiceCT) have facilitated a more detailed understanding of muscle microanatomy, musteloids represent a promising sample to further explore myological adaptations to dietary ecology. In this study, we employ DiceCT to conduct a detailed in situ analysis of the masticatory muscles in seven musteloid taxa, spanning the entire size range of the superfamily. Measured fascicle lengths corresponded closely to previously published gross dissection data, with digital dissection allowing the examination of fascicle orientation and tortuosity. As previously shown in primates, musteloid species showed high levels of variation in orientation between adjacent layers of the masseter, but low variation between temporalis layers. Folivorous species exhibited obliquely-oriented masseteric fascicles, compared to more vertical fibers in frugivores/generalists. Finally, we observed notably greater magnitudes of fascicle tortuosity within the jaw adductor musculature than in the jaw abductors. These insights highlight the value of DiceCT for the detailed study of muscle microarchitecture.

Insight into a Sexually Dimorphic Ostracod Crustacean by Mass Spectrometry and Microtomography

Cindy Davis, Ajna Rivera

Sexually dimorphic eye loss in females and eye maintenance in males of the ostracod *Euphilomedes carcharadonta* is likely driven by ecological selection. It has been observed that males migrate from the sand into the water column more frequently than females and stay for a significantly longer duration, displaying a multitude of swimming behaviors. This increased exposure to predators appears to be a selective pressure for males to maintain complex lateral eyes. The neurochemical pathways driving these behaviors are unknown. Ostracoda, basally branching arthropods, are not well characterized at the molecular level and little is known of their genetics and physiology. To close this gap of knowledge we have developed a technique to analyze ostracod tissue by mass spectrometry, establishing a preferable sample size and solvent for an in-solution tryptic digestion. We use shotgun mass spectrometry to characterize a proteome of the cephalon. Comparative analysis of the male and female proteomes may provide insight into potential pathways driving the sexually dimorphic behavior observed in these animals. This research can provide a starting point for proteomic analysis of related ostracods.

Mussel Poop and Climate Change: The Effects of *Mytilus trossolus* Biodeposition on Ocean Chemistry

Kai Davis, Aaron Ninokawa

Many marine bivalves are ecosystem engineers who promote species richness and diversity by providing habitat and shelter while also ameliorating environmental stressors. In addition to physically modifying the environment, these organisms can drive chemical changes via processes like respiration and calcification and the production of particulate organic matter such as feces and pseudofeces. These biodeposits can host microbial communities that perform their own metabolic processes (i.e., respiration) and have the potential to further alter seawater chemistry dynamics. Here we explore the role these biodeposits serve in driving seawater chemistry within mussel aggregations and how this role might change as seawater chemistry is altered due to ocean acidification. We collected 150 *Mytilus trossolus* mussels and exposed them to a range of ocean acidification (OA) scenarios, with six pH treatments ranging from 7.10 to 7.90, and 5 replicates per treatment with one control. We then conducted two 2-hour incubations to observe chemical changes (pH, dissolved oxygen) within the system, with one incubation with mussels and their biodeposits, and the other for mussels only. Biodeposition rates were independent of seawater pH and the biodeposit contributions to chemistry were minor. In-situ biodeposition dynamics are likely to be more complex than observed in this short-term experiment, necessitating further research into the development of biodeposit microbial communities and the chemical alterations that they drive.

Birds in the Cacao: Examining How Agroforests Provide Habitat for Tropical Birds

Rebecca Davis, Shawn McCracken

But I'm Not a Microscopist: How a Profession Became a Tool

Elizabeth Davis-Berg

The American Microscopical Society, founded in 1878, is one of the first formally organized American scientific societies and progenitor of the fourth longest continually published scientific journal in the United States. However, in recent times, many of us do not consider ourselves microscopists. As biologists, most of us use the tools of microscopy, whether it's light, fluorescence, SEM, microCT, Xromm, or a combination of these techniques, to visualize otherwise hidden systems. So how in today's world do we teach and train budding and established scientists on cutting-edge as well as relevant-but-forgotten skills in microscopy? How does one mount insect genitalia to the head of a pin for scanning? How can we best view our study organisms? From K-12 to college, students learn how to use a microscope as a way to see things that are small, but how do we connect these skills with their research? How do

we teach and document the techniques for subsequent generations? Come to a talk and discuss these ideas and possible ways that AMS and SICB can preserve, broaden, and deepen some of this knowledge.

Parallel requirements for paralogs of transformer during sex determination of the milkweed bug

Arvin Dayao, HaoWen Huang, Nischal Khatri, Lindsey Kim, Mallory Moratori, Dave Angelini

The fate of duplicate genes has important consequences for the evolution of developmental networks. Sex determination mechanisms vary among animals, but in general they share an architecture in which the expression of one gene in one presumptive sex leads to the development of associated sex-specific characters. Such sexual dimorphism may include differences in size and appearance between sexes. Among insects, the genetic mechanisms of sex determination are best understood in fruit flies where the expression of transformer (*tra*) regulates several downstream factors to affect female development. The milkweed bug *Oncopeltus fasciatus* presents a novel case in which transformer has been duplicated. The functions of *tra-a* and *tra-b* in this species remain unknown. We have investigated whether these genes act as master gene regulators of sex determination in the milkweed bug using RNA interference (RNAi) to reduce the expression of *tra-a* and *tra-b*. We quantified sex ratios and analyzed somatic sexual dimorphisms such as abdominal pigmentation, sternite curvature, and genitalia morphology following *tra-a* and *tra-b* knockdown. Research to date has indicated that *tra-a* and *tra-b* knockdowns both result in a significantly larger proportion of adult males. Additionally, *tra-a* and *tra-b* RNAi produced intersex phenotypes with both female and male sexually dimorphic features. These results demonstrate the conservation of *tra* in sex-determining mechanisms among insects. The parallel requirement for these paralogous genes also presents an interesting case of variation in the evolution of this developmental network.

The role of melanin in driving physiological responses to temperature in a terrestrial salamander

Bráulio de-Almeida-Assis, Eric Riddell

Understanding how animals respond to changing environments remain an important question in the era of human-induced global change. Predicted warming temperatures from climate change are expected to significantly constrain the fundamental niche of the most susceptible organisms, such as amphibians, due to their high susceptibility to desiccation and low thermal tolerances. Melanin can mitigate some of the effects of warm temperatures, including buffering reactive oxygen species and regulating skin permeability to water loss, but few studies have explored the physiological role of melanin in salamanders. Here we studied the role of melanin in driving physiological responses to warm temperature in an acclimation experiment on a terrestrial salamander (*Plethodon metcalfei*). We specifically evaluated the production of melanin in the liver between cool and warm temperature treatments and used RNA-seq data from the skin to evaluate whether any melanin-associated genes were correlated with acclimation in water loss physiology. We found a significant increase in the production of melanin in the liver in response to warm temperatures. We also discovered a significant correlation between acclimation of water loss rates and tyrosinase in the skin, the precursor of melanin synthesis. Melanin appears to have several functions in salamanders, such as a waterproofing skin. Future studies might integrate melanin expression into an ecophysiological framework to better understand amphibian niche constraints in the face of environmental change.

Sex-specific transcriptomics of seasonal adiposity gain and loss in the 13-lined ground squirrel

Cole Deal, Kathryn Wilsterman, Cory Williams

Physiological adaptations to periods of food abundance or scarcity are widespread amongst taxa, with extreme examples occurring in mammals that live in seasonal environments. Decades of prior research have demonstrated these adaptations rely on neuroendocrine signaling, but the underlying neurobiological mechanisms are unknown. Ground squirrels are highly seasonal

and have acquired mechanisms to rapidly accrue energy in the late summer to meet the demands of a subsequent 8-month hibernation fast – gaining 30% of their body mass in fat in just 30 days. Prior to hibernation, ground squirrels reduce food intake and lose body weight prior to entering a hypo-metabolic state; how they cease appetitive drive while resources are present is unknown. To gain insight into mechanisms of this transition, we microdissected a region of the hypothalamus involved in energy balance control (the arcuate nucleus, median eminence and third ventricle) and conducted bulk RNAseq in male and female thirteen-lined ground squirrels. We show that males have a higher number of differentially expressed genes compared to females between a fattening and post-fattening phenotype. Functional enrichment analysis revealed that biological processes related to positive regulation of cell differentiation and proliferation were upregulated in males, whereas these processes were downregulated in females, suggesting sex differences in cellular processes across this transition. This suggests sex-differences in hypothalamic plasticity occur prior to hibernation, despite similarities in seasonal metabolic patterns.

Using whole genome data to characterize introgression in Malagasy gemsnakes

Dylan DeBaun, Frank Burbrink, Christopher Raxworthy

Using whole genome sequencing data, we validate previously discovered hybrids and explored the landscape of introgression in several genera of Malagasy gemsnakes (Pseudoxyrhophiidae). Given features of genetic linkage and the recombination landscape, window size selection is an important parameter choice for genomic studies. So, we first explored the effects of window size on introgression detectability. Following this, we were able to explore differences in introgression detection between our whole genome sliding window approach and results obtained previously, using anchored loci. We explored how detection and estimated proportion of introgression differed given the new, larger, and more complete dataset. We quantified variation in the susceptibility of both macro- and micro-chromosomes, as well as auto- and sex chromosomes to introgression. We expected introgression to be more prevalent on micro- than macro-, and even less prevalent on sex chromosomes. We used several hybrid groups to present multiple lines of evidence and to additionally explore differences as a result of timing of introgression.

Enhancer evolution at follistatin paralogs underlying the genetic assimilation of winglessness

Kevin Deem, Jennifer Brisson

The marvelous diversity of plant and animal forms observable in nature stems from eons of accumulated morphological innovations. Despite prolonged scientific interest, the evolutionary processes that give rise to novel phenotypes remain shrouded in mystery and debate. One promising model is genetic assimilation, in which new phenotypes that emerged from environmentally induced variation (phenotypic plasticity), can later evolve to be genetically induced. However, it remains unclear how such a switch from environmental to genetic control might occur. My work aims to characterize the molecular mechanisms facilitating the genetic assimilation of a novel phenotype, to further our understanding of morphological novelty, diversity, and evolution. I utilize the pea aphid, in which ancestral female wing polyphenism has been genetically assimilated in males via duplication of the gene *follistatin* (*fs*). My ongoing projects focus on dissecting the cis-regulatory evolution at *fs* paralogs that may have facilitated the genetic assimilation of wing polyphenism. This work aims to further our understanding of the genesis of novel phenotypes and the molecular underpinnings of morphological evolution.

Manual curation of three firefly genomes reveals odorant receptor diversification in Lampyridae

Susan Deering, Sarah Lower

Odorant receptors (ORs) are an excellent model to study gene family evolution, as they represent among the largest gene families in insects. Although thousands of ORs genes have been identified for species across the class Insecta, their rapid sequence divergence necessitates analyses of more recently

diverged clades to provide insight into OR evolutionary patterns in different insect groups and potential relationships to ecology and behavior. To advance the understanding of the diversity and evolution of ORs in one of the most speciose insect groups, the beetles, we annotated putative odorant receptor encoding genes in three firefly species, *Lampyris yunnana*, *Abscondita terminalis*, and *Photinus corrugatus* (Coleoptera: Lampyridae). These three species diverged approximately 140 million years ago and employ contrasting mating signaling modalities (bioluminescent glow or flashes, or non-bioluminescent pheromone mating signals, respectively). Iterative BLAST followed by manual curation of OR intron/exon boundaries found that, while *A. terminalis* and *P. corrugatus* both have around 100 ORs, similar to other fireflies, *L. yunnana* has only 36 ORs, despite its larger genome size. Further, the adjacent positioning of many *A. terminalis* and *P. corrugatus*, but not *L. yunnana*, ORs on scaffolds suggests that tandem duplication plays a role in OR diversification in this group. Continued genome sequencing efforts in this charismatic beetle group will enable future testing of potential relationships between OR diversification and ecology, specifically mating signaling modality, in fireflies.

Determining the impacts of symbiotic state and environmental pollution on wound recovery in corals

Kyra DeGroat, Jonathan deMontagnac, Justin McAlister, Liz Burmester

A key component to understanding coral reef health is to understand the biological processes that promote live coral cover. In nature, corals are regularly abraded by different sources and must rebuild lost tissue in order to maintain colony health, which has meaningful consequences on the health of coral reef ecosystems as a whole. Additionally, for animals (like corals) that harbor photosynthetic symbionts, the different relative contributions of autotrophy versus heterotrophy to energetic requirements have direct impacts on fitness. Distinguishing the individual effects of both environmental stress and symbiotic state on coral health is difficult to investigate experimentally in most tropical corals because the symbiosis is obligate (i.e. bleaching). However, determining the interactions between nutritional dynamics, environmental stressors, and health are key to understanding coral resilience. In order to better parse the relative contributions of host and symbiont, we explore the vital coral-algal symbiosis and stress tolerance of a particularly hardy, temperate and facultatively symbiotic coral (*Astrangia poculata*) and its photosynthetic symbiont (*Brevium psygmophilum*). Unlike most tropical corals, *Astrangia poculata* is capable of healthily maintaining a spectrum of symbiotic states— from fully symbiotic to fully aposymbiotic, with mixed states in between. In this experiment, we determine the ability of corals of different symbiotic states (aposymbiotic, symbiotic, and mixed) to recover from controlled experimental lesions after exposure to environmental pollutants (tire dust).

Assessing patterns of convergence in burrowing crayfish

Emmy Delekta, Matthew Kolmann

Evolutionary habitat transitions can provide ecological opportunity for organisms, but only if the organisms can adapt to their new environment. Strong selective pressure on phenotype can arise when these habitat transitions fundamentally alter the physical media animals live in, like the invasion of land by lobe-finned fishes and insects. When environmental gradients differ drastically among habitats and multiple lineages transition between these habitats, we expect phenotypic convergence to be prevalent. One transition where widespread convergence has been observed is the shift from aboveground to subterranean environments in fossorial animals. All North American crayfish (Cambaridae) have some burrowing ability, but the degree to which they burrow is split into three categories: primary, complex burrows; secondary, less-complex burrows; and tertiary, simple burrows. Research suggests burrowing promotes morphological convergence in crayfish, with tertiary burrowing forms having a dorsolaterally compressed carapace and long, slender claws while primary burrowing forms have a dorsoventrally compressed carapace and shorter, more powerful claws. However, earlier comparisons relied on qualitative, rather than quantitative assessments of morphology. This study tests for convergence in North American crayfishes using a geometric morphometric approach. We photographed the carapace

and claw for representative species across thirteen North American genera. We hypothesize that crayfishes which occur in similar habitats and exhibit similar burrowing behaviors will converge in their carapace and claw shapes.

Is sea anemone venom fundamentally different

Alonso Delgado, Marymegan Daly, Charlotte Benedict

Venom is of significant interest because it points to a common evolutionary problem and a solution. Venomous organisms (spiders, cone-snails, and snakes) have venom-coding genes which evolve under positive selection. However, sea anemones offer an intriguing contrast as their genes are perceived to evolve under negative selection. This distinction can be attributed to the evolutionary age distinction with sea anemones arising nearly 500 million years ago whilst other well-studied venoms (spiders, cone-snails, and snakes) range from 80-45 million years old. Meaning, more evolutionary time has been able to act on these venom-coding genes potentially skewing the signs of positive selection on those genes. The venom families in which venom has been studied lack phylogenetic perspectives and are greater in evolutionary age than all other venomous species.

To understand if venom evolved differently in sea anemones, we examined venom rates of evolution in a sea anemone family, Aiptasiidae, which has an estimated age of 55 million years. We sequenced species in the family by using UCE target baits and reconstructed phylogenomic relationships. For each species in that tree, we sequenced transcriptomes and then extracted the putative venom profiles using existing pipelines. For known and comparable venom families, we examined DN/DS ratios to understand broad patterns of evolution in a phylogenetic lens at an age comparable to all other evolutionary rate studies.

The effects of heat waves and subsequent hypoxic 'reprieve' on mussel metabolism

Andi Delgado, Animaya Arkills, Wes Dowd

Intertidal mussel beds host biodiversity and provide valuable ecosystem services, making them important to study as more extreme atmospheric heat waves occur more frequently and coastal oceans deoxygenate. While intertidal species' responses to variations in temperature and oxygen have been studied both in isolation and when imposed simultaneously, little is understood about their interaction in sequential scenarios, which are likely to become more common in coastal regions. The experience of aerial sublethal heat stress leads to the production of reactive oxygen species (ROS), which can cause macromolecular damage. This damage is often repaired during periods of immersion (high tide) when oxygen is available. However, if the surrounding seawater is hypoxic, mussels may be forced to rely on anaerobic metabolism, leading to the production of ROS. In this study, *Mytilus californianus* mussels collected from Starfish Point, Washington were subjected to a fully-crossed set of tidal cycle scenarios wherein air temperature during the day-time low tide (18, 25 or 32°C) and dissolved oxygen (DO) during both high tide periods (1.12, 2.1 or 6.0 mg L⁻¹) were manipulated for 1 or 5 days. By analyzing changes in the activities of aerobic (citrate synthase), anaerobic (phosphoenolpyruvate carboxykinase), and antioxidant (catalase) enzymes in adductor and gill tissues, we can better understand the sublethal metabolic responses to realistic, seldom-studied climate scenarios to elucidate the consequences of sequential exposure to multiple stressors.

Epigenetic insights into the evolution of form using Lake Malawi cichlid fishes

Leah DeLorenzo, Kara Powder

While changes to DNA sequence are critical for adaptations, there is growing evidence that epigenetic changes, including differences in DNA packaging and posttranslational modifications of histones, may drive morphological diversity. To investigate the role of epigenetic factors in the phenotypic divergence of an adaptive radiation, we used Lake Malawi cichlids, focusing on the craniofacial morphologies that are associated with their feeding mechanisms. We first manipulated histone acetylation using the HDAC inhibitor Trichostatin A throughout craniofacial development. We used two different species with

distinct adult craniofacial morphologies, *Maylandia zebra* and *Tropheops* sp. 'red fin', to examine the species-specific epigenetic regulation of development. We found both a time- and species-dependent sensitivity to changes in histone acetylation. *Maylandia* embryos treated during early facial development demonstrated increased disparity and paedomorphic phenotypes (short and wide face). In contrast, late stage *Maylandia* were robust to changes in acetylation, as were *Tropheops* at all timepoints. To examine the molecular underpinnings that might explain the species-specific response to treatment, we examined the natural variation in chromatin structure in these fishes. We utilized ATAC-seq and RNA-seq at a developmental timepoint when species diverge in facial shape to identify differences in open chromatin regions and gene expression. Together, our research demonstrates that epigenetic modifications are vital to understanding how phenotypic diversification arises. This work was supported by NIH P20GM121342, NIH R15DE029945, and NSF/IOS #1942178.

Effects of perturbation by vertical gusts upon the kinematics of gliding flight in doves

R  my Delplanche, Kathryn Greil, Ruowen Tu, Henry Sodano, Daniel Inman, Bret Tobalske

The locomotor adaptations exhibited by flying vertebrates have been shaped by the fluid properties of the air through which they navigate. Real-world airflow is complex, and perturbations due to turbulence both present significant challenges for bioinspired flying vehicles and have likely served as selective pressures upon flying organisms. To elucidate useful mechanisms of gust rejection, we measured the 3D kinematics of wing morphing in Barbary doves (*Streptopelia risorii*, n=3) in response to simulated upward gusts during gliding flight in a wind tunnel at an airspeed of 10m/s, and compared the morphing responses to those observed during other flight styles. Gusts, 1 m/s in magnitude, were generated using 2 airfoils mounted at the front of the flight chamber; by increasing the angle of those airfoils relative to the horizontal airflow was deflected up into the bird from below. In response to simulated gusts, doves elevated their wings and decreased their wingspan and wing angle of attack, similar to responses recently reported by other researchers for gliding owls. The doves exhibited significant variation in response characteristics among trials and individuals; nevertheless, in all cases, the magnitude of wing morphing in response to gusts was significantly less than that observed during flapping flight, and was not much greater than that observed during steady-state gliding. This shows that gusts approximately 10% of flight speed do not necessarily require large-scale wing morphing responses, potentially highlighting the importance of other sources of turbulence mitigation such as feather deformation. NSF EFRI 1935216.

Demographic resilience and host evolution shape diverse recovery paths under different disturbances

Joe DeMarchi, Mark Wilber, Cheryl Briggs, Roland Knapp, Thomas Smith

Resilience, encompassing resistance and recovery, is vital for understanding ecological responses to disturbances. Recent recoveries in disease-affected populations emphasize the need to understand recovery dynamics. The mountain yellow-legged frog (MYL), serves as a case study, having suffered disease (Bd) and invasive fish-induced declines. MYL populations independent of disease recovered when invasive fish were removed, specifying baseline recovery trajectories absent of perturbations. Contrarily, some MYL populations persist with Bd, recovering via evolved resistance or tolerance. We developed an epidemiological-ecological-evolutionary model to investigate the mechanisms underlying observed transient recovery trajectories of MYL frogs under different perturbations (invasive fish, disease). We ask: 1.) How do population recovery trajectories differ between press (Bd) or pulse (invasive fish) disturbance? 2.) What eco-evolutionary mechanisms are leading to the observed recovery trajectories in Bd recovering populations? Our models predicted that MYL's demographic structure innately leads to faster transient recovery dynamics (i.e., reactivity) in populations experiencing trout-like compared to Bd-like disturbances, once the disturbance has been removed. Moreover, when we allowed for the evolution of host resistance or tolerance in the continued presence of Bd, maximum recovery rates differed substantially for different defense strategies and compared to recovery from

trout-like, pulse disturbances, consistent with empirical data. Overall, we show that all recoveries are not created equal, and demographic resilience, size-structured perturbations, and evolutionary strategies can synergistically shape the recovery dynamics of populations.

Wing Morphogenetic Fields as Tools to Understand Cellular Shape and Growth

Kyle DeMarr

The evolution of wings has been described as a key innovation linked to the ecological dominance of terrestrial arthropods, with these structures themselves diversifying into an assortment of forms bestowing distinct functional benefits and advantages. An important axis in which wings vary is their pelage, or the presence of single cell-derived structures scattered across their surface in the form of ‘macrochaetes’—setae, bristles, or scales. These structures, analogous to mammalian hair, avian feathers, or reptile scales, contribute to coloration, thermoregulation, and wettability by virtue of their individual morphologies and collective arrangement. Previous studies have investigated fitness contributions of either single or bulk macrochaetes exclusively, for instance in terms of overall color pattern formed on a wing or the thermal properties of a particular scale architecture, with little attention given to the positional and relative context of these features with respect to each other. Viewed within a more local context, macrochaetes seem to fall into gradations of morphologies much resembling transitional series with intermediates filling the gap between any two or more extreme forms. I echo and bring to the fore a observations from the first investigations of heterogeneity in wing macrochaete shape and present examples of modern questions that benefit from this perspective of viewing macrochaete morphologies not as discrete bins but as intergrading series and contextualize this evidence in the hierarchy of biological organization.

Adaptive value of plasticity from interspecific variation in transcriptomes during thermal stress

Jimmy deMayo, Jasmine Vidrio, Greg Ragland

Phenotypic plasticity, where the phenotype is determined by environmental change without genetic change, can both support adaptation (i.e. adaptive plasticity) and oppose it (i.e. maladaptive plasticity). Recent studies suggest that differences in plastic gene expression among recently derived or geographically variable populations are primarily driven by selection against maladaptive plasticity. However, it is unclear whether and how these patterns extend to species-level diversity. Here we explore how transcriptional plasticity influences thermal adaptation following long-term evolution in contrasting thermal environments of *Drosophila* flies with tropical versus temperate geographic distributions. For each species, we measured gene expression via mRNA-seq at key temperatures during a thermal ramp from benign conditions to the critical thermal minimum (CT_{min}) to temperatures approaching the lower lethal temperature (LLT). In this presentation we use whole-transcriptome patterns to test hypotheses about whether long-term evolution in a warmer environment tends to enhance, suppress, or not affect plastic responses along this thermal continuum. We compare these results to previous intraspecific studies and discuss implications for the role of plasticity in adaptation to relatively extreme environments. This study demonstrates the role that plasticity can play in driving thermal adaptation as it relates to phenotypic divergence among species.

Modeling Multisensory Integration in Weakly Electric Fish in Relation to Sensory Salience

Alp Demirel, Selin Özel, Ismail Uyanik

Multisensory integration is a hallmark of animal movement. It is a dynamic process, which allows the animal to adapt to different sensory conditions by continuously changing the contributions of each sensory modality. Here, we investigate the impact of sensory salience on the weights associated with different sensory systems. To achieve this, we developed a custom-built experimental setup that enables studying multisensory integration in weakly electric fish. These fish track the movements of a refuge using their visual and

electrosensory systems. Our nested refuge structure allows separate stimulation of these systems during tracking. Here, the inner transparent refuge only stimulates the electrosensory system. Likewise, the outer visual refuge only provides visual cues. Here, we manipulate the visual and electrosensory salience of these refuges using refuges with/without windows and varying the water conductivity. We used a multisensory feedback model of the fish to estimate the weights assigned to each modality under different sensory conditions. Our results with $N=4$ fish showed that the minimum variance unbiased estimator (MVUE) model captures the changes associated with the variance. However, the classical MVUE model fails to explain the increase in tracking gain when both sensory stimuli are available. Our goal is to extend the MVUE model to understand the impact of sensory salience on the dynamic sensory reweighting observed during multisensory integration. Supported by EU-MSCA-IF (MultiSense).

Bio-Inspired Hummingbird Robot Navigating Environmental Disturbances

Xinyan Deng, Yiming Zhou

Flying animals have demonstrated remarkable adaptability and recovery capabilities in the face of adverse conditions. In this work, we investigate the resilience of a hummingbird inspired robot under environmental challenges including collision in cluttered spaces and wind gust disturbances.

The at-scale hummingbird robot has two flapping wings with independent actuation from two motors. We tested its flight resilience when navigating through a cluttered path obstructed by random obstacles with varying dimensions randomly placed as barriers along the flight trajectory. When encountering obstacles, the wings of the robot exhibited a passive rebounding behavior. The robot effectively navigated through the spatial gaps between barriers after multiple rebounds. The flexible wings, mounted on the reciprocal thorax joint with torsional spring, were able to negotiate with the obstacles without crashing the vehicle or breaking the wings.

We constructed a horizontal wind tunnel to generate wind gust disturbance to the hovering hummingbird robot. A honeycomb configuration was incorporated into the outflow section of the wind tunnel to ensure uniform flow. The robot was commanded to hover at a set position in front of the wind tunnel. Both frontal wind gusts ranging from 1.5 to 3.3 m/s and lateral gusts ranging from 1.5 to 2.5 m/s were introduced. The robot was able to adjust its posture and kinematics to maintain position and attitude stability. Under frontal gust, body pitch angle tilted proportionally to the wind gust speed. Lateral wind gusts caused higher perturbation of the robot, compared to the frontal wind gust.

Pilifers provide proprioceptive feedback about mouthpart movement in hawkmoths, *Manduca sexta*

Tanvi Deora, Mauro Torres, Alison Weber, Bingni Brunton, Tom Daniel

How animals reach for and target objects determines their ability for goal oriented tasks like grasping. Moths and butterflies use their proboscis, a long and flexible mouthpart to systematically explore floral surfaces and target a tiny hole in the center which leads to the nectary reward. How insects sense and control proboscis motion to achieve these remarkably precise movements remains an open problem. Pilifers, paired bristled organs on the left and right proboscis base are a likely source of proprioceptive feedback. To characterize the response properties of this organ, we drove lateral motions of the proboscis with a band-limited white noise mechanical stimulus in anaesthetized head-fixed moths. Using multisite extracellular electrodes, we recorded neural responses from the pilifer nerve. Our recordings reveal that pilifer mechanosensory neurons are sensitive to lateral motions, either to the left or to the right. Like other mechanosensory organs, they respond extremely rapidly, often within a few milliseconds. Our white noise stimulus allowed us to build neural models that characterize the stimulus features that elicit pilifer mechanosensory action potentials. These properties are strikingly similar to other insect mechanosensors, including the abdominal mechanosensors and strain sensors on wings and halteres. Similar neural filtering across all mechanosensors suggests an important role for sensor mechanics and motion in encoding relevant information.

Impact of dietary protein on growth, body composition, and performance in *Peromyscus californicus*

Elissa Derrickson

Dietary protein is a primary component of diet quality and is critical to successful growth and reproduction. This study examines the relationship of maternal and offspring dietary protein on juvenile growth, body composition and performance. Prior to pairing, *P. californicus* were placed on isocaloric diets varying in protein and followed for three litters. Pups were maintained on this diet after weaning and followed for 12+weeks; body composition and swim endurance of juveniles was measured throughout this period. The impact of dietary protein on juvenile postnatal growth had a threshold effect with growth rate lowest on the lowest protein diet but similar on all other diets containing 8% or more crude protein. Lean mass and fat components of juvenile body composition were impacted by dietary protein. Lean mass was correlated with total mass and was greater in pups on higher protein diets; however lean mass as a proportion of fat-free mass was not significantly different across diet treatments, indicating that growth may be constrained by protein intake. Contrary to the protein leverage hypothesis, total fat was greater on high protein diets. Pup swim endurance was correlated with dietary protein, indicating total protein and/or total fat impacted endurance. Dietary protein constrains growth when limited but in excess is correlated with increased growth and fat deposition.

Non-parallel behavioral responses to soundscape perturbations during the COVID-19 pandemic

Elizabeth Derryberry, Graham Derryberry, Ruth Simberloff, Amy Luo, Michael Blum, David Luther, Jennifer Phillips

The COVID-19 pandemic triggered unprecedented environmental perturbations—sometimes referred to collectively as the Anthropause—that have progressed rapidly and over broad spatial extents, affording novel quasi-experimental opportunities to measure outcomes of human-environment interactions. Here we evaluate the resilience of a common songbird (white-crowned sparrows *Zonotrichia leucophrys*) to noise pollution by comparing soundscapes and songs across the San Francisco Bay Area prior to, during, and after the Spring 2020 statewide shutdown. Restrictions on human movement during the shutdown reduced noise pollution, relaxing auditory pressures on animals that communicate via sound. Birds quickly responded by producing wider bandwidth songs at lower amplitudes, effectively increasing signal efficacy and salience. In contrast, behavioral responses have lagged behind increasing noise levels as restrictions on human movement have loosened over time. Although song amplitude eventually returned to pre-pandemic levels, paralleling noise levels, birds have continued to produce wide bandwidth songs, with consequences for signal masking in noise. These findings illustrate that behavioral traits are slower to change in response to newly adverse conditions, indicating non-parallel responses to noise pollution removal and re-introduction.

Egg size, the forgotten life history trait: using the Asian lady beetle as a model

Genavieve Desjardin, Tony Williams

Individual quality (variable and correlated with fitness) is determined by phenotypic (e.g., body size) or life-history traits (e.g., fecundity). Although many studies have focused on phenology and fecundity, few studies have researched the causes and consequences of intraspecific variation of egg size. Fewer studies have considered the impact of warming (cf. cooler) temperatures due to climate change on ectotherms and their future fecundity. Climate predictions suggest insects are more sensitive to warming temperatures. Here, we investigated individual variation of egg size in *Harmonia axyridis*, the Asian ladybird beetle, a globally introduced predatory generalist, at 22°C and then at higher temperatures of 26°C (as predicted by IPCC 2022 climate projections) and 32°C. We hypothesized that a) *H. axyridis* egg size will decrease under high temperatures as individual females will allocate more resources to larger clutch size and fewer resources to individual egg size (maximizing female fitness), but b) smaller egg size will not come at a cost in terms of offspring fitness because larvae from smaller eggs will have

higher viability at higher temperatures. Our findings will contribute to a better understanding of the trade-offs and the mechanisms behind egg size variation within the context of climate change.

Measuring Effects of Stress on *Sepia bandensis* through the Judgment Bias Task

Sarah Detmering, Robyn Crook

Judgment Bias Tasks (JBT) are used to assess the emotional state and welfare of animals in zoos, farms, and laboratories, as well as in rodent studies of human health and disease. JBTs attempt to measure the emotional or affective state of an animal based on its interpretation of an ambiguous or intermediate cue, after a training period where distinct cues represent positive and negative outcomes. In theory, animals in positive affective states are more likely to interpret the ambiguous cue positively, reflecting optimistic bias. Alternatively, animals with negative affect are more likely to interpret ambiguous cues pessimistically. While JBTs are commonly used in vertebrate animals, their use in invertebrates has been limited. Concern for the welfare of cephalopods is growing, as is a broader recognition of the presence of emotional and affective neural circuits in invertebrates. Here, we developed a JBT assay for *Sepia bandensis*, to determine whether cuttlefish exhibit negative affective states resulting from chronic and acute stress. We show that cuttlefish learn to associate food with a visual cue, and that responses to an ambiguous cue are affected by prior exposure to chronic and acute stress produced by impoverished housing and simulated predation threat. Our data suggest that cuttlefish may experience cognitive bias, the first indication of this capacity in cephalopods. JBT may therefore be an effective tool for assessing cephalopod welfare.

Cold, wet winters: How climate change may impact reproductive investment in captive crossbills

Jalyn Devereaux, Jessica Karr, Thomas Hahn, Jamie Cornelius

Climate projections indicate changes in winter weather in some regions of North America that may impose higher thermoregulatory costs on birds. Species that invest in migration or reproduction during this time may be the most impacted. Crossbills are opportunistically breeding finches that seem to breed less frequently in winters characterized by cold rain than snowier conditions. Using 48 captive red crossbills (*Loxia curvirostra*) we investigated how temperature, precipitation, and daylength may impact energy costs and reproductive competence from November to February. Birds were randomly divided into warm (21°C) or cold (6°C) treatment groups that experienced wet (rain 30 min/day) or dry conditions. Cold birds were housed on two different photoperiods (Corvallis, OR, USA; Fort Nelson, BC, Canada). We used gonadal measurements and plasma corticosterone and testosterone (males only) to quantify the effects of these treatments on glucocorticoid and reproductive physiology. Our data suggest that rain increases circulating corticosterone and food intake compared to dry treatment groups. Wet mass of ovaries was higher in warmer temperatures but was not impacted by rain. Testis length was smaller in the cold rain treatment, though not in those housed on a shorter daylength. Testosterone showed no response to weather treatments, but increased significantly as winter progressed. These results indicate that winter rain may be a cost to birds, but the impact of these costs on reproductive investment is nuanced.

Energetics and Biomechanics of Hovering in Fishes

Valentina Di-Santo, Xuewei Qi

Hovering, a crucial locomotor behavior in fishes, serves as a foundation for a range of activities including foraging, environmental exploration, conspecific interaction, and nest tending. This behavior involves a fish's capacity to maintain a stable position within the water column with minimal directional movement. Particularly intriguing is the hovering gait observed in almost neutrally buoyant fishes, as those with swim bladders inherently possess dynamic instability. This prompts them to continually generate forces countering rolling and pitching due to the separation of the center of mass and buoyancy. In this comparative analysis, we used 14 near-neutrally buoyant fish species to delve into the energetic expenditures and three-dimensional

kinematics of fin and body movement during hovering. By measuring metabolic rates of individual fish during both hovering and resting periods, we quantified the net energy costs linked to this specific behavior across species. For an in-depth understanding of fin and body kinematics during hovering, we employed orthogonal high-speed cameras to capture fish movements. Our findings show the presence of varied motion patterns, involving the simultaneous activation of multiple propulsors during the hovering gait. Furthermore, we quantified a substantial elevation in metabolic rates during hovering compared to resting states. This study underscores the substantial energetic investments associated with stabilizing the body while “swimming in place” for fishes.

When to go against the flow: lessons from migrating fish

Kelly Diamond, Heiko Schoenfuss, Richard Blob

How does an organism overcome environmental selection pressures for which they have no prior experience? This challenge is faced by both migratory species, such as the waterfall climbing goby fish, ‘o’opu nōpili (*Sicyopterus stimpsoni*), and first-generation college students navigating academia. The first part of this talk will focus on how migration-related performance of ‘o’opu nōpili changes over time. ‘O’opu nōpili migrate upstream from marine to freshwater environments in migration pulses, where thousands of fish swim upstream each day for several days following a flash flood. We examined how performance varies over the course of migration pulses for two major selection pressures: evading piscivorous predators and climbing waterfalls. Both pressures are expected to decrease over a migration pulse as predator hunger decreases and flood-level stream flows recede. Results suggest that escape performance does not vary over the course of a migration pulse. In contrast, climbing performance peaks 3-5 days after a flooding event. These results could help to identify when the largest numbers of fish are able to successfully migrate after a flood and improve our understanding of how diversity in performance is maintained in upstream populations. The second part of the talk will translate the lessons learned from this study and discuss when to listen to mentors who have experience in various academic specialties and when to go against the flow.

Contemporary convergent evolution of physiological traits in response to urban heat islands

Sarah Diamond, Ryan Martin

Whether shared selection pressures lead to shared evolutionary responses is a long-standing question in evolutionary biology. It has taken on new relevance under recent climate change, with evidence both for repeatable, rapid evolution of climate-sensitive traits among a number of species, but also evidence of limits on evolutionary change for other species. Despite the critical nature of understanding how broadly convergent evolution of climate-sensitive traits occurs, and whether exceptions to these broad patterns are predictable based on species’ traits, our inferences remain limited. In particular, while there is widespread evidence of contemporary evolution, support generally comes from non-climatic agents of selection such as evolving antibiotic or pesticide resistance. And, much of the data for convergent climatic niche evolution comes from studies of responses over historical, geological time scales. Cities, through their urban heat island effects, provide a venue for exploring convergent contemporary evolution to climatic warming. Here, we examine the repeatability of evolution of higher heat and diminished cold tolerance traits across urbanization gradients to assess the potential for convergent contemporary evolution to climatic warming. We examine whether the magnitude of evolutionary divergence in these traits is related to the magnitude of climatic change across the urbanization gradient and whether species traits might predict exceptions to convergence. In general, we find support for convergent evolution, with exceptions relating to generation time and behavioral thermoregulation.

Spin and Slap: High-Speed Prey Capture of the Bolas spider *Cladomelea akermani*

Candido Diaz, John Long, John Roff

Spiders use combinations of silks, adhesives, and behaviors to ensnare and trap prey. A common but difficult to catch prey in most habitats are moths. They escape typical orb-webs because their bodies are covered in sacrificial scales that flake off when in contact with the web’s adhesives. This defense is defeated by spiders of the sub-family of Cyrtarachninae, moth-catching specialists who combine changes in orb-web structure and predatory behavior. The most extreme changes are shown by bolas spiders, who create a solitary capture strand containing a few glue droplets at the end of a single thread. Here, we use a high-speed video to observe the spinning prey capture behavior of the bolas spider, *Cladomelea akermani*. We measure kinematics of the spiders, bolas, and moths to begin to quantify the physical and mechanical properties of the bolas during prey capture, the behavior of the moth, and how these factors lead to successful prey capture. We found spinning provides two advantages. Firstly, spinning spreads pheromones and creates a wider alert area for searching male moths; most often they fly past the spider from upwind, then reverse and fly against the wind towards the spider. Secondly, we found spinning elicits the moth escape response of dropping, causing them to fall directly into the spinning bolas below. This research is supported by the National Science Foundation project #2031962.

Is feather color a signal of cognitive ability in Eastern bluebirds?

Danae Diaz, Steve Nowicki, Sonke Johnsen

Cognition may be important to assess in the context of mate choice, but individuals are unlikely to be able to directly observe the cognitive abilities of potential mates, relying instead on correlated assessment signals. I am testing whether the chestnut-colored pheomelanin-based breast patch of Eastern bluebirds (*Sialia sialis*) serves as an indicator of cognitive abilities in this species. A positive relationship between cognitive abilities and melanin-based coloration may be expected because pheomelanin and the central nervous system (CNS) both require cysteine, a limiting resource, and resources should be preferentially allocated to more critical processes (i.e., the CNS). Thus, color may serve as a reliable indicator of CNS development. I am also assessing whether variation in coloration and/or cognitive ability corresponds to reproductive success, as measured by extra pair fertilizations in this socially monogamous species. I measured breast patch area and coloration (spectral reflectance) of 24 individuals across four study sites in central North Carolina, and assessed how they performed on one cognitive task (a nest box entry puzzle). Using generalized linear models (GLMs), I asked whether more colorful individuals perform better on these cognitive tasks. Using GLMs, I also assessed whether there was a relationship between coloration and/or cognitive ability and mate choice, using extra-pair paternity as a measure of reproductive success.

Using RNA Interference to Understand Selective Flight Muscle Histolysis in *Gryllus lineaticeps*

Tomas Diaz, Caroline Williams, Jacqueline Lebenzon

In the wing polymorphic field cricket, *Gryllus lineaticeps*, the cost of flight can outweigh the benefits of dispersal. In this flight-for-reproduction trade-off, flight-capable adult crickets can selectively break down (“histolyze”) their flight muscle to use those resources for reproduction. This selective histolysis involves the break-down of dorsolongitudinal flight muscle while maintaining the neighboring dorsoventral muscle. The mechanism behind this insect flight muscle histolysis is not well understood. We hypothesized that autophagy is the mechanism by which histolysis is achieved because we previously observed two important autophagy-related genes, Beclin and ATG5, increase in dorsolongitudinal muscle during the course of histolysis. To identify the extent to which autophagy drives muscle histolysis, we will use RNA interference to knock down Beclin and ATG5 transcript abundance in adult crickets with functional flight muscle, before histolysis begins. We hypothesize that knocking down these genes will prevent/delay histolysis and oogenesis in flight-capable crickets which can be measured via gene expression of selected genes, quantified muscle color, and ovary size. Our

study will be the first to experimentally determine autophagy's role in selective muscle degradation in these crickets and will give insight into how the pathway of autophagy has evolved to be used as a cellular response in a multitude of contexts.

Observations of planktonic gastropod larvae in turbulence indicate surfing behaviors

Michelle DiBenedetto, Rémi Monthiller, Christophe Eloy, Karl Helfrich, Lauren Mullineaux

Benthic species often have a free-swimming planktonic larval stage. Successful survival, dispersal and settlement of the larvae depend on their behavior and their environment. Coastal and surface ocean waters are often turbulent, therefore the effects of turbulence on plankton transport are of interest. Our study investigates the behavior of the slipper snail (*Crepidula fornicata*) veliger larvae in a jet-stirred turbulence tank, employing particle-image-velocimetry and particle tracking to measure co-located larval and fluid velocities. Our findings suggest that larvae have complex responses to the local fluid gradients, particularly by rotating counter to the flow. These behaviors suggest that some plankton may "surf" turbulence as theorized by Monthiller et al. (Phys. Rev. Lett., 2021), i.e., allowing them to leverage upwelling turbulent currents for enhanced upward transport beyond their sole swimming capability.

Selection for maximum speed explains patterns of posture and energetics across body sizes

Taylor Dick, Friedl De-Groote, Christofer Clemente

An unusual pattern among the scaling laws in nature is that the fastest animals are neither the largest, nor the smallest, but rather intermediately sized. Because of the enormous diversity in animal shape, the mechanisms underlying this have long been difficult to determine. To address this we challenged novel predictive human musculoskeletal simulations, designed on the human body form and scaled in mass from the size of a mouse (0.1 kg) to the size of an elephant (2000 kg), to move as fast as possible. Our models replicated patterns observed across extant animals including: (i) an intermediate optimal body mass for speed; (ii) a reduction in the cost of transport with increasing size; and (iii) crouched postures at smaller body masses and erect postures at larger body masses. Finally, we used our models to determine the mechanical limitations of speed with size, showing that larger animals are likely limited by their ability to produce muscular force while smaller animals are limited by their ability to produce larger ground reaction forces. These replicated patterns observed across extant animals, including quadrupeds, suggest these biological phenomena likely emerge from selective pressures to maximize movement speed and are not the result of phylogenetic or other ecological factors that typically hinder comparative studies.

A computational map of mechanosensory feedback for *Drosophila* flight control

Bradley Dickerson, Serene Dhawan

Sensory input to the brain is organized into maps that are arranged based on either the anatomical location of individual afferents or some computational principle. Such maps are particularly important for monitoring and rapidly processing timing differences to control complex behaviors, such as insect flight. Many insects, including the fruit fly *Drosophila melanogaster*, rely on wingbeat-synchronous mechanosensory feedback to achieve both stability and maneuverability. This is in part due to specialized mechanosensory organs unique to flies, halteres, both help dynamically regulate the wing steering system and also function as biological gyroscopes. The halteres are equipped with nearly 150 strain sensors called campaniform sensilla that are arranged into five stereotyped groups along the haltere's dorsal and ventral aspects. Although we have a sense of the gross projection patterns of the haltere afferents, the principles governing how haltere information is centrally organized remains an open question. I will present our progress from our efforts tracing the haltere's circuitry with electron microscopy-level resolution. We leveraged the Female Adult Nerve Cord (FANC) dataset to identify and fully reconstruct the haltere's primary afferents. Morphology-based clustering

analyses revealed 5 neuronal subtypes—putatively corresponding to the different haltere campaniform sensilla fields and chordotonal organ—each of which synapses onto a distinct subset of wing-steering motor neurons (MNs). We additionally reconstructed the contralateral haltere interneurons and described their synaptic inputs from different haltere afferent subtypes, as well as their output onto the wing MNs. By tracing the haltere and its targets, we are developing an atlas of the structure-function relationship between rapid mechanosensory feedback and motor systems.

Ontogeny and ecological correlates of grip strength across lemurs

Edwin Dickinson, Melody Young, Michael Granatosky

The ability to generate strong grip force is integral to the successful navigation of an arboreal environment. Across tetrapods (including amphibians, reptiles, birds, and mammals), grasping forces are increased among arboreal taxa relative to their terrestrial counterparts. However, these forces are near-exclusively collected from adult individuals, and data on how these forces vary during development are sparse. To address this shortfall, we collected *in vivo* grasping forces from left- and right-sided forelimbs and hindlimbs across an ontogenetic sequence of 7 lemur species (aye-ayes, ring-tailed lemurs, crowned lemurs, blue-eyed black lemurs, ruffed lemurs, mongoose lemurs, and sifakas). Body mass data were also collected from each individual to calculate relative grip strength as a proportion of body weight (%BW). Across all species, relative grip strength increased through early development, peaking in early adulthood and declining with senescence. Our relatively strongest individuals were sifakas, highly arboreal animals specialized for vertical clinging and leaping. Collectively, our data provide a new perspective on grasping forces, highlighting that both relative and absolute gripping potential varies considerably as a consequence of age. Contrary to initial expectations, infants were not relatively stronger than other age groups, despite their need to cling to their mothers prior to attaining locomotor independence. We also note that, from a comparative perspective, relative grip forces in adult lemurs (~40-50%BW) are comparable to humans, and show similar ontogenetic trajectories.

In vivo gripping forces and their anatomical correlates in lemurs

Edwin Dickinson, Melody Young, Michael Granatosky

Arboreal animals must navigate complex three-dimensional environments while maintaining a strong grasp to mitigate the high fitness risks associated with falling. Indeed, grasping forces measured in several tetrapod lineages demonstrate that arboreal animals generally exhibit stronger grasps than their terrestrial counterparts. However, primate grasping differs from other mammals by: 1) the ability to oppose their first digits across the palm or sole; 2) having nails instead of claws; and 3) possessing large intrinsic and extrinsic limb muscles associated with digital flexion. In this study, we employ a two-stream approach to analyze gripping abilities in lemurs, the most basal primate lineage. First, *in vivo* grip forces were collected from the hands and feet of more than 60 individuals across 8 species. Subsequently, anatomical estimates of grip force were collected by gross dissection of extrinsic and intrinsic muscles in the forelimb and hindlimb of representative individuals from each taxon. We show that, among lemurs, the most arboreal genera (e.g., *Propithecus*) show the strongest grasping forces as well as the largest muscles associated with digital flexion. Ontogenetic analyses of grasping forces also indicate that lemurs show similar age-related strength curves to humans. We conclude that evolution in an arboreal milieu has driven strong manual and pedal grasping abilities in primates, though these relative magnitudes are still low compared to arboreal birds.

Unhinged: a futile obsession to uncover the mystery of insects' most essential innovation.

Michael Dickinson

Insects are arguably the most successful radiation of metazoan animals in the history of life. The key innovation that underlies the success of insects—and the main subject of my talk—is the tiny mechanical hinge that attaches the

wings to the body, thus enabling the animal to fly. The wing hinge is a miniature mechanical machine that automatically transforms the small, high frequency oscillations of the powerful flight muscles into the small, precise sweeping motions of the wings. Despite over 200 years of interest among biologists and engineers, little progress has been made on elucidating the function of the wing hinge because conventional techniques have not provided the spatial and temporal resolution necessary to visualize its tiny internal mechanical elements as they operate during flight. Within the last few years, my lab has tried to develop techniques that collectively make it possible to resolve the structure and function of the wing hinge and reverse-engineer its salient properties. Our integrative, multi-disciplinary approach reveals the mechanical logic of the insect wing hinge, arguably among the most sophisticated and evolutionarily impactful mechanical structures in the natural world.

Does exposure to artificial light at night (ALAN) affect spring migratory departure in a songbird?

Samantha Diedrich, Ellen Ketterson, Sarah Wanamaker, Alex Jahn

Temperate-zone birds use photoperiod as a primary cue for timing daily activities and life-history events such as molt, migration, and breeding. However, as artificial light at night (ALAN) becomes more prevalent through urbanization, the difference between daylight and dark nights has become less distinct, which could influence evolved patterns of timing. Dark-eyed juncos, like many other songbirds, rely on the vernal increase in the photoperiod to trigger migratory behavior and seasonal reproductive development via activation of the hypothalamus-pituitary-gonadal (HPG) axis. Previous research in juncos shows that exposure to ALAN advances the timing of gonadal growth. Unknown is whether ALAN influences migratory timing.

We captured juncos ($n = 56$) overwintering in Indiana and housed them in an indoor aviary. We exposed the treatment group ($n = 28$) to ALAN (~3 lux) while the control group ($n = 28$) experienced dark nights. We quantified migratory readiness by measuring individual activity levels using accelerometers, and migratory departure using Lotek nanotags upon release in early March. Three MOTUS towers situated around the release site detected nanotag presence, allowing an estimate of migration departure date; additional nationwide towers registered their migratory paths after departure. We are processing the results to assess the effects of ALAN on behavior, physiology, and migratory timing.

Evolution of mitonuclear mismatched *C. elegans*: Effects of adaptedness, mating system and mitochondrial mutations

Zachary Dietz, Suzanne Estes, Vaishali Katju, Ulfar Bergthorsson

Mitonuclear coevolution is essential for energy metabolism and organismal survival because both nuclear (nDNA) and mitochondrial (mtDNA) genomes encode products necessary for energy metabolism (e.g., components of the mitochondrial respiratory chain; MRC) and other processes. How intergenomic coevolution is maintained given the radically different population biologies experienced by nDNA and mtDNA is not well understood. The nuclear compensation hypothesis suggests that adaptive evolution in nDNA-encoded MRC genes has been accelerated to compensate for degradation of their mtDNA counterparts. Recent molecular evolution studies provide little support, but direct tests are lacking. A related hypothesis—the mitonuclear sex hypothesis—seeks to explain the evolution and maintenance of sexual reproduction. It suggests that sexual reproduction is maintained because it increases the rate at which new combinations of nDNA alleles can compensate ongoing mitochondrial genome decay. We recently provided the first controlled study of how rates of outcrossing evolve in response to mitonuclear mismatch by allowing replicate lineages of *C. elegans* nematodes containing either mitochondrial or nuclear mutations of MRC genes to evolve under three sexual systems: facultatively outcrossing (wildtype), obligately selfing, and obligately outcrossing. We present new results from this set of evolved MRC mutant lines and interpret them in light of evolutionary theory and evidence for the evolution of male frequency in some facultatively outcrossing lines. This talk will address questions including how ancestral fitness and rates of outcrossing correlate with adaptive fitness outcomes, and how the mtDNA genome may contribute to such adaptation.

Influence of Putative Developmental Networks on the Development of Dermal Armor in Armored Catfish

Wesley Dillard, Julia Bailey, Gareth Fraser

Stem vertebrates had dermal skeletons consisting of bony plates covered in odontodes made of dentine and enamel. Most extant bony fish lineages have reduced or lost the dental element of their armor with only the derived bony element remaining, present either as plywood-like scales or dense, dermal scutes. However, nine disparate lineages of teleost fish have re-evolved odontodes as part of their dermal armor, including Loricariidae, armored catfishes that have both bony and dental elements despite having evolved from a scaleless ancestor. Armored catfish have gained recent interest as models of extra-oral odontode evolution. However, we know little about the development of these structures beyond the influence of a dozen genes present in dental tissues of other classes. In this study, we investigate the development of the dermal skeleton of the bristlenose armored catfish, *Ancistrus* spp. Their armor forms in separate subunits, a single underlying dermal scute or fin appendage and several distal odontodes, linked developmentally by shared genes and networks. We use gene expression, small molecule inhibition, and Computed Tomography (CT) to observe the influence of putative pathways on the interdependent development of bony and dental elements in dermal armor. Understanding the interactions of these pathways in this highly-derived group of teleost fishes can contribute valuable insights to the evolution of the dermal skeleton in vertebrates.

Characterizing male dominance behavior in a polygynous breeder, the northern elephant seal

Hayden Dillon, Jake Roth, Heather Liwanag, Katie Saenger

Polygynous breeding is characterized by one male mating with many females, and the northern elephant seal (*Mirovanga angustirostris*, NES) is a well-known polygynous species, with males defending harems including as many as 100 females. Male-male competition during the breeding season is well-documented in NES, but there is little to no information about this behavior at the Piedras Blancas rookery, the largest NES mainland breeding colony. This study aims to better understand the dominance relationships among male NES at the Piedras Blancas rookery and how these relationships affect mating and beach behavior. The goals of this study were to (1) establish dominance rankings for focal NES males based on their displacement interactions and (2) correlate aggressive behaviors and copulations with those different dominance rankings. To do this, we tracked individual males ($n=114$) throughout the breeding season, identified by a unique hair dye mark on their side, and observed their behavioral interactions via visual surveys twice per week during the 2021-2023 breeding seasons. For each observation, we recorded the specific dominance behaviors (e.g., vocalization, rearing up, chasing) of each individual in addition to factors such as the beach location, mating success, and age class. We found that aggressive behaviors and copulations were positively correlated with dominance ranking. Understanding behavioral dynamics during the breeding season will help us better understand how these animals use their breeding beaches.

The importance of winter microclimates for terrestrial ectotherms

Michael Dillon, D. M. Shayne Dodge, Jordan Glass, Ellen Keaveny, Sarah Waybright, Sabrina White

Microclimatic variation is increasingly recognized as an important determinant of ectotherm physiological ecology. With ongoing climate change, microclimates may provide a critical resource allowing ectotherms both to avoid thermal extremes and to maintain preferred body temperatures despite increasing mean ambient temperatures. Winter microclimates are much less understood but may be just as important given the duration of winters in temperate regions and the “gatekeeper” effect winters have for annual organisms – winter survival is critical for success the following growing season. And experienced winter temperatures likely determine timing of spring emergence. Unfortunately, empirical data on microclimatic variation in winter across scales are limited. Here, we characterize winter temperature variation from local (meters) to regional (kilometers) to continental (US) scale. Using published

data on ectotherm physiology, we ask how winter temperature variability may alter survival, energy use, phenology, and post-winter condition for representative ectotherms at these different scales. Finally, we discuss some key determinants of variability in above- and below-ground winter temperatures by combining model estimates of microclimate with empirical measurements. Microclimatic variation in winter likely profoundly alters success of terrestrial ectotherms.

Using CRISP-Cas9 to test thermal tolerance genes in Rainbow Trout

Bradford Dimos, Michael Phelps

Understanding the mechanisms underlying organismal thermal tolerance are essential for predicting the capacity of species or populations to adapt to climate change. Genomic techniques have made the identification of genes association with thermal tolerance straight forward, however moving from associative to causative relationships is more challenging. To address this issue, we sought to generate Rainbow trout harboring loss of function mutations in genes previously shown to be associated with tolerance using the CRISPR-Cas9 genome editing system. In this study we demonstrate that CRISPR is capable of generating biallelic loss of function mutations at sufficiently high efficiencies to test thermal tolerance in the F0 by generation crispant animals deficient for three thermal tolerance-associated genes *aldob*, *atf5*, *bag3* in addition to the marker gene *slc45a2*. Overall, we find that crispant fish have altered thermal tolerance confirming the role of these genes in the temperature stress response of Rainbow Trout.

Insights from a Novel Membrane-Based Stretching Device

Pervin Dincer, Nilufer Duz, Hasan Kilic, Erdem Goral, Yasin Gulsum, Waleed Odeibat, Harun Artuner, Ismail Uyanik

The harnessing of mechanical stimuli, such as stretch dynamics, shear stress and applied pressure, holds the capacity for modulating extracellular functions through the initiation of mechanotransduction pathways, thus affecting gene and protein expression. The resultant changes modify cellular function or morphology in response to these mechanosensory prompts. The mechanical cues is vital to numerous cellular aspects encompassing growth, development, differentiation, and cellular interplay. Moreover, conversion of mechanical force to biochemical signalization is instrumental in homeostatic maintenance of tissues including skeletal and cardiac muscle, bone, and vascular structures. Interruptions in mechanotransduction mechanics often instigate gross physiological disequilibria. In this study, a membrane-centric cellular stretching apparatus has been engineered to induce uniaxial or biaxial mechanical stress on cells through sinusoidal stretch modulation of varying levels (5-20%), frequencies (0.2-2 Hz), and patterns. The innovative mechanics and membrane design of the device facilitate uniform stretch distribution across membrane's surface, a fact corroborated by image processing-based calibration. System functionality was evaluated through q-PCR analyses of mechanosensitivity-associated genes (CTGF, C-MYC, MYL9), revealing a marked upregulation of MYL9 and CTGF gene expressions. Furthermore, the device augments our understanding of mechanical stress impacts on cells. Owing to its broad applicability across diverse cell types and tissues, it can enable elucidation of disease pathophysiology, regenerative processes pertinent to tissue damage, and the identification of targeted therapeutic strategies. Supported by HU-BAP (TOA-2023-20338) and TUBITAK (120E472).

Investigating drivers of trophic preference and symbiotic state across depth in a temperate coral

Clara DiVincenzo, Stephanie Peak, Karl Castillo

The coral-dinoflagellate symbiosis is essential for the survival of coral reefs but is highly vulnerable to rising sea surface temperatures. For tropical corals, this relationship is obligate, as dinoflagellates provide the corals with up to 95% of their nutrients. In contrast, temperate corals can exist without symbionts, and can rely on heterotrophic consumption for nutrient acquisition. The temperate coral *Oculina arbuscula* (*Oculina*) naturally exists in both symbiotic and aposymbiotic (symbiont-free) phenotypes off the coast of North Carolina. The downward-sloped reef provides both shallow (~5m) and deep

(~10m) environments, inhabited by predominantly symbiotic and aposymbiotic *Oculina* colonies, respectively. A reciprocal transplant of *Oculina* fragments between shallow and deep sites was conducted at Radio Island, NC. In this study, we investigate environmental factors that influence symbiotic phenotype, mechanisms of nutrient acquisition, and trophic adaptation in *Oculina*. We examine variations in gene expression, C/N isotopes, energy reserves, and symbiont density at four time points (0w, 2w, 3m, 1y) to capture both short- and long-term changes. In addition, we will measure environmental factors (temperature, light, and nutrient availability) as predictors of trophic state across depth. Over the year-long experiment, we expect shifts in transplanted corals' symbiotic phenotypes and trophic ecologies towards those of native corals. These findings will provide key insights into the environmental conditions suitable for coral heterotrophy and the molecular, cellular, and physiological basis for symbiotic state.

The mechanics of spoke regions in tesserae and their role in energy absorption in shark cartilage.

Molly Dobrow, Maria Laura Habegger, Mason Dean, Stephen Stagon

Unlike the stiff bony skeletons in mammals, sharks have skeletons of cartilage, a material with low stiffness. Despite this mechanical disadvantage, sharks have evolved to be apex predators, with high-performance ecologies. Shark jaws are of particular interest as they undergo high cycle loading without failure. It has been argued this is due to the unique material and structural organization of their cartilage, in particular the superficial mineralized elements (tesserae), which form a shell surrounding the unmineralized hyaline cartilage. Tesserae are thin, geometric tiles arranged in pavement-like layers, with laminated hypermineralized zones (spokes) at their points of contact. Although spokes are theorized to be key mechanical features in tessellated cartilage performance, investigating their mechanics and the synergistic effects of variable mineralization in spokes has been limited due to these structures' small scale. This research investigates the role of spokes in stress reduction by constructing larger-than-life tesserae models, derived from microcomputed tomography data of *Carcharhinus limbatus* tesserae, incorporating a range of material gradients and spoke designs using multi-material additive manufacturing (AM). Models were mechanically tested to examine energy absorbed by the material and the variation in strain rate within spoke regions of individual and neighboring tesserae. We discuss results relative to theoretical models and within an ecological framework (e.g. how reduced biting stresses affect cartilage biology), while framing biomimetic implications for the design of long-life composite architectures.

Determining the Expression Patterns of Ultraviolet Sensitive Opsins in *Gonodactylaceus falcatus*

Noah Doeden, Mireille Steck, Megan Porter

Stomatopod crustaceans have the greatest expressed opsin diversity observed among animals, including three ultraviolet-sensitive (UV) opsins; *uv1*, *uv2*, and *uv3*. Previous transcriptome work identified the same three UV opsins in both adult and larval stomatopod eyes, despite inhabiting different environments, utilizing UV light for different functions, and completely reconstructing their retinas between stages; however, the scope of that project did not include the UV opsin localization patterns in either life stage. Using a combination of reverse transcription (RT) PCR and immunohistochemistry (IHC), RNA expression and protein localization of the three UV opsins was investigated in the eyes, head, and bodies of larval and adult *Gonodactylaceus falcatus* individuals. This is the first study to investigate UV opsin localization patterns across life stages and explore the role of UV opsins beyond the eye in stomatopods. All three UV opsin transcripts were expressed in larval eyes, while only *uv1* was expressed in the body. Preliminary IHC trials suggested that *uv1* and *uv2* were present in the eye and brain of adult individuals. The presence of these opsins outside of the eye implies a previously unexplored, secondary, nonvisual function for UV light detection.

The fa(c)ts that matter: Bees differentially allocate and modify common fatty acids in pollen

Adi Domer, Eran Levin

The body lipids of many hymenoptera are relatively saturated, with low proportions of polyunsaturated fatty acids (PUFA). Pollen serves as the primary lipid source for bees, and dietary PUFA are considered important for these insects. Nevertheless, the digestibility and absorption of different fatty acids (FAs) by bees remains inadequately understood. We enriched pollen with ¹³C labeled FAs with varying saturation levels and investigated the assimilation, oxidation and allocation of these FA by bumble bees (*Bombus terrestris*). We found that palmitic and stearic acid, the most common saturated FAs in pollen, were poorly absorbed by bees even when emulsified, while monounsaturated and polyunsaturated FAs were absorbed at a higher rate. Intriguingly, a metabolomic, analysis conducted on bees fed with isotopically labeled polyunsaturated linoleic acid, unveiled that the absorbed PUFAs were extensively reassembled as saturated FAs, suggesting there is an unknown mechanism for FA saturation in the bees' cells. This study implies that the evolutionary process shaping the lipid composition of pollen does not solely rely on the nutritional requirements of pollinators. Instead, the plants' dependence on insect pollinators has influenced the nutritional composition of pollen, albeit not optimized it. Furthermore, the study suggests that the chemical properties of lipids and their digestibility partially explain their essentiality in animal nutrition and raises some questions about the function of PUFA in the bodies of these insects

Chronobiology of urban life: new insights from parallel recording of activity and body temperature

Davide Dominoni, Barbara Helm, Claire Branston, Pablo Capilla-Lasheras

Increasing worldwide urbanisation has been associated with changes in the daily rhythms of wildlife. In birds, several studies have shown that some, but not all, diurnal species can shift their daily rhythms into the night in urban areas, with suggested fitness benefits. However, it is unclear whether this increased nocturnal activity is compensated by a decreased diurnal activity. Moreover, all previous work focused on recording behavioural rhythms, such as that in singing and locomotor activity, while no data is available on how physiological rhythms might respond to urbanisation in the wild. To fill these gaps, we used automated biotelemetry on six different songbird species inhabiting urban and forest areas in Scotland, to obtain activity and skin temperature recordings every 3-min around the clock. The analysis of activity data confirmed previous results, as we found species-specific response to ALAN. European robins and Eurasian blackbirds advanced their morning onset of activity in urban vs forest areas of up to 3 hours, while the other species did not. The increased nocturnality was not compensated by decreased activity during the night, as urban robins and blackbirds were more active during the daytime hours than forest conspecifics. Moreover, the advancement of morning activity in urban robins and blackbirds was not paralleled by a similar advancement in body temperature, suggesting desynchronisation of behaviour and physiology. We anticipate that our contribution will offer an important new insights on the effects urbanisation on the daily lives of wild animals.

Integrating Morphology, Environment and Glucocorticoids into Estimates of Reproductive Success

Beverly Domschot, Paul Lukacs, Creagh Breuner

Glucocorticoids (GCs) can indicate how individuals balance trade-offs among vital rates (i.e., survival, growth, and fecundity) and, therefore, hold promise as a tool to identify early signs of demographic decline. GCs integrate internal and external factors experienced by an individual and, in turn, regulate suites of traits. This study investigates the interconnections of GCs, morphological/energetic characteristics, and environmental conditions and their effects on the reproductive output of a breeding population of mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*) using structural equation modeling (SEM). Disentangling these intricate relationships is crucial for developing GC measures in conservation. SEM facilitates this inquiry by allowing for the identification of direct, indirect, and intermediate links among environment, morphology, energetics, GCs, and reproductive success. Preliminary findings

indicate that unbound (free) GCs perform better in the model. Specifically, 1) energy stores inversely predict free baseline GCs, 2) temperature and open ground directly predict free baseline GCs, 3) free stress-induced GCs inversely predict if an individual successfully fledged offspring within a year, 4) free baseline GCs positively predict the total number fledged. By unraveling the connections between GCs and their influencing factors, researchers can pinpoint the most predictive external/internal variables that influence GC levels and identify the most informative GC measures for conservation purposes.

How armor shape and arrangement affects the mechanics of a composite system

Cassandra Donatelli, Eric Chier, Megan Vandenberg, Matthew Kolmann, Adam Summers

An overlooked function of dermal armor in vertebrates is locomotor enhancement. We usually think of armor as an adaptation for defense, but it serves multiple functions and likely is under several selective pressures. Here we investigate armor's role in locomotion; specifically, the role of armor shape, structure, and organization using fishes as our model group. Armor diversity within fishes is outstanding, ranging from smooth rhomboid scales (i.e. Lepisosteidae), to interlocking bony plates with protruding spines (i.e. Agonidae), to highly mineralized embedded scutes (i.e. Acipenseridae). Though gross morphology differs, there are some recurring characteristics such as scale overlap and the presence of tongue and groove mechanisms that allow scales to move only in prescribed directions while swimming. In this work we first characterized armor diversity using CT scans. Then, we designed and manufactured idealized armor models to test the function of specific shapes and orientations (i.e., hexagonal-overlapping, circular-spaced, etc.). These models were 3D printed using a fused filament method with a fabric support sandwiched between layers. We used a universal testing machine to explore how different armor shapes and arrangements affect body mechanics and energy storage using both idealized models and real specimens. Our results reveal the multifunctional role of dermal armor and may inform future bio-inspired designs.

Environmental stressors as trigger of dynamic color change

Evan Donnachie, Matthew Fuxjager, Doris Preininger, Susanne Stücker

In some anuran species (frogs and toads), males rapidly change their body color during brief mating aggregations. In such explosive breeding events, many males compete for a limited number of females. From a mechanistic perspective, we know that stress hormones are able to elicit this rapid color change. We hypothesize that dynamic color change has evolved as a sexual signal that is triggered by rapidly changing ecological conditions (start of monsoon rain and/or change of temperature), mediating fast mate recognition in dense breeding aggregations. We tested the activation of coloration changes from a cryptic brown to a bright yellow elicited by environmental factors in males of the Indian Common toad, *Duttaphrynus melanostictus* at the Vienna Zoo. In three experiments we explored whether heavy rain, a drop in temperature of $\approx 7^{\circ}\text{C}$, or a combination of both trigger color change in reproductively active adult males. Results show that yellow saturation of skin color increased after 6 min of heavy rains (before rain vs. after rain: $p < 0.05$), and differed from the control group ($p < 0.01$) that received no rain. We suggest that monsoon rains associated with the onset of explosive breeding act as abiotic stressors triggering catecholamergic response that mediates male color change.

Oh Me Oh Meiofauna! Analyzing Western Antarctic sediment community structure through meiofaunal DNA

Kyle Donnelly, Pamela Brannock, Candace Grimes, Andrew Mahon, Kenneth Halanach

The Western Antarctic Peninsula is home to an astonishing diversity of invertebrate species. This display of biodiversity is easily seen by observing the Antarctic seafloor, and, unsurprisingly, the diversity extends into the benthic sediments themselves. Microscopic eukaryotes known as meiofauna inhabit sediments where they fulfill a critical role in sediment aeration, nutrient cycling, and other important benthic-pelagic coupling processes. Previous studies

have been conducted to observe Western Antarctic meiofaunal community composition over broad geographic ranges. We complement these previous studies but focus on a more limited geographical region with increased sampling density and resolution. To assess the composition of these communities, the hypervariable V1 and V9 regions of the 18s rRNA subunit were used to conduct a meiofaunal metabarcoding analysis of sediment cores taken from 19 sites around the tip of the Western Antarctic Peninsula. Sediment cores were collected via a Multi-corer device from September through December of 2020 aboard the RV/IB Nathaniel Palmer. Resulting sequences were grouped into operational taxonomic units (OTUs) based on 97% similarity. Community composition and diversity values were calculated using alpha and beta tests including Chao, Simpson's, Bray-Curtis and Jaccard's indices, as well as a nMDS analysis. Dominant taxa observed across the 19 sites were comprised of various annelid families, nematodes, hemichordates, and crustaceans, highlighting consistencies between this study and previous work done in the region.

Springs as Islands: Island Biogeography and Conservation Genomics of Two Endemic Pleurocerid Snails

Samantha Donohoo, Paul Johnson, Nathan Whelan

Spring-associated species often share similar characteristics with island species, including limited distributions, low levels of genetic diversity within springs, high levels of genetic structure among springs, and an increased extinction risk from anthropogenic influence. To better understand the unique properties of spring endemics, we examined population genomics and molecular ecology of two species of Pleuroceridae snails that are endemic to Alabama, USA: *Elimia bellacrenata* (Princess Elimia) and *Elimia cochliaris* (Cockle Elimia). Specimens were collected across the historical range of both species. Genome-scale data was generated using a 2bRAD approach. We examined historical migration events, estimated demographic history for each species, and used phylogenetic analysis and species tree inference to infer the relationships within populations and among species. Our analyses recovered four distinct lineages: 1.) *E. bellacrenata* sensu stricto from Ebenezer Spring Complex; 2.) *E. cochliaris* sensu stricto from Tannehill Parkway Spring; 3.) one distinct, undescribed species from Valley Church Spring; and 4) a second undescribed species from the Watercress Darter National Wildlife Refuge. These results not only provide further insight into pleurocerid biogeography and speciation but also support the idea of treating springs as aquatic islands in a terrestrial landscape. Both island and spring species are often rare, which makes determining their distributions and evaluating genetic diversity critical to defining their conservation needs.

Seasonal changes in ecosystem engineering of marine sediments by infauna

Kelly Dorgan, Chesna Cox, Nina Stark, Grace Massey, Carl Friedrichs, Adrian Rodriguez-Marek, Md Rejwanur Rahman

Infaunal animals modify their sediment environments through burrowing, tube construction, and ingestion and egestion of sediments. Infauna can weaken sediments by disrupting cohesive bonds and excavating burrows, or can strengthen sediments through compaction during burrow construction. These impacts have been shown to affect bulk properties of sediments such as porosity, permeability, and shear strength. Here, we explore the impacts of infauna on sediment strength and erodibility by sampling sediment communities and physical properties in the York River Estuary, VA, in spring, when infaunal abundance is high following spring recruitment, and in fall, when abundances are much lower. We used a Portable Free Fall Penetrometer to measure quasi-static bearing capacity, a Gust chamber to measure surface erodibility, a custom probe to measure profiles of subsurface cohesion, and took X-ray images to visualize bulk density of sediments. We expected impacts of infauna on sediment structure to be driven by abundances, with greater impacts in the spring. However, our data showed more indications of potential infaunal impacts in the fall, indicating that there may instead be a lag between the presence of infauna and their impacts on sediment structure. Understanding the relationships between infaunal abundance and activities and sediment structure and stability will improve interpretation of geotechnical data in productive coastal sediments.

Ornaments indicate parasite load only if they are dynamic, or parasites are contagious

Liam Dougherty, Faith Rovenolt, Alexia Luyet, Jukka Jokela, Jessica Stephenson

Choosing to mate with an infected partner has several potential fitness costs, including disease transmission and infection-induced reductions in fecundity and parental care. By instead choosing a mate with no, or few, parasites, animals avoid these costs and may also obtain resistance genes for offspring. Within a population, then, the quality of sexually selected ornaments on which mate choice is based should correlate negatively with the number of parasites with which a host is infected ('parasite load'). However, the hundreds of tests of this prediction yield positive, negative, or no correlation between parasite load and ornament quality. Here, we use phylogenetically controlled meta-analysis of 424 correlations from 142 studies on a wide range of host and parasite taxa to evaluate explanations for this ambiguity. We found that ornament quality is weakly negatively correlated with parasite load overall, but the relationship is more strongly negative among ornaments that can dynamically change in quality, such as behavioral displays and skin pigmentation, and thus can accurately reflect current parasite load. The relationship was also more strongly negative among parasites that can transmit during sex. Thus, the direct benefit of avoiding parasite transmission may be a key driver of parasite mediated sexual selection. No other moderators, including methodological details and whether males exhibit parental care, explained the substantial heterogeneity in our dataset. We hope to stimulate research that more inclusively considers the many and varied ways in which parasites, sexual selection, and epidemiology intersect.

A muted proteomic response to thermal stress in a stenothermal Antarctic fish

Wes Dowd, Dietmar Kueltz

Stenothermal Antarctic fishes including the emerald rockcod (*Trematomus bernacchii*) share a long history of isolation in extremely cold waters and may lack the classic "heat shock response." We comprehensively assessed the proteomic response of this species to elevated temperatures in three tissues (gill, kidney, and brain). Animals were first acclimated to one of three constant acclimation temperatures, after which a subset were sampled. The remaining individuals experienced an acute heat stress with or without recovery ($n=5-6$ per treatment). Protein expression was quantified with curated, tissue-specific, data-independent acquisition (DIA) assay libraries on a UPLC-MS system. The proteomic response was consistently muted, in terms of both numbers of differentially expressed proteins and the magnitude of their fold change. In some treatments and tissues no single protein (out of 2721-3421 per tissue) was differentially expressed. Furthermore, there was little thematic consistency across similar treatments in the identity or functional categorization of differentially expressed proteins, suggestive of a lack of appropriate transcriptional or translational regulatory mechanisms. The promoter regions of the corresponding genetic loci were not consistently enriched for any transcription factor binding motifs (such as Hsf1 or HIF). Subsequent bioinformatics approaches indicated strong conservation within the Antarctic notothenioid lineage of several protein domains that coordinate the response to thermal stress, although other steps in this cascade might plausibly explain the muted proteomic response to heat. Overall, our data offer further insight into the physiological underpinnings of stenothermy in Antarctic fishes.

Risk-taking behavior and defensive morphology of marine three-spined stickleback

Steven Downs, Stephanie Crofts, Kevin Neumann

Defensive morphology, such as spines or armor plates, is ubiquitous across multicellular life and can co-evolve with a host of other traits, including modifications to risk-taking behavior, or metabolism, to form an Armor Syndrome. While armor syndromes have mainly been studied in plants and terrestrial mammals, the goal for this project is to expand this taxonomic coverage. Here we focus on armor syndrome traits in a marine population of Three-Spined Stickleback (*G. aculeatus*), as correlations between behavior and armor are well studied in freshwater populations. We collected fish from waters

surrounding Friday Harbor, WA and measured 5 risk-taking behaviors: activity, inspection/time inspecting, orientation, and time sheltering. Following behavioral trials, we microCT scanned each fish. We used the Slicermorph module in Slicer3D and ImageJ to measure dorsal and pelvic spine morphology and lateral armor plate coverage and density using. To detect any correlation between our behavioral and morphological data, we ran linear regressions in R. We found no significant correlation between armor measurements and behaviors, but saw a significant negative correlation between both the 1st and 2nd dorsal spine aspect ratios and number of inspections, one of our inferred risky behaviors. Based on findings from freshwater *G. acuaetus*, we might expect to see greater variation between marine populations than observed here for both morphology and behavior.

Diverse Anatomical Peak Shifts Underlie Body Elongation in Zoarcoid Fishes

Summer Duba, David Collar

Evolutionary shifts towards elongate body shapes have occurred repeatedly in ray-finned fishes and led to a variety of eel-like forms across diverse lineages. However, whether elongation events have been shaped by similar evolutionary processes remains an open question. Transitions in body shape can occur through multiple combinations of vertebral and cranial skeleton changes, and overlap in anatomical evolution can shed light on commonalities in the circumstances underlying elongation. In our investigation of the suborder Zoarcoidei, we identified seven instances of body elongation that varied in their associated anatomical peak shifts. The most elongate species, *Ptilichthys goodei* (quillfish), experienced extensive head elongation and increases in precaudal and caudal vertebral numbers, but other body shape transitions involved only subsets of anatomical shifts. For example, increasing precaudal vertebrae numbers drove elongation in the Pholidae (gunnels), while increasing caudal vertebrae numbers were more salient in the Zoarcidae (eelpouts). These distinct anatomical shifts suggest elongate bodies evolved in association with different functional capacities that likely contributed to divergent habitat use patterns. For example, gunnels inhabit rocky, shallow intertidal zones, where additional precaudal vertebrae may confer advantages in respiratory function at low tide for temporary air breathing. Eelpouts, by contrast, have some of the deepest habitat ranges of the suborder, where greater numbers of caudal vertebrae may confer greater flexibility and swimming efficiency under high pressures and cold temperatures.

The impacts of climate change on secondary seed dispersal in dung beetles

Nathan Duerr, Kimberly Sheldon

Dung beetles, which move and bury the feces of vertebrates, are major drivers of ecosystem processes and provide crucial ecosystem services, including secondary seed dispersal. Dung beetles bury seed-containing dung in food caches or in brood balls used for breeding purposes, but little is known about how this behavior will be affected by climate change. We utilized field manipulations to investigate the effect of simulated climate change—including simultaneous increases in temperature mean and variance—on the seed dispersal behavior of two tunneling dung beetle species, *Phanaeus vindex* and *Onthophagus taurus*. We placed single adult females into either control or greenhouse treatments along with temperature loggers. We mixed glass beads of three sizes into cow dung to mimic seeds, provided beetles with the dung, and then allowed them to bury dung for either six or nine days. At the end of each trial, we recorded information on dung deposits, including the type (i.e., food cache or brood ball), number, size, burial depth, and the amount of each bead size found in the deposit. We found differences in burial depths of brood balls and food caches within species, as well as differences in the size and amount of beads buried between species. Exposure to higher temperatures resulted in brood balls being buried deeper across species, but did not change the burial depth of food caches.

GABA and lactate preconditioning increases cell division in annual killifish cell line during anoxia

Devan Duey, Chelsea Hughes, Riley Roth-Carter, Daniel Zajic, Jason Podrabsky

Annual killifish (*Austrofundulus limnaeus*) live in temporary ponds in Venezuela and experience drastic changes in their environment that cause their ponds to dry up. This species survives by producing drought- and anoxia-tolerant embryos that are deposited in the mud. Embryos survive these conditions by entering metabolic dormancy (diapause) until environmental signals break their dormancy and they continue developing. In order to survive anoxia, embryos rely exclusively on anaerobic metabolism, which leads to abundant lactate accumulation. Previous research has shown that the production and degradation of the neurotransmitter γ -aminobutyric acid (GABA) is crucial for long-term anoxia survival. This study explores the role of lactate and GABA metabolism in anoxia tolerance. To test this, we exposed embryo-derived cells (WS40NE) in anoxia to three treatments: anoxia preconditioning, lactate preconditioning, and GABA supplementation. For all treatments, cell survival was monitored, and extracellular lactate levels were measured. Compared to the control, cells exposed to lactate and GABA proliferated at a higher rate, whereas the anoxia preconditioned treatment proliferated at a lower rate. Rate of lactate accumulation was dependent on time spent in anoxia as well as whether cell media was changed. Media changes led to higher rates of lactate production compared to cells in static media. Understanding survival of cells during anoxia may give insight to how human conditions, such as strokes, can be avoided or damage can be reversed.

Salinity and diet influence ranavirus infection outcomes and gut bacterial communities in wood frogs

Alexa Dulmage, Robyn Reeve, Grace Curtis, Kourtnie Whitfield, Myra Hughey, Erica Crespi

Salinization of freshwater habitats poses a growing threat to amphibians. Exposure to high salinity conditions can increase ranavirus infection intensity and nutrient availability. However, the mechanisms through which elevated salinity alter disease dynamics are still largely unknown. One hypothesis is that environmentally induced alterations to gut bacterial communities could influence infection outcomes. We explored the effects of salinity and diet on ranavirus infection outcomes and gut bacterial communities in larval wood frogs (*Rana sylvatica*). Larvae were reared in outdoor semi-natural mesocosms at two salinities (300 and 1500 mSi/cm) crossed with two diets: baseline diet (leaf litter/alfalfa pellets) and baseline + *Chorella* algae. Larvae were exposed to either culture media or ranavirus in individual containers for 6 days before euthanization. Viral titers in liver tissue were determined using qPCR and gut bacterial communities were analyzed using 16S sequencing. Relative to mock exposure, ranavirus-exposed larvae lost weight and accelerated development in the baseline diet, but not in the algae-supplemented diet. Elevated salinity and algae supplementation increased ranavirus titers. Ranavirus exposure and algae supplementation reduced bacterial diversity in the gut, while algae supplementation and elevated salinity altered bacterial community structure. The environmentally induced shifts in bacterial abundance identified in this study set up specific hypotheses that can be tested in the wood frog-ranavirus system to better understand interactions between the environment, host bacterial communities, and pathogens.

Characterizing invasive *Watersipora* (Bryozoa) populations in the central California rocky intertidal

Meredith Duncan, C. Sarah Cohen, Benson Chow, Kevin Myron, Jaden Stone

Several taxa within the bryozoan genus *Watersipora* are successful global marine invaders that are common in marine fouling communities in bays and harbors. Two species within this genus, already known to be abundant within San Francisco Bay (California, USA), have recently been recorded spreading to natural intertidal and subtidal habitats on the central California outer coast near the Bay, which suggests a high invasive potential in pristine coastal habitats. However, the species composition and genetic diversity of these populations has not been previously characterized. We produced COI

barcoding sequences for *Watersipora* specimens sampled from four rocky intertidal sites, as well as for specimens from several dock sites in and around San Francisco Bay. The two *Watersipora* species previously recorded within the Bay continue to persist there, and both species were also identified at rocky intertidal sites. Field observations of intertidal populations support year-round persistence of *Watersipora* at rocky intertidal sites, with seasonal shifts in population abundance. Our results emphasize the importance of continued sequencing and seasonal monitoring of outer coast *Watersipora* populations in order to further characterize the genetic diversity and population dynamics of *Watersipora*'s outer coast spread.

A transcriptomic study of the lone star tick's eyes and mating behaviors for disease mitigation

Mitchell Dyen, Robert Fitak

The lone star tick (*Amblyomma americanum*) is a zoonotic disease vector found in the southern and midwestern United States but has been rapidly expanding northward. Its population growth has caused a concurrent increase in tickborne illnesses, such as tularaemia and ehrlichiosis, affecting humans and animals alike. This increased prevalence of tick-borne illness calls for more understanding of preventing the spread of lone star ticks and controlling their populations within their habitat. Tick mitigation strategies, such as CO₂ traps, and controlled burns are used to slow disease expansion. These methods can be costly to implement and incur unnecessary health risks to humans; therefore, a new alternative prevention strategy for tick-borne illnesses is needed. Developing a more effective methodology for preventing tick disease transmission requires a robust understanding of tick sensory systems. The large, iconic spot on the back of the female lone star tick is a visually distinct sexual dimorphism, yet no studies have investigated if the spot acts as a mating signal. If vision plays a role in mate-seeking in the lone star tick, it could be a starting point for developing a biocontrol strategy that disrupts mate finding in ticks. We plan to characterize genes expressed in the eyes of the lone star tick to decipher which sensory systems are utilized in mate-seeking behavior, which can contribute to innovating future tick bite mitigation strategies.

Spying connections: using a word game to integrate concepts in physiology education.

Jonathan Dyhr, Joel Roberts

Physiology is fundamentally integrative, combining concepts from different scientific fields such as physics, biology, and chemistry to understand body function. Students struggle to learn these concepts independently, much more so when connecting them together. The difficulty is compounded by acquiring knowledge of the many different molecules, cells, tissues, organs and organ systems that compose the body. In this poster, we discuss how to adapt the word game Codenames to create a fun, easy-to-implement activity that pushes students to the higher Bloom's levels of learning. The game requires minimal preparation by the instructor, primarily a list of course-related terms. The class is divided into groups of approximately 8-12 students, with each group being further divided into two teams. The groups are given a 5x5 grid of words, and each team is assigned 8-9 random words from the grid. Each team elects a spymaster, who provides one-word clues to link the assigned terms based on their meanings, while also differentiating them from the other terms in the grid. The other students on the team guess the terms related to the clue. In order to succeed, students must discuss the meaning of the terms, how they relate to the clues and the thought process of the spymaster. The presentation will also address strategies for getting the most out of the activity, overcoming implementation challenges, and encouraging student participation.

How social stress affects the gut in the socially monogamous prairie vole

Jack Dziubek, Eva Lindner, Madeline Roth, Jennie Stevenson, Mark Haussmann

The bi-directional communication that occurs between the gut and the brain, termed the gut-brain axis, plays important physiological roles in digestion, metabolism, immunity, and cognition. Stress can have wide-ranging effects on

the gut-brain axis. Socially living animals, like the prairie vole (*Microtus ochrogaster*), are greatly impacted by social stressors. Here we explore how social stress in prairie voles affects the gut-brain axis. To explore the relationship between social isolation stress and the gut-brain axis, we measured corticosterone, nutrient absorption, and gastrointestinal transit time in paired and isolated prairie voles throughout a six-week period. We found that while there was not a significant difference between gastrointestinal transit times in the paired and isolated groups, corticosterone concentrations were significantly greater for the isolated voles. We also will report data on plasma glucose and triglyceride levels to examine potential differences in gut absorption. Current ecological factors including climate change, habitat destruction, and even pandemics can create social stressors for socially living animals, including humans. Our work provides insight into how social stress alters aspects of gut functioning that can affect organismal performance and health.

Do nestling European starlings "exercise" in anticipation of fledging?

Kate Earle, Brett Hodinka, Joshua Allen, Tony Williams

Humans benefit from physical exercise (e.g. increasing aerobic capacity) in anticipation of increased physical effort or workload (e.g. running a marathon). Given that free-living animals face similar rapid transitions in workload, do they also exercise? Here we predicted that nestling European starlings (*Sturnus vulgaris*) would utilize activity in the nest (as a form of "voluntary exercise"?) in anticipation of the increase in workload (i.e. onset of active flight) associated with the rapid transition from sedentary nestling to volant, active fledgling. We recorded nestling behaviour for 5 days prior to fledging (days 17-21) including a) "active behaviours", e.g. wing flapping and stretching, walking and jumping, and b) "passive behaviours" e.g. sitting, standing, and perching. Additionally, we measured somatic developmental traits (mass, wing, tarsus) and obtained blood samples from the nestlings one day before their predicted fledge date to assess physiological development. We predicted that a) active behaviours would increase in frequency and duration approaching fledging, b) that if this reflected "voluntary activity" it would be independent of brood size (cf. simple social crowding effects which should correlate with brood size), and c) that, if activity functions as "exercise" then increased nestling activity will positively correlate with somatic and physiological developmental traits at fledging.

Anaplasma marginale alters metabolic rate of adult male Dermacentor andersoni ticks

Kayla Earls, Shelby Jarvis, Susan Noh, Kennan Oyen

Anaplasma marginale is a tick transmitted bacterial pathogen that causes bovine anaplasmosis, a production limited disease of cattle found worldwide. Upon acquisition, *A. marginale* enters and replicates in the midgut. During the acquisition feed, *A. marginale* migrates to the salivary glands, undergoes a second round of replication and is transmitted to a new host. The metabolic cost of acquiring and hosting *A. marginale* is unknown and may impact tick fitness and longevity. For the acquisition feed, adult *D. andersoni* fed on either an uninfected or *A. marginale* infected calf. A subset of ticks were incubated at 26°C for 7 days and allowed to transmission feed on a naïve calf. We then measured VCO₂ and body mass both acquisition and transmission fed ticks at 1, 4, and 7 days post feeding. Body mass of infected and uninfected ticks decreased over time, but at a greater rate in uninfected ticks ($F_{2,133} = 10.4$, $p < 0.0001$). Absolute metabolic rate of ticks peaked at 1 day post feeding and declined through day 7 ($F_{2,247} = 62.9$, $p < 0.0001$). Infected ticks had significantly lower mass-specific metabolic rates following both acquisition and transmission feeding ($F_{1,218} = 22.8$, $p < 0.0001$). Unexpected changes in metabolic rate of infected ticks could be due to interactions between blood digestion and pathogen development in the tick or suggest a role for infection in tick metabolic suppression.

Automated tracking of behavioral responses to stressors across populations and species in *Bombus*

August Easton-Calabria, Madalyn Laskowski, James Cral

Pollinators and other insects across the globe now face a variety of novel anthropogenic stressors, many of which have no historical analog. Bumblebees are important native and generalist pollinators among bees and are integral components of ecosystems across the globe, but recent studies suggest that their populations are declining; these declines pose significant risks to ecosystems and food systems worldwide. Not all bumblebees have responded to these anthropogenic stressors the same way, with some species even increasing in abundance. It is therefore critical to understand why and how sensitivity to environmental stressors differs across bumblebee species and populations. Here we present the BumbleBox - a low-cost, open-source and high-throughput tracking system used to monitor individuals within bumblebee colonies. This system allows us to quantify individual and colony behavioral dynamics in response to anthropogenic and environmental stressors, and to study the potential for population-level adaptation across species. In particular, we examine the effects of extreme temperature (10°C-37°C) and pesticide exposure (imidacloprid, 10 ppb) on the behavior of bumblebee colonies (*Bombus impatiens* and *Bombus bimaculatus*) reared from wild-caught queens of two distinct populations - one with a history of high imidacloprid use, and the other with much lower historical usage. Broadly, our results highlight the importance of behavioral research across *Bombus* to predict the nuances of how different species and populations will respond to intensifying climatic and anthropogenic stressors.

Multiple origins of tuber formation - Evolution of a unique storage organ

Patrick Edger

Plant galls are unique, often complex organs formed by plants in response to signals from a range of parasites. They are generated by the parasite's ability to alter the host plant's gene transcription, reorienting developmental and differentiation trajectories of cells and tissues. While gall development arises from transcription of plant genes, the initiation and formation of plant galls are completely controlled by the parasite. Galls are said to be an example of an "extended phenotype" of the parasite and as such the gall is an adaptive trait of the gall maker. The ability to elicit gall formation has evolved independently many times over 300 million years. Galls share many traits. At the most fundamental level they provide specialized nutrition and protection from environmental threats. At another level, transcriptomic analyses are revealing molecular mechanisms shared across insect orders. And many galls elicited by unrelated parasites exhibit nearly identical phenotypes, suggesting either an advantage to or constraints on such forms. While our understanding of gall development is in its infancy, we will discuss factors likely influencing convergence in gall form and provide a preview of mechanisms likely involved in their development.

Salish Sea Biodiversity Genomics: Over 100 species sequenced with outside genomes used in assembly

Eric Edsinger, Michael Kieras, Stacy Pirro, Leonid Moroz

Recent large-scale initiatives in Biodiversity Genomics utilize advanced sequencing technologies to generate chromosome-scale reference genomes across diversity. Released assemblies have enabled transformative insights and are rapidly growing in number. However, financial costs can be significant and technology access and DNA requirements can be challenging, which can make feasibility for local species problematic. Parallel strategies offering simple lower cost alternatives can enable diverse research communities to sequence local biodiversity. One approach is to leverage accurate yet inexpensive and accessible short-read sequencing and then improve fragmented assemblies using outside genomes of closely-related species. Here, we report on student and community-collected, Illumina-sequenced, and Spades-Zanfona reference-assembled draft genomes for over 100 marine eukaryotes from the Salish Sea (Pacific Northwest, North America). We find small tissue samples of diverse species transferred to 70% ethanol and stored at room temperature did well in Illumina sequencing despite likely DNA

fragmentation, allowing easy preservation, even for remote field samples, and enabling use of ethanol-stored specimens from research collections. Assessing Zanfona reference assembly performance in snail, urchin, barnacle, hermit crab, and octopus clades, we find improvements are limited to cases where high-quality reference genomes are closely-related, and where there is optimized short-read coverage, but can produce reasonable draft genome assemblies. Overall, our work provides new genomic resources to advance knowledge, conservation, and use of Salish Sea species and highlights accessible approaches in Biodiversity Genomics.

Head rotation impacts the force required for penetration in damp granular media

Charles Edwards, Henry Astley

Increased penetration force makes damp granular media a challenging environment for burrowing organisms, but cohesion between particles allows for permanent deformations such as the creation of tunnels. Some limbless squamates use head rotations during locomotion to excavate and/or reduce the forces required for penetration through cohesive granular media. However, these animals and their motions are difficult to study. To examine the effect of head kinematics on burrowing forces, a robophysical model was constructed with a cylindrical body and a moveable head. Head rotation and shaft extension are actuated by hydraulic pistons driven by stepper motors, while load cells placed between the motors and driving pistons measure the forces involved during actuation. Horizontal head rotation coupled with forward penetration through damp sand resulted in a 23% decrease in the required penetration force compared to penetration without head rotation. However, increasing the rotation angle from 17.4 degrees to 27.5 degrees had little additional effect, suggesting a diminishing benefit to increasing head rotation amplitude beyond an optimal excursion angle. Ongoing experiments are examining the effect of rotation amplitude and intermittent motion on penetration force. Overall, the mechanics involved in locomotion through damp granular media are understudied, and this research helps to address that knowledge gap.

Using phylogenies to connect genomic and phenotypic variation under convergent evolution

Scott Edwards

Connecting genotype and phenotype is of ongoing interest in evolutionary biology. Comparative genomics is now allowing us to map genes for traits using phylogenetic approaches ("PhyloG2P"), which leverage phenotypically unique lineages or convergent evolution to provide surprisingly precise mapping of loci underlying evolutionarily labile traits. The growing family of statistical models known as PhyloAcc can help identify genomic regions associated with evolution or loss of a phenotypic trait using phylogenetic trees. Three statistical models in the PhyloAcc family include associating rates of genome change and change in a binary or continuous trait, with and without the assumption of incomplete lineage sorting. New software implementations on Github greatly ease data analysis and allow rapid screening of thousands of loci. Such models will improve our power to detect associations between genome and phenotype evolution, particularly in situations of convergent evolution. An example focusing on loss of flight in birds reveals a strong role for non-coding regulatory evolution in the origin of key adaptations of birds. Functional tests, including measuring chromatin states using ATAC-seq and high-throughput enhancer screens help sift through hundreds of potential candidate enhancers whose evolution could influence traits associated with loss of flight.

A distributed visual system of pigmented eyespots arose within a genus of tropical New World chitons

Douglas Eernisse, Daniel Speiser

Recent experiments have demonstrated that pigmented aesthetes sensory organs, termed eyespots, are found in Caribbean species of Chiton and provide these chitons with visual capabilities, possibly to seek shelter when threatened. More typical chitons have unpigmented aesthetes in their shell

plates that are still photosensory; they are effective in detecting shadows. However, unlike eyespots, they apparently cannot detect spatial information about light. The visual capabilities of distributed eyespots are surprising given that these organs are so much smaller and simpler than the more elaborate shell eyes that likely evolved independently in more distantly-related genera of chitons. However, the phylogenetic distribution of pigmented eyespots within the approximately 85 species of the subfamily, Chitoninae, which includes Chiton, is unexplored. We have undertaken a survey of pigmented eyespots coupled with molecular estimates of phylogeny across Chitoninae and have found eyespots only in a monophyletic grouping of tropical New World members of Chiton. We decalcified shell plates from selected worldwide Chitoninae species and visualized whether the aesthetes systems included pigmented eyespots. One caveat is that the pigment appears to be somewhat labile, becoming less apparent in older specimens. Preliminary evidence suggests that the pigmented eyespots are restricted to a monophyletic grouping of only about 11 New World tropical species of Chiton, implying a very recent origin of eyespots and providing opportunities for comparative investigations of these distributed visual systems.

Investigating glucocorticoid abundance across development in glassfrogs (*H. fleischmanni*)

Emily Terrill, Abigail Stevenson, Eva Fischer, Lisa Surber

Glucocorticoids are steroid hormones that mediate many physiological processes including metabolism, development, and the stress response. There are two main glucocorticoids in vertebrates: cortisol and corticosterone. Historically, animal taxa have been labeled either cortisol or corticosterone dominant. Due to the abundant literature on corticosterone dominance in amphibians and the well-established role of corticosterone in amphibian metamorphosis, most studies on amphibians exclude cortisol. However, given mounting evidence for complexity in glucocorticoid patterns within and across taxa, we were curious whether and how glucocorticoid abundance might change across life stages of our focal species, the glass frog *Hylinobatrachium fleischmanni*. Using non-invasive waterborne hormone measurements, we measured cortisol and corticosterone from the same individuals across six life stages: early, mid, and late stage tadpole, froglet, juvenile, and adult. We find that glucocorticoid abundance varies across life stages with a surprising reversal in the balance of cortisol versus corticosterone following metamorphosis. Our results showcase the dynamic and nuanced role of cortisol and corticosterone throughout development.

Global coordination using local information in fire ant pontoon bridges

Noah Egan, Haolin Zeng, Ram Avinery, Hosain Bagheri, Shengkai Li, Takao Sasaki, Daniel Goldman

Fire ants (*S. invicta*) can collectively entangle to create free-floating rafts which allow the colony to survive upon nest flooding. Here we demonstrate that they can also create structures which allow foraging across a water surface. Laboratory experiments using sub-colonies of 8000 fire ants demonstrate that when the ants detect food in the center of a 14 cm diameter bowl filled with water, they can reach the food by self-assembling into a floating pontoon bridge consisting of approximately 500 ants. Multiple proto-bridges initiate from the boundary; the ants lengthen proto-bridges by directly entering the water. Ants also use the water meniscus at the bowl rim to propel themselves into the water, aggregating into floating rafts that later attach to proto-bridges. Within two hours, most proto-bridges retract and a single final bridge forms, suggesting global coordination. However, our agent-based model suggests that a local behavior, the tendency for individual ants to join proto-bridges more often in areas with stronger food scent, can cause the colony to form a single bridge. This implies that the formation of single bridges can be modulated by environmental conditions. The model prediction that ants form multiple bridges in a bowl with nonuniform distance between the food and rim was verified in experiments, thus giving insights into how collectives can coordinate over long distances using only local information to create functional structures.

High infectivity and waterborne transmission of seagrass wasting disease

Morgan Eisenlord

Seagrass wasting disease (SWD), caused by *Labyrinthula zosterae* (Lz), impacts temperate seagrass beds worldwide. We quantified SWD transmission in eelgrass, *Zostera marina* (Zm), meadows through field and laboratory experiments. Although transmission pathways are fundamental to understanding the epidemiology of infectious diseases, they are poorly understood in the ocean, and little is known about the natural transmission of SWD. To test transmission modes in nature, we ran three field experiments out-planting sentinel eelgrass shoots within and 100-120 meters adjacent to natural eelgrass beds. Infection rates and severity did not differ significantly among outplant locations, implicating significant water-borne transmission. The temperature-dependent infectious dose of Lz through waterborne exposure was assessed in a controlled laboratory experiment. The dose to 50% infection was only 6 cells mL⁻¹ and did not differ significantly with the temperatures tested (7.5°C and 15°C). SWD prevalence and severity also did not differ significantly with temperature. Our results show Lz is highly pathogenic and readily transmits through water without direct contact with infected plants. Understanding the complex transmission dynamics of this disease in the context of changing ocean conditions will improve eelgrass protection and restoration in critical coastal habitats worldwide.

A preliminary analysis of tag-derived buoyancy suggests basking sharks do not drawdown lipid reserve

Jaida Elcock, Martin Arostegui, Simon Thorrold, Camrin Braun, Gregory Skomal

Basking sharks (*Cetorhinus maximus*) are large, filter-feeding sharks that are known to make seasonal, large-scale migrations from temperate waters of the North Atlantic Ocean to the (sub)tropics, sometimes making trans-Atlantic and trans-equatorial movements spanning more than 17,000 km. However, the functional role(s) and energetics of these migrations remain unknown. Based on an existing dataset of pop-up satellite archival tag deployments on basking sharks in the Northwest Atlantic, we used 845 days of high-resolution depth time series measurements from a subset of 10 individuals to test whether drift dives isolated from these data can be used to detect buoyancy changes over the course of migration. Our results suggest drift dives can be isolated from depth data but are sensitive to the temporal resolution of these measurements. While all individuals exhibited extensive use of the mesopelagic during winter, poor data recovery among transmitted datasets (up to 95% of days missing high resolution data) and variable migration timing hindered our ability to quantify drift rates over a representative portion of the migration cycle. Our results do not indicate significant changes in drift rate during migration, suggesting basking sharks do not drawdown lipid reserves to fuel migration.

Effects of body size on the energetics of weight-bearing roaches

Be Eldash, Rudolf Schilder

Most animals carry loads during their lifetime. Due to unequal scaling of body mass and force-generating muscle architecture, carrying a load proportional to body mass may be more demanding for large animals than for small animals. We ask if the ability of animals to adjust to external loads scales with body mass. Weight-loaded mammals can make skeletal muscle adjustments and expend energy at levels similar to that of unloaded mammals during low-intensity activity. Whether this is true at high-intensity activity is unknown. We hypothesize that plasticity will be inversely related to the intensity of activity so that, at rest, loaded animals can maintain energetics comparable to that of unloaded ones. As intensity increases, plasticity will be limited resulting in an increased energy expenditure in loaded animals. Hemimetabolous insects are convenient models to test this hypothesis as their body mass increases significantly during development while maintaining a similar shape and mechanics of locomotion. In this study, we explore how metabolic rate during rest and high-intensity activity of *Blaberus discoidalis* roaches change in response to the addition of a load proportional to body mass across several stages of development.

Using diceCT to describe the musculature of the mystacial pad in Harbour seals (*Phoca vitulina*)

Alyx Elder, Robyn Grant, Elizabeth Evans

Pinniped whiskers are the most sensitive and specialised of all mammals. Like most mammals, pinnipeds have the capacity to control whisker movements, suggesting the presence of intrinsic muscles - sling-like muscles that drive whisker protractions. While intrinsic muscles have been observed in whisker follicles of some pinniped species, and extrinsic muscles defined in Bearded Seals (*Erignathus barbatus*); it is challenging to describe the arrangement of muscles across a whole pinniped mystacial pad using histology, due to the pads large size and curvature. However, intrinsic and extrinsic muscles can vary in size and arrangement between species, so descriptions are needed for more pinniped species.

For the first time, we adopt diffusible iodine contrast-enhanced Computer Tomography (diceCT) to describe the three-dimensional structure of whisker muscles throughout the mystacial pad in Harbour seals (*Phoca vitulina*). Frozen, dissected pads from three Harbour seals were scanned in the Nikon High Flux Bay. Images were compared between unstained and iodine-stained scans (immersed for 28 days). Muscles were observed using both techniques, although contrast was significantly improved when stained, enabling easier segmentation. Using diceCT we describe the mystacial pad muscles in Harbour seals for the first time, validating the importance of 3D-visualisation in mystacial anatomy. Such detailed descriptions can be used to conduct comparative analyses and test associations of muscle size and arrangement with foraging style or depth in pinnipeds.

Intraspecific variation in thermal tolerance in the endangered Caribbean coral, *Acropora palmata*

Holland Elder, Sibelle O'Donnell, Sophia Lee, Eleftherios Karabelas, Daniella Leon, Maria Ruggeri, Courtney Klepac, Erin Muller, Carly Kenkel

The region-wide decline of *Acropora palmata* has significant implications for the structure and function of Caribbean reefs as this is the only species capable of building the reef-crest habitat zone. Nursery bred coral can be used to restore these habitats, but the ultimate success of these efforts depends on whether there is sufficient genetic diversity and thermal resilience in restored populations to survive and reproduce in a rapidly changing climate. We quantified thermal tolerance of 150 sexually produced *A. palmata* genotypes in response to a one-month long tank-based thermal stress experiment. Survival was significantly reduced in the heat treatment, with heat-treated genotypes incurring a 4.7 times higher mortality risk on average. However, there were also significant differences in survival among genotypes under heat treatment, with an order of magnitude difference in hazard ratios between the worst-performing and best-performing genotypes. Photophysiological data suggest that differential mortality was largely driven by host susceptibility as photosynthetic ability of the algal symbionts did not appear impaired. Genetic differentiation in coral hosts and dinoflagellate endosymbionts may also drive variation in heat susceptibility among genets and integration of these additional data will shed light on underlying mechanisms with the overarching goal of improving techniques to support future interventions through identifying stress tolerant coral individuals and genes.

Chronotype varies but is repeatable across the breeding season in a female songbird

Emily Elderbrock, Geoffrey Brown, Ned Dochtermann, Holland Galante, Michaela Hau, Timothy Greives

Wild animals organize their activity around a 24-hour day and optimizing daily timing across the year may be advantageous for both survival and reproductive success. Variation in chronotype, or when individuals begin or end their active day relative to a cue such as photoperiod, often exists within a population. Both intrinsic and extrinsic factors contribute to this variation and activity patterns may vary further during reproduction as investment in young changes. Here we describe population levels changes in female great tit (*Parus major*) activity patterns of onset and offset of activity as well as assess variation and repeatability in daily activity both within- and across-breeding stages. We fitted individuals with accelerometers to track activity beginning

prior to egg laying through chick rearing. Prior to clutch initiation females began their active day before sunrise, however just prior to laying activity was delayed until after sunrise. Females ceased activity prior to sunset across the monitoring period but ended activity earliest during egg laying and incubation. Female great tits exhibited the most consistent behavior pre-laying with greater variance in chronotype during parental care. Individual female daily activity was moderately repeatable within breeding stages and strongly correlated across several breeding stages, indicating consistency across parts of the season. These findings contribute to our understanding of both individual variation during reproduction and the fitness implications of chronotype in wild animals.

Scaling of induced power from dragonflies suggests that griffenflies were under-powered

Olaf Ellers, Caleb Gordon

Paleozoic skies were ruled by extinct odonate insects called "griffenflies", some which had wingspans three times that of the largest extant dragonflies and ten times that of average extant dragonflies. Previous studies have suggested that flight was made possible for larger fliers because of higher atmospheric oxygen levels that would have increased air density. We use actuator disk theory to evaluate this hypothesis. Actuator disk theory gives similar estimates of induced power as Ellington (1999) estimated for micro-air vehicles based on insect flight. We calculate that for a given mass of griffenfly, and assuming isometry, the contribution of oxygen to a higher density would only have reduced the power required to fly by about 11%, which would be enough for a flier 3% larger in linear dimensions. Further accounting for the higher power available due to high oxygen air, and assuming isometry, we calculate that the largest flyer would have been only 1.2 times larger in linear dimensions than extant dragonflies. We also consider known allometry in dragonflies (May, 1981) and estimated allometry in some extinct griffenflies (Clapham & Karr, 2012). But such allometry only increases the possible larger flyer size to 1.23 times linearly larger. We conclude that Meganeuran griffenflies had different flight patterns than modern dragonflies.

The influence of calcium regulation on migratory and thermoregulatory phenotypes of songbirds

Cory Elowe, Alexander Gerson, Maria Stager

Migratory birds undergo substantial physiological changes to prepare for long-duration endurance flight, including hyperphagia, fat deposition, a reliance on fat as a fuel source, and flight muscle hypertrophy. Similar changes may appear in birds acclimated to cold winter conditions. Sarcolipin (SLN) is a protein that binds to sarco/endoplasmic reticulum calcium-ATPase (SERCA) and in mammals this has been shown to uncouple calcium transport from ATP hydrolysis, exacerbating energetic costs and increasing heat production or signaling for mitochondrial biogenesis, fatigue resistance, and a shift to fatty acid oxidation with reduced obesity. We have been examining the role of SLN and calcium signaling in the cold-acclimated and migratory phenotypes through transcriptomic analysis of flight muscle in migratory condition and cold acclimation and measurement of SERCA activity and calcium pumping efficiency in the muscle. Contrary to expectations, muscle SLN transcription appears to increase in migratory songbirds, but is suppressed in response to cold temperatures. SLN is strongly associated with fat stores, but we did not find direct functional changes to SERCA calcium pumping efficiency, suggesting that SLN may be involved in metabolic shifts that either lead to fattening or respond to lipid oxidation. Given that SLN is associated with reduced obesity in rodent studies, it appears that the role of SLN differs in birds. Furthermore, the suppression of SLN transcription in the cold suggests that active shivering exercise and long-day photoperiods alter physiology through different mechanisms despite similar phenotypic outcomes.

Applying the Phenotype-Performance-Fitness Paradigm to Explore Aerial Combat in Hummingbirds.

Rosalee Elting, Md Zafar Anwar, Donald Powers, Bo Cheng, Haoxiang Luo, Bret Tobalske

The “Phenotype-Performance-Fitness” paradigm introduced by Arnold continues to motivate study of the complex covariance among morphology, physiology and fitness. Most applications of this paradigm focus on escape from predation, and measures of fitness are often elusive. Here we expand use of the paradigm to explore combat in territorial hummingbirds. Combat outcomes between individuals are regularly assumed to reflect success at obtaining mates and/or food resources. There may be tradeoffs between these benefits to fitness versus energetic costs of fighting and of growing and maintaining flight morphologies for maneuverability. These tradeoffs have been studied extensively in some taxa (e.g. ungulates), but remain largely unstudied in hummingbirds. We predict energetic tradeoffs to be important in this clade as their flight requires extremely high mass-specific power. Using calliope hummingbirds (*Selasphorus calliope*, $n = 4$) in the lab, we measured phenotypes (mass, span efficiency, load lifting, kinematics) and their predictive power on outcomes: metabolic rate before and after fights, resource use and dominance hierarchy. We also measured flight kinematics in the field where new resources were not introduced and it is expected that dominance hierarchies were pre-established (to study if fights differ from those used to establish hierarchies). In contrast with escape maneuvers that are stereotypic and last < 200 ms, combat in hummingbirds occur over multiple seconds and feature a rich array of maneuvers. ONR N000141912540

The metabolic cost of fighting-related injuries

Zachary Emberts, Ummat Somjee

Many animals often fight with other members of their species. When individuals engage in these fights, injuries can occur. Most theoretical fighting models suggest that the costs associated with these injuries should influence an individual's decision to retreat from a fight. Thus, damage from intraspecific combat is frequently noted and quantified. However, much remains unknown about the actual costs associated with fighting-related injuries. Here, we investigate whether the number of fighting-related injuries and/or the location of fighting-related injuries influence their metabolic cost in the giant mesquite bug, *Pachylis neocalifornicus*.

Identifying shared and host-specific transcriptomic characteristics of the cnidarian-algal mutualism

Madison Emery, Emily Van-Buren, Kelsey Beavers, Laura Mydlarz

Throughout the cnidarian phylogeny a mutualistic intracellular symbiosis with photosynthetic dinoflagellates in the family Symbiodiniaceae has independently evolved multiple times. Given these instances of convergent evolution, the mechanisms of symbiosis establishment and maintenance within symbiotic cnidarians may vary. The symbiosis mechanisms are likely most disparate between symbiotic scyphozoans and symbiotic anthozoans, as they host their symbionts in different cell types. However, even distantly related symbiotic cnidarians share some characteristics, such as the suppression of immunity while hosting symbionts. Here we compare gene expression data from two facultatively symbiotic cnidarians representing two independent evolutions of the cnidarian-dinoflagellate symbiosis, the upside-down jellyfish *Cassiopea xamachana* and glass anemone *Exaiptasia diaphana*. To compare these distantly related species we identified orthologous genes that were then used to contrast differential gene expression analyses and weighted gene co-expression networks to identify similarities and differences in the species' strategies for hosting Symbiodiniaceae intracellularly. Our preliminary results indicate that of the overlapping differentially expressed genes, over half of them have differing directionality. Despite these clear differences, both species show evidence of innate immune genes being suppressed in a symbiotic state. These data can help differentiate core characteristics of the cnidarian-algal symbiosis from host or taxa specific characteristics.

Developmental diversity among brittle stars from the Caribbean coast of Panama

Richard Emlet

Along the Caribbean coast of Panama, shallow water ophiuroids occur abundantly in wide range of habitats including coral reefs, seagrass beds, and mangrove sediments. In late summer and fall many species of brittle star spawn soon after collection, and I used this opportunity to examine development of three species with reduced larval forms and several others with non-feeding, vitellaria larvae. Reduced larvae of two species had mouths and complete guts but did not ingest algal cells; their development was so rapid that facultative planktotrophy seems unlikely. The formation of a feeding larval body that does not need to feed suggests these species may have recently lost the ability to feed and are transitioning to nonfeeding forms. Comparisons with descriptions of other species with modified development reveals repeating (and likely convergent) patterns within this echinoderm class and with other echinoderms in tropical shallow water habitats.

Singing in the heat: Transcriptomic signatures of thermal resilience in a songbird

Tara Empson, Melissa (Misty) Proffitt, Susan Cantonwine, Maëlle Lefeuve, Emily Levy, Kimberly Rosvall, Elizabeth Derryberry

There is an urgent need to understand how warming temperatures affect animal behavior. Mating signals may be particularly vulnerable. Even endotherms like birds, are experiencing sublethal heat effects. For example, we know that heat can reduce the rate at which male zebra finches sing. However, some males are more behaviorally resilient than others. Here, we take an organismal approach to identify the mechanisms underlying individual differences in behavioral resilience. Using male zebra finches (*Taeniopygia castanotis*), we measured song production during a standardized heat challenge. We then measured gene expression via RNAseq in both the brain and syrinx (vocal control musculature). We predict behavioral resilience will covary with transcriptional patterning, particularly in neural pathways of reward and syringeal pathways of muscle function. Results will lend insight into how at least some individuals are evading the deleterious effects of heat on adaptive behaviors.

Enhancing 3D Models with RIPPLE: Overcoming Overlap Constraints for Accurate 3D Reconstruction

Catherine Eno, Christin Murphy, Brooke Flammang, Audrey Kellogg, Mackenzie Damon

Photogrammetry is a measuring technique that assembles a 360° series of overlapping photos of an object and reconstructs a highly accurate three-dimensional digital model. A fundamental photogrammetry rule is that a higher degree of photo overlap leads to a more precise 3D model. Most photogrammetry software requires at least 60% overlap in the photos, and potentially up to 80% overlap depending on the software. However, obtaining this overlap is not always possible due to constraints in capturing enough images around the object, particularly during fieldwork. One way the overlap issues can be mitigated is through the use of RIPPLE, an open source toolbox of presets, developed by our team, currently available on GitHub for use in Adobe Photoshop. that enhances clarity of the images by eliminating glare, haze, shadows, and background noise. To evaluate the effects of RIPPLE, images of an inflatable mammalian model with varying percentages of overlap were taken and analyzed with and without RIPPLE presets in the photogrammetry software Meshroom. We found that the RIPPLE models required less overlap to produce more morphologically accurate 3D models compared to those without RIPPLE. Consequently, our findings illustrate the benefits of incorporating RIPPLE as an intermediate step when developing photogrammetric models as it reduces the amount of overlap necessary to create accurate 3D models.

Oncogenic RasV12Induces Cellular Senescence in Avian Species

Reihane Eric, Joshua Shirazi, Amir Hosein Sanjari-Nia, Chidambaram Ramanathan, Yufeng Zhang

One basic process that may contribute to age-related dysfunction and chronic sterile inflammation is cellular senescence. Cellular senescence refers to the essentially irreversible growth arrest and undergo distinctive phenotypic alterations, including profound chromatin and secretome changes, and tumor-suppressor activation. Activation of certain oncogenes can cause senescence-inducing genotoxic stresses, which resulted in cellular senescence in primary cells. RasV12is oncogene Ras carries an alanine to valine mutation at codon 12, where overexpression of RasV12 induces premature senescence rather than transformation in primary mammalian cells. Even though overexpression of RasV12 has been intensively studied in laboratory rodents and human cells, but its effects on primary cells from avian species is unknown. Here, we first generated an inducible lentiviral plasmid vector of RasV12using chicken Ras sequences. Then we infected primary dermal fibroblast from Chicken Gallus gallus and Zebra Finch Taeniopygia guttata with lentiviral system, and induced cellular senescence for these cells. Cellular senescence was quantified by measuring the β -galactosidase levels, cellular metabolic function and SASP profile. We found that cells from these two species could be induced senescence by RasV12overexpression similar to their rodent and human cells counterparts. However, the secretory and metabolic profile between species varies.

Evidence for independent origins of avian iridescence

Yasmeen Erritouni, Alejandro Rico-Guevara

Iridescent plumage is present in nearly all major radiations of birds, but it remains unknown if this trait is the result of shared ancestry or of independent evolution. Disparate avian clades produce iridescence using the same basic components – melanin and keratin – but use unique morphologies to do so. This study uses a transcriptomic approach to determine whether patterns of gene expression are shared across groups of birds that have converged on iridescence. Similar transcriptomes may suggest either strict genetic requirements for iridescence or conserved ancestral genetic characters. We first identified the genes responsible for iridescent nanostructures through a transcriptomic comparison of developing iridescent and non-iridescent feathers in wild Anna's (Calypte anna) and Rufous (Selasphorus rufus) hummingbirds. We then compared these hummingbird transcriptomes to those of superb starlings (Lamprolornis superbus) from a previous study to elucidate whether distantly-related groups of birds produce iridescent feathers using similar genetic mechanisms. The transcription profiles of the two hummingbird species were more similar to one another than either was to the starling transcription profiles. These results lend support for independent origins of iridescence in the Trochilidae and the Sturnidae despite similar morphology and function. Continued collection of feather transcriptomes from distantly-related species will cultivate an understanding of the genetics and evolution of iridescence in birds.

Recovering from voluntary bodily damage: Robust locomotion in Opiliones

Ignacio Escalante

Animals have evolved behavioral and morphological adaptations to deal with environmental challenges. However, these adaptations may have long-term consequences that could impact an animal's performance and fitness. I will focus on the arachnid group of Opiliones, which voluntarily release their legs to escape predators. These animals use their legs for locomotion, sensory perception, and reproduction. In field surveys of the tropical species of Prionostemma, I have found that more than half of the individuals are missing legs. I will review recent work on the ultimate and proximate implications of leg loss in these arachnids. First, with field experiments, I found that Opiliones did not experience fitness consequences, as leg loss did not affect their survival or mating success. Second, I explored the energetics of locomotion and found that oxygen consumption increased for animals that lost three legs but not one leg. Third, using biomechanical tools, I found that leg loss compromised

the kinematics of locomotion, but individuals recovered initial velocity and acceleration quickly. These findings demonstrate robustness and behavioral plasticity to compensate for bodily damage. With current and future work, I aim to demonstrate the biomechanical and neural mechanisms that allow Opiliones to compensate for leg loss. Ultimately, understanding how animals have adaptations to deal with environmental challenges has broad implications for biological systems, robotics, and biomedical design.

Astragalar and calcaneal shape predict locomotor mode in caniforms

Lauren Essner, Victor Munteanu, Andrew Orkney, Brandon Hedrick

Mammals have diversified into a wide array of different ecological niches and have evolved a diversity of locomotor modes for traversing those niches. Locomotor mode impacts the entirety of an animal's morphology and the ankle is of particular importance because it creates a flexible yet stable joint capable of supporting immense pressure and a versatile range of motion. Caniform carnivores are a model system for understanding the evolution of locomotor mode, with a rich variety of locomotor modes ranging from semi-aquatic otters to arboreal red pandas, making them an excellent clade within which to examine the relationship between morphology and locomotor ecology. We studied two bones within the ankle, the astragalus and calcaneus. The astragalus allows for distal limb rotation and flexion while the calcaneus creates torque used in high-powered movement. We examined a wide range of paired caniform astragali and calcanei ($n = 30$ species) spanning six locomotor modes (semiaquatic, semifossorial, terrestrial, cursorial, arboreal, scansorial). We hypothesized that ecological adaptation would be concentrated in the calcaneus, and that the astragalus would be more strongly related to phylogeny. We microCT scanned individual ankle bones and performed 3D geometric morphometric analyses in a phylogenetic context. We found that calcaneus and astragalus shape are integrated with one another and both depend significantly upon locomotor mode, which explains 41 and 34% of shape variation respectively.

Using the endemic Nesophrosyne leafhopper as a model system to Predict Adaptation to Climate Change

Miguel F Estrada-Caballero, Gordon Bennett

Insects are a primary component of terrestrial biodiversity, but this diversity still remains both poorly understood and at risk of climate-induced collapse. To understand how insects will be affected by climate change, we will leverage the Nesophrosyne leafhopper genus endemic to the islands of Hawaii. Individual in this genus are narrowly adapted to specific host plants and ecological ranges (e.g., desert, rainforest, and subalpine regions). Nesophrosyne further rely on the integrated contributions of essential amino acids from two obligate bacterial symbionts, Sulcia and Nasuia, which have undergone extreme genome reduction and require cellular support from their host. To understand how hosts and their symbioses jointly adapt to climate change, Nesophrosyne are sampled from across environmental gradients. Transect regions running from arid host coastal regions to subalpine environments will be run comparatively sampled on the islands of Maui and Hawaii. The two islands are ecological and evolutionary replicates, containing the same habitats but with distinct species that have convergently adapted to the same environmental conditions. This natural laboratory framework permits the study of processes leading to organismal adaptation and evolution in populations under selection. For each sampled population, genomic skimming techniques are used to monitor changes in allele frequencies across host and symbiont genomes. We will further measure physiology metrics to determine shifts in organismal and physiological responses to their environments.

Exposure to traffic noise changes baseline auditory neural activity and decision-making in crickets

Erik Etzler, Darryl Gwynne, John Ratcliffe, Hannah ter-Hofstede

Exposure to anthropogenic noise has been shown to be detrimental to a variety of animals. Evidence of whether exposure to road traffic noise over development affects crickets is conflicting. Females reared in traffic noise

have been reported to be either faster or slower to locate mates than those reared in silence. These behavioural studies did not identify if the results were due to hearing or decision-making changes. Here, we reared *Teleogryllus oceanicus* (Gryllidae) females in either traffic noise or silence, and then had them locate speakers playing male song with either traffic noise or silence as adults. We then recorded the activity of the AN2 auditory interneuron of adult females reared in both environments when listening to male song under traffic or silent conditions. Regardless of rearing condition, crickets were slower to leave the shelter in silence than traffic. Crickets were slower to leave the shelter if they were reared in traffic compared to in silence. In silence, crickets reared in traffic noise had higher baseline activity levels in their AN2 interneuron than those reared in silence, but rearing condition did not affect AN2 activity in response to song. Our results indicate that anthropogenic noise can not only impede mate locating ability, but also change hearing. Further, they highlight the idea that silence is an unnatural acoustic condition which may be perceived as a threat.

Comparative Anatomy of Otomorph Epibranchial Organs

Allyson Evans, L Patricia Hernandez, Josh Egan

The ability to extract small prey items from large volumes of water is widespread among microphagous fishes, all of which face the same metabolic hurdle of consuming enough food particles to meet their nutritional needs. Certain planktivorous and detritivorous fishes possess an epibranchial organ (EBO) in their posterior pharynx that facilitates the aggregation of small prey. Morphologically complex and phylogenetically diverse, EBOs are novel trophic structures that have evolved independently in at least six families across teleosts with the majority of both morphological and taxonomic diversity occurring within otomorph fishes. Otomorph EBOs range from the small slits in the roof of the pharynx to medioventrally expanded, papillae-lined pouches. Despite their phenotypic diversity, they share several anatomical components including an internal tube with an epithelium rich in mucus-producing cells, a surrounding layer of skeletal muscle, and support from the posterior branchial arches. Here, we present a comparative study of EBOs from thirteen otomorph species. Using gross dissection, histology, and scanning electron microscopy, we describe the degree to which architectural components of the EBO exhibit patterns of convergence in shape, size, muscularity, adiposity, and the morphology of the papillae that line their internal surface. Grounded within both a functional and phylogenetic framework, we discuss whether differences in this intricate anatomy can best be explained by specializations to a similar feeding ecology or by phylogenetic relatedness.

Backing that Wrasse up to the Next Generation of Morphological Data Collection and Analysis

Kory Evans, April Hugli, Mayara Neves

The ability to observe and quantify phenotypic variation and diversity is a core and central need for the fields of biology, functional morphology, biomechanics, and macroevolution. Within these fields, discovery has frequently tracked technological advancements associated with visualizing and analyzing morphological data. Over the past decade, and especially the last five years, micro-CT scanning has allowed researchers to collect high-resolution morphological datasets to explore the intricate and hidden features of organismal design to answer a wide variety of questions ranging from muscle architecture in archosaurs, to mosaic evolution in fish skulls. In this talk, we will explore the many ways that micro-CT scans have been employed to build a large, phylogenetically diverse dataset within an awe-inspiring family of fishes; the wrasses (family: Labridae) to answer a diverse range of questions ranging from convergent evolution, modularity, biogeography, ontogeny and more. We show striking cases of convergent evolution across the wrasses, we delimit the evolutionary and functional modules of the wrasse skull while also illustrating the unique and novel ways that the skull develops. We will also illustrate how the striking diversity of wrasse morphology has evolved across the world's oceans and where the morphological diversity has clustered over their 80-million-year history. Lastly, we will highlight future directions for this dataset and encourage others to build phylogenetically and morphologically diverse datasets of their own.

Comparing the Impacts of Human Disturbance and Marine Preserve Status in the Intertidal Community

Logan Evans, Christine Mantegna

Exploring the impacts of human disturbance on the rocky intertidal community has implications for how human interaction can affect community composition, organism abundance, diversity, and species richness. The efficacy of marine protected areas' role in preserving biodiversity has been widely debated. We found that areas of increased human disturbance lacking protective status appear to be less biodiverse. This finding provides context for implementing future marine preserves. We performed quadrat and abundance surveys on Yellow Island, a marine preserve in the Salish Sea to characterize the biodiversity of the intertidal. Utilizing community science data that used equivalent survey methods to ours from the Multi-agency Rocky Intertidal Network (MARINE) for two urban intertidal sites in the Salish Sea with intermediate and higher disturbance, we aim to answer the question of how protected status and human disturbance level impact community composition. Statistical analysis of meiofauna percent-cover and phyla counts reveals that the intermediate and higher disturbance areas have lower total phyla presence compared to the marine preserve low disturbance site. Increased disturbance sites are dominated by algal cover while the marine preserve site has increased non-algal phyla and algal phyla. With further research in eDNA and other biological traits, we can get a fuller picture of how disturbance and protection status impacts the intertidal community. This work highlights the importance of long-term monitoring projects in the marine environment.

Specializations of the chameleon pectoral and pelvic girdles to differing levels of arboreality

Cydney Even, Dakota John, Christopher Anderson

As organisms transition between terrestrial and arboreal lifestyles, their locomotor systems experience disparate functional demands. Chameleons are known for their array of distinct adaptations, including their ability to change color, their ballistic tongue projection, and their pincer-like feet and prehensile tails. The chameleon family, however, is incredibly diverse in their size, morphology, natural history, behavior, and the degree to which they inhabit arboreal vs. terrestrial environments. In fact, while chameleons are often generally regarded as arboreal specialists, many species spend considerable amounts of time, particularly during the day, on the ground, and some are even almost entirely terrestrial. We examined the impact of relative arboreality on the anatomy of the pectoral and pelvic girdles of 22 chameleon species using μ CT scans. These species represent all twelve described chameleon genera and, when possible, both highly arboreal and largely terrestrial taxa within each genera. Using phylogenetic comparative methods, we found consistent patterns of size and shape differences in girdle elements between arboreal and terrestrial species across the family. Beyond deepening our understandings of chameleon adaptations, this research also provides insights into how the pectoral and pelvic girdles adapt as species shift between arboreal and terrestrial habitats.

Which Genes Influence the Migratory Timing of Great Reed Warblers?

Emily Fackler, Robert Fitak, Dmitry Kishkinev, Petr Prochazka

Many organisms migrate, however there is a lack of knowledge on how, and which, genes play a role in migration timing. Great reed warblers (*Acrocephalus arundinaceus*), a migratory bird that breeds in Eurasia and overwinters in Africa, are an excellent model to study the genetics of migration. Each season, some individuals migrate early, and some migrate late. In other words, they exhibit differences in their migratory chronotypes. Past research has found that there are three candidate genes (TOP1, PEAK1, and CPNE4) that influence the migratory chronotypes of American kestrels. In this study, we first explore the relationship between these three candidate genes and the migratory chronotypes of great reed warblers. However, we have not seen a relationship between migratory chronotype and the three candidate genes, as seen with kestrels. Subsequently, we have taken a whole-genome approach to identify additional candidate genes. Utilizing a method called pool-seq, we have combined DNA of our earliest spring migrants into one pool and DNA

of our latest spring migrants into a second pool. Whole genome sequencing has been completed on these two pools to compare the genetic variation between the early and late chronotypes. We will present our findings on whether there are additional candidate genes that influence the migratory timing of great reed warblers. Our results will provide insight into how genes influence migratory timing behavior in birds.

Discovering the Dark Side of Fireflies: Fishing Around for Pheromones

Greg Fahrner, Zhangyi Wu, Katie O'Connor, YIYU ZHENG, Nathan Peot, Sean Halloran, Jocelyn Millar, Douglas Collins, Gregory Pask

Insects have evolved highly specialized mating communication strategies using a variety of sensory modalities, such as acoustic, visual, or olfactory signaling. A subset of firefly species has lost the ability to produce light as adults and are believed to attract potential mates using volatile sex pheromones. One of these species found in the eastern United States, *Lucidota atra*, is day-active and can serve as a model for understanding the evolutionary mechanisms underlying the switch from visual to olfactory communication. Here we describe our efforts to determine if *L. atra* females are using volatile pheromones to attract mates and to isolate the active component. Using field-collected female *L. atra*, headspace volatiles were collected in an active aeration apparatus and screened for bioactivity in a multi-choice behavioral assay with males. Promising chemical extracts were further tested using coupled gas-chromatography–electroantennographic detection (GC-EAD) to identify the active molecule among the mixture of headspace compounds. Further work to characterize these candidate pheromones in the field are ongoing and will provide deeper insights into the evolutionary processes associated with shifting mating communication strategies.

Neurons to movement in Hydra

Adrienne Fairhall

The cnidarian Hydra is a fascinating model organism for neuroscience. It is transparent; new genetic lines allow one to image activity in both neurons and muscle cells; it exhibits a quite rich suite of behaviors; and it continually rebuilds itself. Hydra's fairly simple physical structure as a two-layered fluid-filled hydrostat and the accessibility of information about neural and muscle activity open the possibility of a complete model of neural control of behavior. We have developed a biophysical and biomechanical model of Hydra's muscles and body that allows us to transform measured neural activity into behavior, and a neural model that proposes a mechanism driving its contractile oscillations.

Investigation of muscle and nerve tissue resiliency in mice, *Mus musculus*

Samantha Falcone, Samantha Gartner, Thomas Roberts

Both skeletal muscle and peripheral nerves generate force passively when stretched. A collagenous extracellular matrix (ECM) contributes to passive force in both tissues, but the organization of the ECM differs between muscle and nerve. A comparison of muscle and nerve mechanical behavior might inform our understanding of how ECM structure determines function. We compared the resiliency (fraction of energy returned after stretch) of muscle and nervous tissue, with a specific focus on locomotor muscles and peripheral nerves. Soleus muscles and sciatic nerves were isolated from six mice and attached to a 50g servo motor. The isolated tissues underwent periods of sinusoidal length changes ranging from 1-15% strain and 1 to 10 Hz. Both nerve and muscle showed significant loss of energy with each cycle, with preliminary results indicating greater resiliency in the sciatic nerve compared with the soleus muscle. The mechanisms of passive force production in these tissues are not fully understood, in particular the relatively low resilience of passive muscle has not been explained. A future aim of this work is to determine whether differences in ECM architecture underlie differences in mechanical behavior of muscle and nerve.

The genetic basis of sexual dichromatism: hints from a female-polymorphic hummingbird

Jay Falk, Georgy Semenov, Scott Taylor

Despite a great deal of attention to the adaptive function of color differences between sexes, little is known about the genetic mechanisms that produce these differences. Identifying the genetic basis of sexual dichromatism is a challenge because in dichromatic species, coloration is tightly correlated with sex, making it difficult to separate the genes associated with sex in aggregate versus those that specifically regulate color differences. In this project, we study a fascinating species that is uniquely able to address these questions, the white-necked jacobin hummingbird (*Florisuga mellivora*). White-necked jacobins females are polymorphic – most females have very different coloration from most males (heterochromic), yet 20% of females are male-like in coloration (androchromic). Thus, dramatic sex-difference and sex-similarity are both expressed, allowing the study of genetic factors underlying sex difference while controlling for other factors. We sequenced whole genomes from 10 males, 10 heterochromic females, and 10 androchromic females and aligned them to the Anna's hummingbird reference genome. We discuss results from this analysis, identifying regions of the genome that may be associated with the two female morphs of this species, and compare these regions between males and androchrome females to identify how genetic differences may evolve between sexes in hummingbirds. Lastly, we discuss the wide potential for using hummingbirds as a model system for understanding sex-biased traits and evolution by cross-sexual transfer.

Deep homology of the cis-regulatory code in brachyury enhancer predates the origin of notochord

Tzu-Pei Fan, Yi-Hsien Su

The Brachyury gene encodes a T-box transcription factor that is crucial for the development of notochord, a novel trait that distinguishes chordates from other animals. Brachyury expression in axial mesodermal cells (notochord progenitors) has been regarded as a chordate innovation, yet it remains unclear how the chordate ancestor acquired this midline expression domain. By compiling data from previous studies of notochord enhancers in several chordate brachyury genes, we discover a specific syntax comprised of binding sites of FOXH1, ZIC, ETS, and Su(H) (FZES) with strict order and orientation in the notochord enhancer. We also identify FZES syntax in active cis-regulatory elements (CRMs) of brachyury orthologs in various non-chordate species. Reporter assays demonstrate that the FZES-containing CRMs from non-chordates exhibited activities in zebrafish notochord, and mutagenesis of the FOXH1 or ETS binding sites reduced the activity. In contrast, in sea urchin embryos, these CRMs drive reporter gene expression in all three germ layers, and FOXH1-site, but not ETS-site, mutations decrease the reporter activity only in the archenteron. Our results suggest that FZES syntax predate the emergence of notochord and serve divergent functions in chordates and non-chordates. We propose that during chordate evolution, the newly established links between trans-factors and FZES facilitated axial mesodermal expression of brachyury, promoting the emergence of the notochord.

Fish gill ventilation as a system for studying intraspecific biomechanical variation

Stacy Farina

Gill ventilation in ray-finned fishes (Actinopterygii) requires the use of nearly every bone in the complex fish skull. It also requires the actuation of three distinct chambers (the buccal chamber and left and right gill chambers) in a cyclical, coordinated pattern. Due to the low oxygen content and high viscosity of water, gill ventilation also must be highly efficient. Therefore, gill ventilation is an ideal system for answering questions about the evolution of functional and anatomical complexity in vertebrates. My research program has focused on this system primarily across three clades of benthic fishes: sculpins, anglerfishes, and flatfishes. Thus far, our focus has been on family and species-level variation in the evolution of form and function. In this presentation, I will discuss how we have recently been gaining insights into intraspecific kinematic and functional variation in gill ventilation and how we can link intra- and interspecific variation to better understand the evolution of biomechanical systems.

Covariation in the cortical and trabecular structure of the ape clavicle

Hannah Farrell, Zeresenay Alemseged

It is widely assumed that in mammalian long bones, there is generally more trabecular bone in areas with thinner cortical bone, like the epiphyses and subarticular regions. Conversely, regions with thicker cortical bone, like the diaphysis, tend to have fewer trabeculae. These assumptions suggest that the two structural components of long bones undergo (re)modeling in response to similar external stimuli, carrying biomechanical implications for bone strength. Here, we use the ape clavicle and its trabecular network throughout the element to test the assumption of covariation between the cortical and trabecular structure. Using micro-CT scans of wild-origin hominoid clavicles ($N = 82$), we tested for significant relationships between measures of trabecular morphology (trabecular bone volume, BV/TV; trabecular thickness, TbTh; trabecular separation, TbSp) and cortical cross-sectional geometry (ratio of minimum and maximum area moment of inertia, IMAX/IMIN; ratio of area moment of inertia about the craniocaudal and dorsoventral planes, IDV/ICC, and relative cortical area, CSA/TSA). BV/TV and CSA/TSA do significantly covary, but the strength and directionality is not consistent across the sampled taxa with some taxa displaying negative correlation (ex., Pan and Homo) whereas others show strong positive correlation (ex., Gorilla). Further, when all trabecular and all cortical variables are analyzed as blocks of traits, there is no evidence of covariation between the trabecular and cortical structure.

A new species of *Dondersia* (Mollusca, Aplacophora, Solenogastres) from the Gulf of Mexico

William Farris, Emily McLaughlin, Carmen Cobo, Kevin Kocot

Solenogastres (=Neomeniomorpha) is a clade of benthic marine mollusks that are worm-like in appearance and covered with small scales or spines called sclerites rather than a shell. Around 300 species have been named to date, but it is estimated that tenfold more await description. Due to their generally small size, rarity, and the necessity of histology to confidently identify most specimens to even the family level, few experts are actively researching this group. Meanwhile, there are many open questions on their diversity, morphology, and evolutionary history. In this project, we sought to improve understanding of the solenogaster genus *Dondersia*, a group including several charismatic and colorful species. *Dondersia* is the type genus of the family Dondersiidae, although the monophyly of the family has been called into question. Using histology, scanning electron microscopy, and DNA barcoding, we are describing a new species of *Dondersia* and redescribing other species of the genus. The new species, collected from the Gulf of Mexico, has a striking purple and yellow coloration and a series of middorsal keels. Results will further our knowledge of the species-level diversity and morphological disparity of *Dondersia* and Solenogastres in general.

Comparing wing venation patterns in non-migratory and migratory dragonflies

Siti Fauziyah, Jessica Ware, Marianne Alleyne

Dragonflies are known for their agility in flight using lightweight, flexible, and strong wings. Some dragonfly species have territories that are relatively small, and they perch frequently (non-migratory species), at the other extreme some dragonfly species can cover great distances such as across the Indian Ocean (migratory species). Previous studies showed that migratory species have different wing characteristics compared to that of non-migratory species, these characteristics include wing shape, wing corrugation, and wing microtrichia. However, our understanding of how the venation patterns differ in migratory from those of non-migratory species, as well as how these characteristics contribute to their aerodynamic efficiency, is still limited. Here, we compare the venation pattern in fore and hindwings between non-migratory and migratory species by imaging museum specimen chosen to represent a variety of species from across the phylogenetic tree. The 2D images were analyzed using ImageJ software and the geometric morphometrics was performed using MorphoJ. We quantified, using canonical variate analysis, the differences between venation patterns of the dragonflies with different flight behaviors. For instance, cells at the leading edge of the wing of migratory dragonflies are

more rectangular and bound by relatively thicker veins, making the wings less flexible. Our work will lead to a better understanding of how insect wings work on the micro- and macro-scale.

Keywords: dragonfly wing, migratory, non-migratory, venation, aerodynamics

Comparative Biomechanics of Feeding Performance in Dusky Salamanders

Raymond Fedrick, Martha Munoz, Henry Camarillo

Lungless salamanders (Family: Plethodontidae) are a diverse group of amphibians (representing more than half of salamander diversity) that are descended from a direct developing, lungless ancestor. Dusky salamanders (genus *Desmognathus*) are an interesting case within plethodontids since they represent the re-evolution of a biphasic life history. This constrains feeding behavior by necessitating that the individual accommodate for different modes of feeding throughout their life, relying on suction feeding as larvae before switching to tongue projection after metamorphosis. Within dusky salamanders, there is also considerable variation in the microhabitats used by adult salamanders between species (ranging from primarily aquatic to primarily terrestrial). While the biomechanics of plethodontid feeding has been generally well characterized, the potential impact of microhabitats (on land vs. in water) on feeding mechanics remains an open question. Here, we aim to characterize the feeding mechanics of different *Desmognathus* species of the mid-Atlantic Appalachians, with a particular focus on variance associated with microhabitats. We conducted feeding trials between salamander species, utilizing markerless pose estimation powered by DeepLabCut. By doing so, we quantify kinematics associated with feeding such as tongue projection velocity, lunge distance, and gape cycle. From this study, we hope to better characterize the feeding ecologies of *Desmognathus* species, while also laying the groundwork for future comparative work examining the potential impact of biphasic life histories on plethodontid feeding ecology.

Trade-offs among cranial soft tissues in avian evolution

Ryan Felice, Andrew Knapp, Taylor West

Birds have highly specialized craniofacial morphology compared to their non-avian dinosaur relatives. Among the many derived cranial features is the enlarged, globular brain, which is thought to be correlated with increased cognitive and sensory capabilities, thus enabling diversification into new ecological niches. However, the brain is just one component of the multistructural, multifunctional head. How does the acquisition of large, globular brains correlate with other cranial tissues such as the eyes and jaw muscles? We use evolutionary causal models to test for trade-offs among cranial structures in crown birds. We characterized the shape of the brain and jaw adductor muscle attachment sites using high-dimensional geometric morphometrics and the dimensions of the bony orbit using linear metrics across extant birds ($n = 50$). We used phylogenetic path analysis to test whether (A) brain shape constrains adductor muscle shape and eyeball volume, (B) brain shape is constrained by jaw adductor muscle shape, or (C) these cranial tissues evolve independently. Our best supported model includes a strong positive influence of brain shape on correlation between eye volume and a strong negative influence of brain shape on jaw muscle shape. Large, spherical brains are correlated with small jaw muscles and smaller elongate brains are correlated with larger jaw muscles. Together, these findings suggest that the cost of encephalization is a constraint on jaw muscle size and shape in birds.

TRPV1 in Burmese python retina: A potential role in magnetoreception?

Lucia Felipe-Gonzalez, Sherri Emer

Birds, mammals, fish, turtles, and some bacteria exhibit abilities used to detect the Earth's magnetic fields to aid in processing directional information required for movement, migrating, and/or homing. Past research showed that Burmese pythons can find their way "home", suggesting they have an innate navigation system. While the mechanism for magnetoreception is debated, it may involve intracellular magnetic particles that are associated with a mechanosensitive membrane receptor capable of converting mechanical signals into electrical

signals usable by the nervous system. Our goal was to use local, wild-caught invasive pythons to test the hypothesis that the snake retina contains a mechanosensitive transient receptor potential protein (TRPV1) that has the potential to function in magnetoreception. Fixed eyes were thinly sliced and used with immunohistochemistry and fluorescence microscopy to evaluate TRPV1 presence and distribution. In our preliminary assessments, we observed TRPV1 labeling in most retinal cell layers. Further investigation into TRPV1 colocalization with potential magnetoresponsive particles is planned. While pythons are not migratory, determining if they have the neuroanatomical components for use with magnetic field information is important to understanding their behaviors, movements, and potential for geographic expansion, which is critical for management of this invasive apex predator.

Effects of Microplastic Uptake on Amphibian Growth & Development

Andre Felton, Brianna Zamarripa, Cristina Mendez, Oscar Hernandez, Meredith Slimp, Jeffrey Hutchinson

Global declines of amphibian populations have primarily focused on classic chemical contaminants and compounds (i.e., pesticides, fertilizers, salts, etc.), but there is a paucity in research addressing the role of emerging pollutants such as microplastics and nanoplastics on amphibian health and contributory role to global declines. Microplastic pollution has been shown to be ubiquitous across freshwater systems yet our understanding of exposure routes and interactions within ephemeral fluvial systems that exhibit high spatiotemporal variability in MP concentrations on co-occurring amphibians that utilize ephemeral pools for reproductive and development purposes is limited. In this study, the effect of chronic microplastic exposure on amphibian growth and development throughout larval and metamorphic stages was examined using *Xenopus laevis*. Tadpoles across four treatment concentrations were exposed to secondary generated polyethylene microplastic fragments throughout pre- and pro-metamorphic development. Results regarding impacts on growth and developmental stages are currently being processed and analyses will be reported during the poster presentation. Amphibians are among the most threatened taxa globally and have served as effective models for other ecotoxicological studies, yet less than 1% of all studies investigating the effects of MP on biota have used amphibians. As global plastic production is expected to continue and increase, understanding MP exposure routes and effects of chronic exposure on amphibian health can provide insight to effective mitigative practices in conservation efforts.

Evolution of the hymen in mammals: phylogenetic distribution, diversity and function

Kayla Fennell, Adrienne Wu, Rachel Keeffe, Patricia Brennan

Despite its broad cultural significance and medical importance in humans, little work has been done on the evolution of the mammalian hymen. This includes its presence or absence in different mammalian groups, its development, its ontogeny, and potential function. We reviewed the literature on mammalian reproductive tracts to evaluate the hymen's presence or absence, its hypothesized function, and phylogenetic distribution. We found that the hymen has been reported as present in 12/17 orders of placental mammals, absent in one, with data missing from four. The hymen is absent in Monotremes, suggesting that the hymen is an ancestral trait of placental mammals. This hypothesis is supported by hymen development, which occurs early in the embryo when the müllerian ducts encounter the tissue of the vaginal floor. It is unlikely that the hymen serves an adaptive function in most clades, but rather it is a byproduct of the development of the mammalian vaginal canal. Based on histological descriptions, we suggest that vaginal closure membranes found in some mammalian groups are co-opted from the hymen, and serve the function of protecting the reproductive tract in immature individuals from superfluous copulation and/or pathogenic agents. Due to the paucity of data on hymens in the vast majority of mammalian families, a fuller picture of hymen evolution can only be described once further work is done on the hymen in mammals.

The conserved role of integrated canonical and non-canonical Wnt signaling during anterior-posterior

Jennifer Fenner, Ryan Range

One of the first events to occur in animal development is the formation of the Anterior-Posterior (AP) axis. This fundamental step in embryogenesis is largely controlled by a deep evolutionarily conserved posterior-to-anterior gradient of Wnt/ β -catenin signaling that patterns the early germ layer (endoderm, mesoderm, ectoderm) gene regulatory networks (GRNs) along this axis. In the sea urchin, AP specification and patterning is controlled by an integrated Wnt signaling network of canonical (Wnt/ β -catenin) and non-canonical (Wnt/JNK and Wnt/PKC) signaling pathways. Here we use mRNA overexpression and siRNA knockdowns to show that many components of the sea urchin AP Wnt signaling network are also critical for AP axis patterning in the in the hemichordate, *Saccoglossus kowalevskii*. In sea urchins Wnt8-Fzd5/8-JNK signaling is critical for the restriction of the anterior neuroectoderm (ANE) GRN to a territory around the anterior pole. Our functional perturbations show that Wnt8, Fzd5/8 and JNK are necessary for ANE restriction in *Saccoglossus*. We also show that knockdowns of the Wnt antagonist Dkk1 leads to the elimination of the ANE GRN. Finally, our data indicate that Fzd1/2/7 in *Saccoglossus* does not antagonize the Wnt/JNK restriction mechanism, as it does in sea urchins, but rather functions synergistically with Fzd5/8 to restrict the ANE GRN around the anterior pole. Echinoderms and Hemichordates are members of the Ambulacrarian phyla that forms the immediate sister group with chordates. Thus, collectively these data indicate that interactions among canonical and non-canonical Wnt signaling pathways during AP axis formation were present in the last common chordate/ambulacrarian ancestor.

TGF-Beta and Wnt interactions coordinate anterior-posterior and dorsal-ventral axis specification

Jennifer Fenner, Ryan Range

In deuterostomes (chordates, hemichordates and echinoderms), the specification and patterning of the anterior-posterior (AP) and dorsal-ventral (DV) axes overlap in time and space. However, we lack a clear understanding of the explicit molecular mechanisms that control these axes as well as the extent of their interactions in any developmental model organism. In sea urchin embryos an integrated network of canonical (Wnt/ β -catenin) and non-canonical (Wnt/JNK and Wnt/PKC) signaling pathways specify and pattern the early germ layers (endoderm, mesoderm, and ectoderm) along the AP axis. During this progressive AP patterning process, ventrally localized Nodal signaling initiates DV axis specification, establishing opposing Nodal and BMP2/4 signaling gradients that activate DV GRNs in all three germ layers. We have previously shown that non-canonical Wnt16-Fzd1/2/7 signaling is broadly active during early AP and DV axis specification, playing a critical role in the sea urchin AP Wnt signaling network. Here, we use functional knockdown experiments to show that non-canonical Fzd1/2/7 signaling and a different broadly expressed Wnt ligand, Wnt6, are also necessary early Nodal signaling activity, as well as the transcription of bmp2/4 and the dorsal ectodermal GRN. Unexpectedly, our data indicate that nodal transcription is normal in Wnt6 and Fzd1/2/7 knockdown embryos, but phosphorylation of its downstream target Smad2/3 is perturbed. Together, our results illustrate that in sea urchin embryos there are direct interactions among components of the early Wnt signaling network and Nodal-BMP2/4 signaling pathways that govern AP and DV formation, respectively.

Telomere dynamics in Tree Swallows: exploring the effects of temperature and microbiome

Jessica Fenners, Sarah Chapman, Jennifer Houtz, Maren Vitousek, Mark Haussmann

As the climate is rapidly changing, many species are facing a more unpredictable and thus challenging environment. Particularly, frequent cold weather swells, known as cold snaps, have posed a significant thermoregulatory challenge that is suspected to shorten telomeres—the non-coding, protective, terminal caps on chromosomes—and ultimately reduce organismal lifespan. However, it is unknown how the composition of the gut microbiome affects an organism's ability to face these thermoregulatory challenges. Here, we

investigated the effect of temperature and antibiotics on telomere length in the widely distributed and tractable Tree Swallow (*Tachycineta bicolor*). Wild nestlings were reared in captivity from days five to twelve post-hatch under both cold (31°C) and control (35°C) conditions and further divided into two groups: antibiotic treated and non-antibiotic treated. Blood samples from twelve days post-treatment were analyzed using the telomere restriction fragment assay. While there was no significant temperature by antibiotic interaction, both warmer temperatures and antibiotic treatment resulted in individuals with longer telomeres. These findings suggest that more variable and colder climates early in the breeding season are likely to have a detrimental impact at the cellular level. Though we predicted that the administration of antibiotics may result in more telomere loss, the longer telomere average for the antibiotic treated nestlings suggests the connection between temperature challenges and the gut microbiome is complex and deserving of further research.

Artificial light at night induces immune-telomere trade-offs in house sparrows (*Passer domesticus*)

Stephen Ferguson, Harrison Williams, Catherine Grey, Elizabeth Danko

During early development, altricial nestlings must allocate a limited energy budget to support rapid growth and physiological change. Stressors like prolonged temperature extremes and water or food restriction may leave physiological signatures, and even mild events can be relevant for developing young that are limited in their ability to adjust behaviorally. Stress during this time may thus induce a reallocation of resources to prioritize different aspects of growth. Artificial light at night (ALAN) represents an increasingly pervasive disruptor that can alter hormonal responses, growth and size, and immune function, among other traits. We hypothesized that exposure to ALAN would induce tradeoffs between the constitutive immune response and telomere dynamics in nestling house sparrows (*Passer domesticus*). To test this, we exposed nestlings to low-level ALAN (~1 lux) or sham lights at the nest between day 5 and 10 post-hatch. Nestlings at day 10 were similar in body size, condition, and hematocrit levels regardless of treatment. Immunocompetence increased from day 5 to day 10 regardless of treatment; however, day 10 ALAN-exposed individuals showed reduced immunocompetence in bacteria killing assays but increased telomere lengths over controls. Such trade-offs without immediate physical growth costs suggest nestlings are resilient to at least minor stressors and may contribute to the house sparrow's success as an invasive worldwide.

MHC and the genome: selection and population genetics vary between migratory and resident songbirds

Esther Fernandez, Danielle Whittaker, Joel Slade

Theory predicts that migratory animals are exposed to a higher diversity of pathogens than resident species. These pathogens can impose strong balancing selection on immune genes, such as at the major histocompatibility complex (MHC). In contrast, sedentary species may exhibit good gene effects at MHC since they likely coevolve with local pathogens. Dark-eyed juncos have been identified as a candidate species for studying evolution-in-action, such as the two seasonally sympatric subspecies, the migratory northern slate-colored junco and the resident Carolina slate-colored junco in the Virginia Appalachians mountains. This system provides a unique opportunity to study the relationship between migratory behavior and the evolution of immune genes. We explored how patterns of molecular selection, allelic diversity, and phenotypic variation at MHC class I and II may be explained by migratory behavior. We hypothesized that MHC class I and II in the migratory juncos are under stronger positive selection and are more diverse than the resident species. We also predicted the genetic structure between both subspecies at both classes of MHC and genome-wide markers. We characterized both classes of MHC and genome-wide markers. Contrary to our hypothesis, we detected purifying selection at MHC class I and positive selection at MHC class II for both subspecies. We found a genetic structure for class I but not for class II MHC, found MHC class I alleles associated with subspecies membership, and found genetic structure using genome-wide markers. Ultimately, our study will advance our understanding of how migratory behavior may drive the evolution of immune genes and population divergence in birds.

Synergistic effects of thyroid hormone and dexamethasone on diverse aspects of frog metamorphosis

Naomi Fernandez, Maryam Zareizadeh, Myana Keusch, Dakota Lazore-Swan, Michael Agyekum, Keith Omane-Agyei, Kaitlyn Barton, Aidan Fauth, Kelsang Chokey, Kyle Abramson, Alexander Schreiber

Metamorphosis of the frog *Xenopus laevis* is accompanied by diverse developmental programs represented by cell death and tissue resorption (tail and gill), proliferation and growth (legs), and organ remodeling (skull, brain, gut, immune system). Thyroid hormones (TH) are known to be essential to virtually all aspects of metamorphosis, and glucocorticoid stress hormones are hypothesized to exert synergistic effects with TH. To evaluate the extent of potential synergy between these two hormones on different developmental programs, we treated Nieuwkoop-Faber (NF) 54 stage prometamorphic tadpoles with triiodothyronine (T3) and/or dexamethasone (DEX) for 5 days to induce metamorphosis. Using 2-factor ANOVA analysis, we found that compared with T3 treatment alone, DEX+T3 significantly accelerated virtually all aspects of development, including resorption of the tail (by 80%) and gill (48%), remodeling of the skull (reduced interocular distance by 80%), remodeling of the immune system (reduced thymus size by 50%), shortening of snout-vent length (by 30%), brain thickening (26%), and shortening of the olfactory nerves (20%). By contrast, rates of leg growth induced by T3 were not significantly accelerated in the presence of DEX. These findings support the hypothesis that glucocorticoid stress hormones synergize with TH to accelerate diverse developmental programs of metamorphosis, potentially allowing tadpoles to optimize rates of development in the presence of varying degrees of environmental stress, such as predation, dehydration, reduced food availability, or increased interspecific competition.

Development of the lung of the Gray short-tailed opossum (*Monodelphis domestica*) investigated by μ CT

Kirsten Ferner

Marsupials are born with structurally immature lungs when compared to eutherian mammals. The Gray short-tailed opossum (*Monodelphis domestica*), a pouch less marsupial from Brazil, is born at the late canalicular stage of lung development. Consequently, the majority of lung development takes place in ventilated functioning state during the postnatal period.

X-ray computed tomography (μ CT) was used to three-dimensionally reconstruct the bronchial tree, the terminal airspaces and the pulmonary vasculature in order to reveal the timeline of lung morphogenesis. The lung development was examined from embryonic day 13, during the postnatal period (neonate to 57 days) and in adults. At birth, the lung of *Monodelphis domestica* consists of large terminal air spaces, which are poorly subdivided and open directly from short lobar bronchioles. During the postnatal lung development, differentiation and expansion of the bronchial tree and a massive increases in air sac number and architectural complexity takes place. Between 28 and 35 days alveolarization starts and a mature bronchial tree, including respiratory bronchioles and alveolar ducts develop. Alveoli located at the walls of large conducting airways might be remnants of the transformation of former respiratory epithelium into bronchiolar epithelium in a ventilated lung and are unique to marsupials. The structural transformation of the lung of *Monodelphis domestica* follows similar patterns as described in other mammalian species. Lung development seems to be highly conservative within mammalian evolution.

Do spatially and temporally varying selection affect species boundaries in sympatric Monkeyflowers?

Kathleen Ferris

Many studies have investigated how temporally and spatially varying selection affect the maintenance of genetic variation within populations and species. However, less is known about how these ubiquitous forces impact the maintenance of genetic variation between species, or in other words whether they erode or reinforce species' boundaries. Here we ask this question using a decade spanning repeated reciprocal transplant experiment between two sympatric Monkeyflower species: *Mimulus laciniatus* and *Mimulus guttatus*. *Mimulus guttatus* is a wide ranging species that typically occupies mesic environments such as wet seeps or streams throughout Western North America.

Mimulus laciniatus is a closely related species that is endemic to the Sierra Nevada, CA and occupies dry rocky outcrops. The two species often occur within meters of each other, but in these distinct microhabitats. We created advanced generation hybrids and measured phenotypic and genotypic selection on them for five years in nearby *M. laciniatus* and *M. guttatus* habitats within Yosemite National Park. We find substantial spatial and temporal variation in natural selection across years and that this variation significantly affects the strength of divergent selection and habitat mediated reproductive isolation. Specifically we find that variation in soil moisture both between habitats and years greatly impacts the strength of divergent selection between the species' habitats, and therefore the maintenance of species boundaries.

Evaluating light environment and display as contributors to color variation in *Phylloscopus* warblers

Kristina Fialko

Understanding the diversity of color in nature has been one of the more elusive evolutionary problems. In the terrestrial environment, comparative analyses have associated color differences among species to light environment, background color and receiver perceptual abilities. However, these only account for a fraction of color diversity, and it has been difficult to explain why a certain species is the color it is. How a color patch is displayed behaviorally also impacts perception and incorporating motion may help elucidate patterns in color diversity. Here we examine color variation across 12 very similar species of warblers belonging to the genus *Phylloscopus*. Most species have pale patches on the wing bars, and some also have white outer tail feathers. We quantify variation in plumage color and model perception of these patches under light conditions found in species habitats. Through playback experiments and high-speed video we also investigate patch use during territorial display. We find no evidence that irradiance or different display motions affect the color of patches across species. We suggest that differences in tail and wing coloration may be a consequence of their potential use in different signaling functions.

Homology of wing morphogenesis in flies and butterflies

vincent ficarrotta, Brian Counterman, Fernando Rodriguez-Caro

Insect wings provide an excellent window into developmental processes and the underlying genetic architecture involved in the origin of evolutionary novelty. Though insect wings are homologous structures, many differences have arisen during the 300+my of winged insect diversification. Most notable are the often brightly colored scales that adorn the wings of butterflies and moths. These wing scales often have elaborate cytoskeletal structures that greatly contrast against simple shaft-like hairs and bristles commonly found on insect wings, such as those in *Drosophila melanogaster*. Butterfly wing scales and fly wing hairs have long been considered homologous structures, however there is limited evidence of the genetic and developmental similarities among the structures to support their homology. Unraveling these similarities and differences is key to understanding how butterflies and moths evolved such complex structures that have been critical in their diversification. Here, we extend on the genetic homologies between two structures, by focusing on two fundamentally important pathways: the lateral inhibition (LI) pathway and the planar cell polarity (PCP) pathways, using rich transcriptomic data from developing wings of *Zerene cesonia* and *D. melanogaster*. We find many of the same genes are expressed in both pathways that contribute to the development of bristle and scale cells and highlight differences of genes in both pathways that may have been involved in this evolutionary transition.

Effects of gonadotropins and intraovarian growth factors on coho salmon ovarian follicles

Chelsea Field, Jennifer Telish, Emma Timmins-Schiffman, Chris Monson, José Guzmán, Kristy Forsgren, Graham Young

The production of viable vertebrate ovum (oogenesis) is a complex process in which maternal provisions support development of the egg cell (oocyte) and surrounding somatic cell layers, together known as the ovarian follicle. Vertebrate sexual maturity is reached at the onset of puberty, demarcated by the shift of late primary growth ovarian follicles into secondary growth. Final

oocyte maturation and ovulation via gonadotropins [i.e., follicle stimulating hormone (FSH), luteinizing hormone (LH)] interaction has been well-studied. Hormonal regulation of stages connected with the transition into puberty is less understood. Our research aims to determine the regulatory role of gonadotropins and intraovarian growth factors at the primary and early secondary growth stages of oogenesis. Coho salmon (*Oncorhynchus kisutch*) explant tissue was exposed to gonadotropins and intraovarian growth factors in vitro. We analyzed ovarian fragments using selected reaction monitoring (SRM) mass spectrometry. Peptides from proteins involved in carbohydrate metabolism, steroidogenic enzymes, and the TGF- β growth factors displayed an elevated, dose-dependent response to FSH, and a decrease in expression in response to LH in both the primary and early secondary stages of development. Additionally, we are histologically investigating phenotype changes and relative gene expression of several genes (e.g., *amh*, *mdh2*, *fshr*, *fth3*, *nppc*, *npr2*). Our research aims to identify important biomarkers of vertebrate egg production which may aid in development of methods to overcome reproductive dysfunction of captivity-reared vertebrates, particularly fishes.

Transcriptomic Responses to Coronavirus Infections in African and North American bats

Ken Field, Sara Talmage, DeeAnn Reeder

Bats are the likely ancestral hosts of nearly all coronavirus lineages but, like most reservoir hosts, do not appear to experience significant illness. Using archived gastrointestinal (GI) tissue samples from two different species of bats, one from North America (*Myotis lucifugus*) and one from Africa (*Epomophorus labiatus*), we have characterized the presence of coronaviruses using viromic and PCR-based approaches. To determine how these hosts do (or do not) respond to infection, we are examining the whole-transcriptome changes in host gene expression that accompany coronavirus infection in the GI tract. RNA was isolated from the GI tracts of 26 North American bats and 150 African fruit bats and RNASeq was performed to a read depth of 40-80 million read pairs per sample. The levels of alpha-coronavirus BtCoV-CDPHE15 detected in the *M. lucifugus* RNASeq reads correlated with their white-nose syndrome status, as expected, and was significantly higher in juveniles than adults. However, no coronaviruses were detected in the RNASeq reads from 150 *E. labiatus* GI samples using this viromic approach. Using consensus PCR, we have identified three of these same African fruit bats as positive for beta-coronavirus BtKY55/BtKY56. We have found changes in host gene expression that correlate with infection, including some that indicate alterations in immune signaling pathways in the host gut.

Assessing physiological and molecular plasticity in the early stages of *Porites astreoides*

Florence Fields, Jill Ashey, Brett Jameson, Chloe Carbonne, Hollie Putnam, Gretchen Goodbody, Yvonne Sawall, Samantha De-Putron

As marine heat waves increase in intensity and frequency, the ability for reef building corals in their early life stages to acclimate and survive is challenged. The mustard hill coral, *Porites astreoides*, is a common weedy species on Atlantic reefs and is a hermaphroditic brooder. To understand how heat stress affects *Porites astreoides* during early life stages, we exposed cohorts of larvae across each day of release, tracked by parental colony, to ambient (28°C) and high (30°C) temperature treatments for two days and assessed respiration, photosynthesis and the effective quantum yield (Fv/Fm). Subsequently, larvae were settled, and spat were exposed for an additional three days to the two treatments and then assessed for size and Fv/Fm. Cohorts of larvae that were released from their parental colonies on later dates had greater Fv/Fm values, regardless of treatment. Fv/Fm from the larvae in the high temperature treatment was significantly lower than in ambient, suggesting a negative effect of temperature on the symbiont photophysiology. Treatment had a greater effect on larvae from certain colonies, illustrating the importance of parental genotype when assessing offspring performance. Our investigation of the physiological and molecular responses of *Porites astreoides* larvae and spat indicates the negative effects of exposure to increased temperature at the earliest life stages is in part influenced by parental adaptations.

Cryopreservation of *Anopheles stephensi* and *Anopheles gambiae* eggs

Chaney Finkeldei, Courtney Gula, Arun Rajamohan

Cryopreservation is a common method of storage where samples are kept in liquid nitrogen and retain viability after thawing. The method of cryopreservation for this study was vitrification, where the samples are rapidly frozen in order to prevent ice formation. To do this, a high concentration of cryoprotectant – a chemical used to prevent ice formation – is necessary. In this study, the cryoprotectant was 100% methanol. *Anopheles* mosquitoes are vectors of malaria, a disease which impacts humans. Cryopreservation of mosquitoes is important to disease research as it can preserve the genotype of mosquitos, facilitate colony maintenance, and improve mosquito control efforts. Cryopreservation of *A. stephensi* eggs has been shown to be successful. The goal of this study is to replicate previous studies, and determine if the protocol is transferable to *A. gambiae* eggs. Our results show cryopreservation is possible with *A. stephensi* but not *A. gambiae*. We observed low rates of hatching for the *A. stephensi* eggs after liquid nitrogen storage, but no survival up to 24 hours. We had no success with the *A. gambiae* surviving liquid nitrogen, and little success after only being placed in the cryoprotectant. We determined the eggs were vitrifying through the use of a cryo microscope to observe the phase change of the eggs as they froze. *A. stephensi* showed signs of vitrification, but results from *A. gambiae* were inconclusive.

Gene regulatory targets of selection for enhanced heat tolerance in *Drosophila melanogaster* embryos

Kylie Finnegan, Brent Lockwood

Sensitive life stages may be a selective sieve through which selection acts to establish the genetic makeup of a population. Previous work has shown divergence in heat tolerance at the embryonic stage among populations of *Drosophila melanogaster*. Yet, we lack an understanding of the molecular mechanisms that underlie genetic variation in heat tolerance in this system. Given that thermal tolerance is a complex physiological trait, and that previous work has demonstrated the critical role of gene regulation for heat tolerance, we hypothesized that gene regulatory variation is the primary basis of thermally adaptive variation in heat tolerance. We utilized a combinatorial approach of lab selection and introgression, along with whole genome sequencing and RNA sequencing, to identify the gene regulatory basis of thermal adaptation in *Drosophila melanogaster* embryos. We identified individual genes and gene networks whose expression patterns were targets of thermal selection. We found that some of these genes colocalize to highly variable polymorphic regions across the genome. We discuss the potential functional roles of these genes in the physiology of temperature adaptation. Our results help to elucidate the mechanisms by which thermal tolerance is maintained and inherited in locally adapted populations.

Using *C. elegans* chemotaxis to understand ant chemistry in an undergraduate laboratory course

Katherine Fiocca, Lauren O'Connell

Undergraduate students enrolled in BIO161: Organismal Biology Lab at Stanford University spent several weeks testing the chemotaxis response of *Caenorhabditis elegans* (*C. elegans*) using chemical extracts of ants found on campus. Argentine ants (*Linepithema humile*) are invasive in the Bay Area of California and produce a defense compound, iridomyrmecin, used in both inter- and intraspecies interactions. Students built low cost aspirators out of common kitchen materials to collect these and other native ants on campus. After successful collections, students extracted ant compounds by sorting ants into glass vials and soaking them in methanol for 24 hours. Students used a *C. elegans* chemotaxis assay to assess worm responses to varying ant species and determined which set of chemosensory neurons are responsible for detection. Students were then encouraged to formulate their own hypothesis on signaling mechanisms and selected genes to knockdown using RNAi. Ultimately, students worked together to submit the finalized manuscript to microPublication Biology, which was accepted for publication. During student evaluations of BIO161, students reported that field work with ants was a valuable experience and that conducting genuine research in a course laboratory

was an affirming experience. Our course demonstrates the feasibility of using low cost materials to engage students in authentic research experiences in chemical ecology within a classroom setting.

Nutritional tuning of tadpole behavior

Marie-Therese Fischer, Camilo Rodriguez-Lopez, Julie Butler, Lauren O'Connell

Communicating nutritional need, interacting with siblings and avoiding strangers are our first social interactions, laying the foundation for a healthy life by acquiring energy, establishing bonds and avoiding risks. While it is widely accepted that neurons controlling energy homeostasis function as important modulators of social behavior, little is known about how diet affects their development. We used tadpoles of the Mimetic poison frog (*Ranitomeya imitator*) to investigate dietary tuning of behavior and neurodevelopment. In the wild, tadpoles grow up in tiny pools where they are provisioned with unfertilized eggs by their mothers. We raised tadpoles on five dietary conditions and tested their affiliative interactions towards a caregiver, aggression towards other larvae, predator avoidance and feeding behavior. After the trials, we sacrificed tadpoles to link behavior to neurodevelopment. We found that feeding was reduced and affiliative behavior was increased in tadpoles reared on a natural diet while aggression and response to predators didn't differ between diet groups. Molecular profiling of neurons active during each behavior revealed Urocortin1 as candidate nutrition-sensing population regulated during affiliative but not aggressive behavior. We characterized diet-induced differences in growth of Urocortin1 and Dopamine neurons and found that numbers of Ucn1, but not TH neurons were significantly increased in tadpoles reared on eggs. Our work provides important insights into how varying nutritional quality during development organizes neuronal circuitry regulating infant social behavior.

Realistic drag coefficients based on accurate 3D geometries of swimming sea lions

Frank Fish, Duncan Irschick, Caitlyn Swiston, Sarah Kerr, Stefani Skrovan, Jennifer Zelig, Megan Leftwich

The morphological design of an animal moving in a fluid determines the drag that must be overcome by thrust at the expense of energy. Accurate measurements of the body shape determine the drag coefficient (CD). Estimates of CD have relied on approximations from artist models and analogies with aerodynamic foil sections. Previous estimates based on the California sea lions, *Zalophus californianus*, were determined from 2D images and compared to 2D foil sections (NACA 06-018, RONCZ 1082, FX S 03-182) without consideration of body contours, the 3D geometry of the body, distortion of the body due to gravity when out of the water, or the presence of flippers. Using 3D photogrammetry from multiple views of digital videos of two trained sea lions swimming in a large pool, physical 3D models could be constructed. The models were tested in a flow tank with a 6-axis force transducer. The lowest CD values for the two sea lion models were 0.021 and 0.024 at a Reynolds number of 375,000. Compared to the 2D aerodynamic foil sections, CD was 1.7-2.2 times greater for the full 3D geometry of the sea lions. 3D models generated using photogrammetry of animals while in water can provide more realistic estimates of hydrodynamic variables to take account of the full 3D geometry of the body.

Temperature impacts on feeding kinematics in the striped barnacle

Savannah Fisher, Rion Reynolds, Clinton Moran, John Zardus

Future climate warming will affect organisms in a multitude of ways, impacting everything from metabolic rate to reproduction. Cold-blooded organisms, whose physiology is subject to environmental temperatures, may be most strongly affected, particularly those that are sessile and cannot move to find thermally optimal temperatures. We aimed to understand how temperature impacts the feeding kinematics of the striped barnacle (*Amphibalanus amphitrite*). This biofouling barnacle can be found in tropical and temperate bays and harbors worldwide. Using fresh-collected adults from Charleston Harbor, SC, we tested the hypothesis that cool and warm temperatures respectively

reduce and increase the speed of their cirral appendages during feeding. We measured average opening and closing velocities of the cirri at 18, 24, and 30°C. In addition, we measured time spent with the cirral fan extended. Cool and warm temperatures both negatively impacted kinematics at certain thresholds which could ultimately result in changes in fitness with changes in climate.

The effects of a commonly used fungicide on honey bee (*Apis mellifera*) health

Adrian Fisher-II, Jon Harrison, Jennifer Fewell, Brian Smith, Jun Chen, Cahit Ozturk, Kynadi Overcash

Honey bee (*Apis mellifera*) pollination services contribute substantially to crop production in the USA. Despite their importance, honey bees endure regional declines and high colony loss rates due in part to pesticide exposure in agroecosystems. Among pesticides fungicides may be particularly prevalent due to their widespread application on various crops during bloom. To assess the effects of the fungicide Luna® Sensation (21.4% fluopyram, 21.4% trifloxystrobin) we provided colonies with pollen containing varying field-relevant concentrations of Luna® Sensation. Colonies fed Luna® Sensation-tainted pollen exhibited temporary reductions in brood production relative to control colonies and increased mortality. However, heat exposure may have obscured other effects of the fungicide and post-treatment recovery rates did not apparently differ between treatment groups.

What are pentastomes, really? And no, they don't have five mouths

Robert Fitak, Taryn Gustafson, Jenna Palmisano

Pentastomes are obligate invertebrate parasites of the enigmatic crustacean clade Pentastomida. Commonly called tongue worms, pentastomes infect a variety of animal hosts causing their namesake disease – pentastomiasis. In Florida, an invasive pentastome parasite is causing catastrophic declines in certain native snake populations. Despite more than 130 described species and previous recognition as their own phylum, pentastomes remain both taxonomically and evolutionarily mysterious. To better understand this obscure group of invertebrates, we generated genomic data for two species within the Pentastomida: *Raillietiella orientalis* and *Kiricephalus coarctatus*. These species represent two distinct orders, the Raillietiellida and Porocephalida, respectively. In this study, we combined both short-read and long-read DNA sequencing to generate draft genome assemblies for each species. Initial results from their mitochondrial genomes support classification as crustaceans and sister to Branchiura. Ongoing work is being performed to further describe the content and organization of their genomes. Overall, these are the first genomic resources for this entire clade and are critical for filling gaps in comparative phylogenetic analysis of crustaceans and mitigating the spread of deadly pentastomiasis in native snakes.

Effects of sex-associated visual and chemical cues on social behavior in a mangrove fish

Brooke Fitzwater, John Bonvillain, Elizabeth Cameron, Raegin Hovin, Faith Kirby, Anna Catherine Meyers, Johanna Nelson, Jenna Stremmel, Ryan Earley

Organisms are immersed in a sea of information and discerning the most useful information can facilitate productive social interaction. Visual and chemical cues that contain information about conspecific sex and genetic similarity can be released by a sender and influence decisions made by a receiver. However, combinations of cues from different signaling modalities, or multimodal signaling, can help to further reduce uncertainty and promote appropriate behavioral responses. We used mangrove rivulus fish (*Kryptolebias marmoratus*) to explore the effects of visual and chemical cues as well as multimodal signaling on behavior towards conspecifics that were the same or opposite sex. Focal fish were presented either with visual cues using hand-painted models, chemical cues, or both cues in combination. In the chemical cues and multimodal signaling experiments, chemical cues were presented on a scale of genetic similarity relative to the focal individual. Sex

of the stimulus animal was an important factor in determining how individuals responded to cues. Notably, when presented with visual cues both sexes bit non-control models significantly more than control models, and both males and hermaphrodites bit male models significantly more than hermaphrodite and control models. Future work will continue to examine how genetic similarity between conspecifics and multimodal signaling impact social behavior.

The impact of temperature and moisture on body size of 13 species of Appalachian Salamanders

Morgan Fleming, Kimberly Sheldon

Body size of organisms profoundly shapes ecology and evolution, and understanding the drivers of body size variation is a central question in biology. In endotherms, environmental gradients often correlate with body size, with approximately two-thirds of studies on birds and mammals finding larger-bodied individuals at higher, cooler elevations. In birds, larger body sizes have also been found in drier habitats. In contrast to endotherms, ectotherm body sizes have been comparatively understudied. However, the mechanisms underlying body size variation in ectotherms may differ from those in endotherms given fundamental differences in physiologies. This is especially true for moisture-sensitive ectotherms, such as amphibians, which face heightened risks of desiccation due to their highly permeable skin.

Here we examined the relationship between body size, temperature, and precipitation, of plethodontid salamanders along elevational gradients in the Southern Appalachian Mountains. We hypothesized that temperature and precipitation interact to drive body size variation and that the magnitude of intraspecific body size variation across elevations differs based on species' habitat preferences. Using structural equation modeling within a Bayesian phylogenetic framework, we examined causal networks connecting elevation, climate, and body size. We found body size trends with elevation that were heavily mediated by temperature and moisture. Our findings deepen insights on biogeographic patterns of body size and enhance our ability to predict species responses to thermal and hydric conditions amid ongoing climate change.

Understanding the impacts of nutrient pollution on an urban coral in an era of global change

Caroline Fleming-Ianniello, Justin McAlister, Randi Rotjan, Grace Beery

Nitrogen is considered the limiting reagent in marine primary productivity, with increases in N bioavailability correlating with increased production. Marine coastal ecosystems that exist downstream from urban centers are subjected to excess dissolved inorganic nitrogen (DIN) via runoff. Increased DIN can overwhelm organismal function, effectively turning nitrogen from a nutrient to a pollutant. One group of urban marine organisms that are vulnerable to nitrogen pollution are corals, due to their endosymbiotic algae (zooxanthellae). Research in tropical corals suggests that excess nitrogen can lead to symbiont overpopulation, generating harmful reactive oxygen species that cause coral mortality. In addition, corals subjected to nitrogen pollution exist in a matrix of local and global stressors, including elevated sea surface temperature and bacterial loads. In a multi-factorial experiment, we leveraged the facultative symbiosis of the temperate urban coral *Astrangia poculata* to probe the effects of symbiotic state and excess nutrient load on symbiotic performance. Symbiotic and aposymbiotic *A. poculata* corals collected in Narragansett Bay were exposed to varying levels of field-relevant nitrate and ammonium concentrations at ambient (20°C) and elevated (30°C) temperatures. Symbiotic health was measured via photosynthetic efficiency and red channel value. Metabolic rate of corals were contrasted with and without an *Escherichia coli* bacterial challenge to understand downstream energetic effects of symbiosis on the coral holobiont. This study is the first to 1) assess the impacts of nitrogen pollution, temperature stress, and combinations thereof on the physiology of symbiotic and aposymbiotic *A. poculata*, and 2) determine the thresholds at which DIN shifts from nutrient to pollutant in a temperate urban coral. More broadly, this research addresses the fundamental question: "How resilient will marine organisms be to persistent urban pollutants in an era of global change?"

Freeze tolerance in a non-model insect, *Tetanops myopaeformis*

Madison Floden, Michael Taylor, Franco Basile, Arun Rajamohan, Kendra Greenlee

Of the 5.5 million insect species, under 300 are freeze tolerant. Freeze tolerance allows insects to survive extreme winter temperatures, but the underlying mechanisms have been determined in few species. Thus, to better understand the mechanisms of freeze tolerance, we need to diversify the species and methods used in such studies. In this study, the sugarbeet root maggot, (SBRM), a freeze tolerant species able to successfully pupate after five years of storage at 6±1°C, was used. We hypothesized that diapausing SBRM use cryoprotectants and control location of freezing initiation to prevent the presence, or uncontrolled formation, of intracellular ice. We measured amounts of the cryoprotectants proline and alanine using GC:MS. We will be comparing freezing initiation location and cold acclimation in field-relevant temperatures between diapausing and non-diapausing SBRM.

Levels of proline, a known cryoprotectant, doubled in diapausing individuals. We anticipate a significant difference in the freezing initiation location and duration to complete freezing between diapausing and non-diapausing SBRMs. Diapausing individuals took an average of 1.5 seconds to complete freezing. We expect to see a dramatic decrease in mass and an increased freezing point when SBRM are kept in conditions simulating the field over a 20-week period. Increasing our knowledge of the SBRM and its freeze tolerance could lead to improved pest management strategies and demonstrate the value of non-model species for understanding freeze tolerance mechanisms.

Influence of muscular mechanical forces in the hallux development of birds and its evolution.

Daniela Flores, Viviana Toro-Ibacache, Alexander Vargas

The opposable orientation of the hallux or perching digit I of birds is acquired throughout development as a result of embryonic muscular activity, involving the torsion of its metatarsal (mt1). Experimental embryonic paralysis results in a non-opposable hallux, with a straight metatarsus, as in basal theropods. However, how muscle activity produces this change is not well understood. Unlike the other (non-opposable) toes, the hallux does not articulate at the ankle, but rather at the mid-ventral surface of metatarsus 2 (mt2). The condition in basal theropods is similar, but the hallux was not opposable and articulated in a more medial position on the mt2. Using immunofluorescence confocal microscopy and molecular markings for the skeleton, muscles and tendons, and radiopaque staining we generated a reliable three-dimensional reconstruction of the embryonic foot of birds, to understand the biomechanical environment, and especially, the effect and magnitude of muscle action on the metatarsal during embryonic developmental changes, and the influence on insertion sites of tendons that affect the orientation of forces. This approach plus the modeling by Finite Element Analysis will allow delimiting the possible mechanisms involved during the evolutionary transition from a non-opposable hallux in basal theropods, to the opposable hallux of birds.

Forelimb and hindlimb bone response to early life exercise training in High Runner mice

Natalie Flores, Diamond Rawlings, Apolo Ibáñez-Rincon, Theodore Garland, Angela Horner

Physical activity is a primary determinant of musculoskeletal health in vertebrates. Exercise early in life may play a pivotal role in later life musculoskeletal health, particularly in bone tissue. To further investigate the role of exercise intensity and duration on bone morphology and strength, we examined the effects of wheel running on bone in a unique model, a line of mice selectively bred for high voluntarily wheel running (high runner mice, HR) for over 90 generations. 80 female HR and Control (non-selected) mice were divided into sedentary and exercise cohorts, with the latter given wheel access for 9 weeks beginning at either at weanling or young adult stage. Animals were euthanized at the conclusion of the training and the carcasses defleshed in a dermestid beetle colony. The femori and humeri were first measured and the bones were subjected to three-point bending protocol in a Material Testing System (MTS) to acquire materials properties. We used a three-way ANOVA

to evaluate the effects of exercise, line type, and age on limb bones. Global morphological differences between HR and non-selected Control line mouse bones were evident in femur and humerus length, epiphyseal widths, midshaft diameters, and bone mass. Effects of age and exercise were fewer, but femoral midshaft was narrower in exercised mice among all cohorts, and femur and humerus length were shorter in older exercised mice. Materials testing results demonstrated that sedentary mice had stronger bones, with higher force and strain at fracture than exercised mice. Taken together, these results suggest that exercise at an early age may impact adult limb bone strength and size and support the growing body of research on the importance of early exercise for musculoskeletal health.

Does the meiotic pachytene checkpoint drive sympatric speciation?

Victoria Foe

The pachytene checkpoint selectively culls gamete-producing cells whose homologous chromosomes are differently organized (e.g. a maternal chromosome with a region inverted compared to the same chromosome from the father). Although not 100% effective, this checkpoint can reduce the frequency of gametes carrying mis-repaired double-strand DNA breaks, mis-repair likely to have destroyed genes by separating proximal and distal gene halves (PMC 8998493). The pachytene checkpoint relies on homolog comparison. Sex chromosomes that lack homologs (e.g. the mammalian Y) are invisible to this checkpoint and accumulate chromosomal reorganizations, lose genes, and degenerate. Most species, even sibling species, differ by chromosomal organization, so hybrid sterility is an additional expected checkpoint byproduct. Recombination will occasionally assemble locally advantageous sets of alleles, and break mis-repair will occasionally capture these alleles within an inversion. In a heterogenous population the benefit conferred by these alleles may offset the reduced fertility inflicted on inversion heterozygotes by the pachytene checkpoint. If the inversion-carriers prosper, inbreeding can generate inversion homozygotes. These will pass on their allelic advantage without triggering the checkpoint, which now imposes a barrier to gene flow between the inversion homozygotes and the parental species, which lacks the inversion. Thus the pachytene checkpoint can accomplish the initial steps of new speciation in sympatry. Furthermore, the need for inversion homozygotes to identify one another to avoid producing sterile offspring should strongly drive sexual selection mechanisms.

Using DNA Barcoding to identify Shark Species off the Lagos and Ondo Coast, Nigeria.

Omolara Fola-Matthews, Olufemi Soyinka, Olayinka Ashiru, Daphne Bitalo

This study was conducted to identify the different species of sharks in Nigerian waters (Lagos and Ondo states) using DNA barcoding of the cytochrome c oxidase subunit 1 mitochondrial gene (CO1). 100 shark fin clips were obtained between February 2021 to January 2022 randomly from landings from artisanal fisheries in Lagos and Ondo states, Nigeria. DNA was extracted at Inqaba Biotec, West Africa following the Conventional CTAB extraction protocol and amplified by polymerase chain reaction (PCR) the primers, FishF1 and FishR2 were used for the reaction. The amplification of the COI gene resulted in standardized 711 bp fragments. A total of 653 positions were observed in the final dataset. The nucleotide frequencies are A=25.04%, T/U=32.08%, C=25.66% and G=17.23%, indicating that T/U has the highest nucleotide frequency among the nucleotides. Only 93 of the 100 samples produced sequences that were informative. The sequence data were edited, trimmed, and submitted to NCBI to assign Gene Bank accession numbers. Matches with blast identified three species from three families *Mustelus mustelus*, (Triakidae, 62.37%) *Rhizoprionodon acutus* (Carcharhinidae, 19.36%), *Sphyrna lewini* (Sphyrnidae, 18.27%) assessed as endangered, vulnerable, and critically endangered respectively according to the International Union for the Conservation of Nature (IUCN) Red List. Accurate species identification will improve species-specific catch landing data in Nigeria.

Precipitation gradient limits range of Texas spiny lizard though altered thermal environment

Edita Folfas, Luke Frishkoff

Climatic constraints are often proposed as limiting species distributions. Several climatic variables correlate with species range limits, but the mechanism behind these are often left unexplored. Particularly, limited thermoregulatory opportunities may constrict distributions of ectotherms that are highly reliant on microhabitat selection, despite correlations suggesting seemingly unrelated climatic variables are the driving force. To examine how thermal constraints limit species distributions, we focus on the Texas spiny lizard which has a strikingly narrow east-west range. Species distributional modeling indicates that rainfall is responsible for this narrow distribution. In the west its range-edge corresponds to drier arid zones, whereas its eastern-edge seems limited by too much rainfall. This precipitation gradient provides a unique opportunity to examine the mechanism behind range limits corresponding with high or low precipitation. While too little water offers a clear physiological mechanism for limitation, understanding why ample rainfall may prevent occurrence is less clear. We propose that rainfall variation alters vegetation such that the thermal environment is no longer suitable for this lizard to persist. Using 120 thermal models across four sites in Texas, we determined operative temperatures within and outside this species' range. Combined with body temperatures of wild-caught lizards, we compare thermal efficiency at eastern and western range boundaries. Our results provide insight into the processes limiting species ranges, information crucial for accurately predicting range shifts due to climate change.

Kinematic analysis of pectoral fin movements in manta ray resting behavior using drones

Vicky Fong, Sarah Hoffmann, Jessica Pate

Energy is a limited resource that all animals need to balance and various energy-conserving strategies have been well documented. Many elasmobranchs are negatively-buoyant obligate ram ventilators that require constant swimming to survive. Using hydrodynamically-efficient strategies, such as surfing updrafts, is demonstrated to reduce energy expenditure and has been described in grey reef sharks. Similarly, manta rays have been observed hovering in strong currents, which may be an energy conservation strategy. Previous research showed that mantas remaining stationary in strong currents inside man-made inlets (hereafter "resting") had significantly slower wingbeat frequencies than "non-resting" mantas. In this study, we explored the potential differences in swimming efficiency between resting and non-resting mantas through kinematic and environmental variables. We analyzed orthogonal drone videos of resting and non-resting mantas using a point-tracking software to quantify pectoral fin kinematics. To evaluate the effective vertical range of motion of the pectoral fins, we measured the instantaneous change in pectoral fin surface area during each upstroke and downstroke. We hypothesized that resting mantas will undergo smaller changes in pectoral fin surface area compared to non-resting mantas, which suggests resting behavior conserves energy. Future research will use computational modeling to investigate how dynamic changes in pectoral fin angle of attack during wingbeats may contribute to hydrodynamic efficiency of resting mantas, and help inform conservation of manta rays resting in areas of high boat traffic.

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To Bite or Not to Bite: The Tongue-Bite-Apparatus in mormyrid fishes (Osteoglossiformes)

Kassandra Ford, L Patricia Hernandez

A large portion of teleost fishes have pharyngeal jaws, a secondary set of jaws located in the throat of the fish that are repurposed from gill rakers. These jaws are used for processing prey items after the prey is captured by the oral jaws. In a smaller subset of teleosts, the Osteoglossomorphs, the pharyngeal jaws are noticeably absent, and instead enlarged hyoid and palatine bones protrude into the oral cavity, a morphology called the Tongue-Bite-Apparatus (TBA). Mormyridae is a family of weakly electric fishes within this group that contains a high amount of craniofacial diversity. While the overall skull shape diversity has previously been quantified in Mormyridae, far less is known about the morphological diversity of the TBA in this family. This study will examine the TBA of Mormyridae using 3D geometric morphometrics and phylogenetic comparative methods. We expect to find correlations in size and shape of the TBA based on phylogenetic relationships, diet, and habitat type, based on examinations of the skull. Future studies will include examinations of integration and modularity of the jaw systems, as well as investigations of dentition patterns and biomechanical function across species.

Hydrodynamic effects of varying number of coordinated appendages in metachronal paddling

Mitchell Ford, Arvind Santhanakrishnan

Numerous aquatic invertebrates swim using a drag-based locomotion strategy known as metachronal paddling, in which a series of appendages (hereafter termed "paddles") are stroked in a coordinated sequence starting from the posterior and proceeding to the anterior. Previous studies have examined how the metachronal coordination of a fixed number of paddles can increase the propulsive forces relative to synchronous paddling. However, another interesting aspect of this locomotion strategy, as observed in nature, is that the number of paddles varies both across species and across stages of development. For example, many crustaceans that use metachronal locomotion undergo larval and juvenile development stages during which they grow additional swimming legs. In this study, we use a self-propelling robot to examine how changing the number of paddles affects the swimming speed, tip vortex dynamics and pressure in the wake of the metachronal paddling system. We found that increasing the number of paddles results in a decrease in circulation in the paddle-tip vortex generated during the paddle's thrust-generating power stroke, likely due to destructive interactions between high- and low-pressure zones on opposite sides of adjacent paddles. Additionally, we found that increasing the number of paddles resulted in linearly increased swimming speed, but a nonlinear decrease in swimming speed per paddle.

Jumping in 3D: Flow characteristics of hybrid metachronal rowing used by escaping copepods

Mitchell Ford, Arvind Santhanakrishnan

Copepods are ecologically important planktonic crustaceans. Copepods are well known for their ability to "jump" or "hop" away from predators with escape speeds of hundreds of body lengths per second, and instantaneous accelerations as large as 20 times the acceleration due to gravity. These high escape speeds are achieved using a hybrid-metachronal drag-based

swimming stroke, in which the swimming legs begin their respective power strokes sequentially but perform their recovery stroke near-synchronously. In this study, we examine the swimming wakes of *Euchaeta* copepods during escape responses using three-dimensional (3D) particle tracking velocimetry (PTV). Additionally, we examined published images of the swimming legs of a variety of copepods and determined that the range of aspect ratios ($AR = \text{leg length} / \text{leg width}$) of the swimming legs fell mostly in the range $1 \leq AR \leq 4$. We developed a robotic model to mimic the hybrid-metachronal leg kinematics of escaping copepods, which was fitted with physical models of swimming legs of varying aspect ratio. Using this robotic model, we perform a parametric analysis of the effect of aspect ratio on propulsive forces and the paddling wake. The 3D structures of the wake and the paddle-tip vortices were quantified using 3D PTV measurements on the robotic model. Forces, tip-vortex circulation, and 3D flow visualization will be presented.

Risk assessment guides nest defense behaviors in female house wrens (*Troglodytes aedon aedon*)

Josephina Fornara, Ross Eggleston, Dustin Reichard

Predation is a critical threat to most animals, but altricial young that rely on their parents for protection are especially vulnerable. According to parental investment theory, parents that modulate their anti-predator responses based on the level of risk should have higher lifetime fitness than individuals that respond to all threats equally. House wrens (*Troglodytes aedon aedon*) are cavity-nesting songbirds that exhibit substantial variation in aggression towards nest predators. Here, we investigated the role of risk assessment in modulating defense behaviors by presenting nesting female house wrens with three predator decoys of varying risk levels and analyzed their anti-predator responses. Eastern chipmunk (*Tamias striatus*) and eastern ratsnake (*Pantherophis alleghaniensis*) decoys simulated nest predators that are “low-risk” to adult wrens. A Cooper’s hawk (*Accipiter cooperi*) decoy simulated a “high-risk” predator for both nestlings and adults. In line with parental investment theory, female wrens defended their nests with equal aggression against the chipmunk and snake decoys but never dove at or attacked the hawk decoy. Despite high inter-individual variation in aggression, we saw population-level consistency in responses to the snake decoy over a two-year period, suggesting stability over time. Our results show that female house wrens exhibit plasticity in their responses to nest predators, indicating that risk assessment plays a role in determining nest defense behaviors in a potentially adaptive way despite risking the loss of current offspring.

Genome-wide coevolution networks point to frequent rewiring of plant plastid proteostasis systems

Evan Forsythe

In plants, nuclear and plastid (chloroplast) genomes experience different mutation rates and levels of selection, yet key cellular functions depend on their coordinated interactions. Several plastid-encoded proteins undergo dramatic accelerations in their rate of evolution in plant lineages. The evolutionary causes and functional consequences of these punctuated periods of rapid protein evolution are unknown, but understanding the full suite of proteins impacted by these mysterious shifts in selective pressures is an important clue. Functionally related proteins often show correlated changes in rates of sequence evolution across a phylogeny (evolutionary rate covariation [ERC]), offering a means to detect previously unidentified suites of coevolving and cofunctional proteins. We developed computational tools capable of detecting ERC signatures across plant genomes and applied these tools to angiosperms to uncover large protein interaction networks. Our networks revealed novel connections between groups of nuclear-encoded proteins that work in concert to maintain plastid protein homeostasis (proteostasis). Some of these coevolving genes form physical interactions within multi-subunit complexes, while others likely instead coevolve due to confunctional connections formed between members of parallel or intersecting pathways, even in the absence of physical protein-protein interactions, highlighting that the ‘interactome’ extends beyond physical enzyme complexes. Our results suggest that accelerated evolution is driven by frequent rewiring of the machinery responsible for maintenance of plastid proteostasis, highlighting the genomic ripple effects of pathway rewiring events in plants.

Reliably different: Aggression is flexible but higher in urban male song sparrows compared to rural

Taylor Fossett, Samuel Lane, Isaac VanDiest, Kendra Sewall

Urbanization poses a novel challenge for wild animals. Animals can respond to this challenge by changing their behavior and increased aggression can be associated with urbanization. It is often assumed that the human built environment has the greatest impact on wild animals, but human presence alone can alter animal behavior. Understanding the relationship between urbanization, human presence, and aggression in urban animals requires comparing behaviors as conditions change over time. The quarantine in response to the COVID-19 pandemic offered an opportunity to study the effects of human presence on animal behavior in urban areas. We investigated how urban ($n=190$) song sparrows (*Melospiza melodia*) responded to simulated territorial challenges over 8 years (2016–2023) including the years before and after the COVID-19 quarantine and compared their behavior to that of rural birds ($n=153$). We found that urban birds were more aggressive than rural birds across all 8 years. Both urban and rural birds were less aggressive during the quarantine than in prior years. However, this was part of a pattern of both urban and rural birds decreasing aggression over the 8 years. This study is consistent with prior work showing that changes in human presence influence the behavior of wild animals. Thorough understanding of the effects of urbanization on wild songbirds requires long term studies across variable conditions.

What do you do again? Developing new techniques in “unconventional” model organisms at Whitney Lab

Brent Foster, Fredrik Hugosson, Cezar Borba, Dorothy Mitchell, Federica Scucchia, Cody Miner, Bailey Steinworth, Mark Martindale

Model organisms are crucial for conducting basic, applied, and translational biology research. Much of cutting-edge biology is possible because of tools established by niche research communities worldwide. Many “ground-breaking” model organisms were marine invertebrates, including the Nobel prize winning systems of the squid axon, the cell cycle dynamics in sea urchin embryos, and the discovery of GFP in jellyfish. The myopic focus of modern-day biologists on a handful of organisms can limit the scope of experimental results, biasing the trajectory of future research towards biological exceptions rather than rules. At Whitney Laboratory for Marine Bioscience, we leverage biodiversity to examine understudied organisms and their potential for investigating basic biology.

Here, we highlight the experimental versatility of three “unconventional” marine invertebrates utilized in our lab: the ctenophore *Mnemiopsis leidyi*, the anthozoan *Nematostella vectensis*, and the scyphozoan *Cassiopea xamachana*. The uniqueness of our study organisms pushes us to develop the techniques that can help us better understand the biological diversity of our world. We are optimizing HCR to dissect wound healing from regeneration and utilizing lightsheet microscopy to explore the development of the ctenophore nerve net and its sensory integration. We are creating methods to selectively isolate aposymbiotic and host-symbiont cells to gain insight into “self-nonself” cell recognition. We are also developing meganuclease-mediated transgene constructs to study biomineralization in a non-mineralizing organism.

Using environmental imaging to interpret and plan behavioural studies

James Foster, Anna Stöckl

Our ever-improving understanding of how anatomy, physiology and behaviour affect what an animal sees at a given moment, alongside improvements in artificial sensors, make measuring real-world light environments both more feasible and affordable. The remaining, not insignificant challenge, is to close the gap between an animal’s physical world and its perceptual one.

We present from a range of habitats, times of day, and perspectives. With these, we demonstrate a set of methods for extracting biologically-relevant information from environmental imaging data, accounting for absolute, spectral and polarization sensitivity, as well as species-specific differences in field

of view, sampling base and resolving power. We propose that available technologies could be further combined to define an emergent field of study that will open a window into animal perception.

The ecomorphology of primates is detected in the long bones

Kathleen Lois Foster, Alessandro Maria Selvitella

Many ecomorphological studies seek to find relationships between the habitat or locomotor behavior of species and their musculoskeletal structure. Both environments that differ in their structural properties and the disparate suite of behaviors that animals in those environments use can lead to differences in the stresses placed on their bones and changes in the size and position of muscle attachment sites on those bones. Habitat specialists may thus be expected to evolve bones whose features increase their strength and reduce the chances of failure in the face of the extrinsic and intrinsic force regimes they regularly encounter. In this paper, we examine how the proportions of primate long bones relate to the habitat and preferred locomotor mode of 45 primate species. We determine that, although primates are often considered generalists, the femur, ulna, and tibia still contain the signal of their primary habitat and locomotor mode. In particular, these bones tend to be longer and more slender and have features associated with an increased range of motion in arboreal, jumping species compared to the more robust bones of semi-arboreal and terrestrial species. Identifying the key skeletal features that vary among species occupying different habitats or performing different locomotor behaviors may help focus future research efforts into the evolution and ecomorphology of this interesting group and provide valuable insights into the natural history of extinct primates.

Bringing wildlife into the lab: Lower division Zoology students use camera traps to observe animals

Alicia Fox

At Allan Hancock College, a community college in California, we have a 3-semester sequence for biology majors that includes a General Zoology class. In an effort to increase time spent in the field and to observe wildlife, students participate in a camera trap project at a local University of California reserve, Sedgwick Reserve in Santa Ynez, CA. Students attend two fieldtrips that give them the opportunity to place and then pick up cameras at multiple water troughs at the reserve. This project offers students the opportunity to make observations in the field, learn animal ID, collect data on animal presence at water troughs, and determine patterns in animal visits at those water troughs. This project has enabled students to view wildlife they may not typically see on their own. Finally, students get involved in putting together slide shows of their favorite pictures and videos for use in outreach (e.g. Friday Night Science, our Spring community outreach event).

Which Factors Affect Thermoregulatory Decisions in Common Wall Lizards (*Podarcis muralis*)?

Logan Fraire, Sierra Spears, Emma Foster, Alyssa Head, Maya Moore, Allison Litmer, Eric Gangloff

Effective thermoregulation is necessary for most ectothermic organisms to perform essential functions critical to survival. However, little is known about what factors affect thermoregulatory decisions in natural environments. Further, few studies consider organismal activity patterns throughout an entire day with respect to temperature and other environmental factors. Such data are particularly useful in understanding persistence and habitat use, especially for invading species in novel environments. We quantified the factors affecting daily activity and thermoregulatory decision-making in introduced populations of the Common Wall Lizard (*Podarcis muralis*) in Cincinnati, Ohio, USA. During summer and fall, we performed standardized surveys every 30 minutes across the entire daily activity period (08:00 to 20:00), recording lizard body temperature using infrared thermography and lizard behavior. We utilized 3-D printed operative temperature models to measure available temperatures throughout the day and collected data on air temperature, humidity, solar radiation, UV radiation, and wind speed. By combining repeated-measures data on the

activity and body temperature of individual lizards in situ with a suite of environmental data, we can identify the factors that affect lizard thermoregulation. We present these data in the context of understanding how these ectotherms can respond to urban environments and rapidly changing climates.

The role of skin temperature in the resistance to fungal infection during torpor

Craig Frank, Joseph Laske, Carl Herzog

White-nose Syndrome (WNS) is a mycosis that kills little brown (*Myotis lucifugus*), Indiana (*M. sodalis*) northern long-eared (*M. septentrionalis*) and tricolored (*Perimyotis subflavus*) bats. It is caused by a cutaneous infection with the fungus *Pseudogymnoascus destructans* during hibernation. Infected bats arouse more frequently from torpor than normal during hibernation which leads to death. *Pseudogymnoascus destructans* was first observed at a single cave in Eastern NY during the winter of 2006-2007 and has since spread to 39 U.S. States and 7 Canadian provinces. This fungus grows at a T_a 18.0°C. Recent studies have revealed that *M. lucifugus* populations in NY state are now increasing, and they no longer suffer severe over-winter mortality while hibernating in areas with *P. destructans*. We hypothesized that this increased resistance to WNS is due to increases in the skin temperatures maintained during hibernation. This hypothesis was tested by conducting temperature-sensitive radio telemetry studies during the winters of 2008-09 and 2021-22 on free-ranging *Myotis lucifugus* hibernating in the same mine in Eastern NY. Mean torpor bout duration increased by 6.12 d during this 13-year period and was associated with an 8.1°C increase in the mean skin temperature maintained during hibernation, raising it to 18.3°C. These findings support our hypothesis.

Developmental and regenerative inconsistencies between tooth-like structures in sharks

Gareth Fraser, Ella Nicklin, Karly Cohen

Sharks and their relatives are typically covered in highly specialized skin appendages called dermal denticles; ancient tooth-like units (odontodes) composed of dentine and enamel-like tissues embedded in the skin. These 'skin teeth' are incredibly similar to oral teeth of vertebrates and share comparable morphological and genetic signatures. We have collected gene expression and morphological data from embryonic sharks to uncover characters that unite all tooth-like elements (odontodes), including teeth and skin denticles in sharks. In addition, we show differences between the skin and oral odontodes that reflect their varied capacity for renewal. Our observations have begun to decipher the developmental and genetic shifts that separate these seemingly similar dental units. We have characterized the regenerative nature of both oral teeth and the emerging skin denticles from the small-spotted catshark (*Scyliorhinus canicula*) and other chondrichthyan models. Ultimately, we ask what defines a tooth at both the molecular and morphological level. Our insights provide a framework to understand how nature develops, replaces and evolves a vast array of odontodes, and how multiple mechanisms of diversity may present in just one species.

Body size is associated with over-wintering diet variation in a migratory bird

Nicolas Frasson, Keith Sockman

The relationship between body size and nutrition is complex and can be mediated by several processes, including size-related intraspecific food competition and preference. Most research on this relationship is conducted on resident species or solely on the breeding grounds of migrants, but little is known about the relationship between body size and diet during other life-history stages of migrants, such as when they are on the over-wintering grounds. We captured Lincoln's sparrows (*Melospiza lincolnii*) early in their breeding season and assessed body size from the first-axis factor scores of a principal component analysis of several morphological measures. We estimated the proportion of dietary C4 plants to dietary C3 plants ($\delta^{13}C$) and trophic position ($\delta^{15}N$) during their period on over-wintering grounds from stable isotope analysis of toenail clippings collected on the breeding

grounds. We found no relationship between body size and $\delta^{15}\text{N}$. However, $\delta^{13}\text{C}$ increased with body size. We do not know the basis or fitness consequences, if any, of this relationship, but future research is aimed at understanding whether dietary variation results from size-based variation in over-wintering location. Our findings add to a growing body of evidence that intraspecific variation in phenotype may have an important relationship with energy and nutrition during multiple life-history stages, including the over-wintering period.

Bacterial polysaccharide triggers settlement and metamorphosis in a polychaete and a coral

Marnie Freckelton, Brian Nedved, Michael Hadfield

Larval settlement and metamorphosis in many marine invertebrates depends on external cues, often originating from bacteria. Lipopolysaccharide (LPS) from the biofilm-dwelling Gram-negative bacterium *Cellulophaga lytica* is an inducer of metamorphosis in larvae of tubeworm *Hydroides elegans*. Larvae of *H. elegans*, however, can be stimulated to metamorphose by multiple marine bacterial biofilm species. To determine whether LPS is a common inductive element for metamorphosis in this species, we tested LPS from inductive Gram-negative bacteria isolated from marine biofilms, including *Tenacibaculum aiptasiae*, and both inductive and non-inductive strains of *Thalassotalea euphilliae* and *Pseudoalteromonas luteoviolacea*. For controls, we also examined commercial LPS from three human pathogens, *Escherichia coli*, *Salmonella enterica*, and *Pseudomonas aeruginosa*. Metamorphosis was triggered by LPS from inducing bacteria, while that from non-inducing bacteria and strains had no effect. Interestingly, some bacterial species that induce metamorphosis of *H. elegans* also induce similar responses in larvae of the broadly distributed Indo-Pacific coral *Pocillopora damicornis*. To determine if this was also due to LPS, we exposed coral larvae to LPS from the same bacterial species and confirmed its inducing role, although from different strains. Additionally, we pinpointed the inducing property of LPS to its polysaccharide (O-antigen) component suggesting that conserved polysaccharide elements are broadly important metamorphic cues. These results offer insights into bacterial involvement in animal development and how marine benthic communities are established and maintained.

Exploring the diversity and the evolution of colour patterns in reef fishes

Bruno Frederich, Laurent Mittelheiser, Amandine Gillet, Vincent Laudet, Alex Dornburg

Reef-associated teleosts are well-known for their astonishing diversity of colour patterns. Various works revealed that pigmentation patterns diverged rapidly and are under ecological and sexual selection. However, the geographical distribution of this diversity was never investigated and thorough comparative analyses among fish families are lacking. Here, we aim to compare the diversity and the evolution of colour patterns in four reef fish families: Acanthuridae, Pomacanthidae, Pomacentridae and Lutjanidae. The colour patterns of species were described by a combination of binary traits translating the presence/absence of various markings (e.g. stripes, spots...), and then summarized using Principal Coordinates Analyses. Geographic data were recorded, and fish species were assigned to five main regions. We firstly observe that the diversity of colour patterns is similar and equally distributed among regions in every family. High divergence of patterns among species and a correlation between disparity and species richness support the hypothesis of a relationship between speciation and colour pattern, and suggest a crucial influence of colour patterns in species phenotypic differentiation. Secondly, we confirm the great evolvability of colour patterns. Finally, we reveal that a high level of colour pattern diversity was produced recently in major subclades of the fish families. Collectively our data highlights the importance of pigmentation patterns as a selective trait in reef fishes and plead for further works integrating ecology, evolutionary and developmental analyses.

Metabolism of ontogeny and biomedical bleed stress in Atlantic horseshoe crabs (*Limulus polyphemus*)

Julia Frees, David Hudson

The Atlantic horseshoe crab (*Limulus polyphemus*) is an important species both to the ecology of the Long Island Sound region, and to ensuring safety of biomedical products (e.g., vaccines, insulin), so it is critical to improve the understanding of how to better utilize this living resource ethically and to advance its conservation. To that end, this project sought to quantify the effects of recurring bleeds in simulation of those made for biomedical purposes on metabolic, behavioral, and physiological stress responses. Animals were also assessed as to whether the reproductive cycle is maintained in bled adults. To advance early life stage husbandry techniques, particularly metabolic demand in earlier life stages, metabolism was measured across early-stage ontogenetic size classes of the animals under two temperature scenarios to determine metabolic demand. *L. polyphemus* does not show detectable changes in stress parameters from two repeated 10% estimated hemolymph volume harvests. Given the need to make a sustainable and ethical harvest of hemolymph for biomedical usage, it is recommended that this lower amount be combined with recent literature knowledge on catheterization of aquacultured animals. Juvenile *L. polyphemus* individuals need more energy and resources in earlier life stages, which changes with temperature, meaning that feeding needs to be optimized on a per-mass basis as the animals grow.

Electrocommunication and steroid hormones covary with individual condition across knifefishes

Megan Freiler, G. Troy Smith

Individual variation, such as reproductive condition or status, can explain variation in signaling and hormonal profiles in vertebrates. Weakly electric knifefishes offer an excellent opportunity to investigate individual variation in communication and steroids in a comparative context. Knifefish modulate the frequency of their electric organ discharges during agonistic and courtship interactions to produce chirps. Chirp use and its hormonal regulation varies extensively across species. Here, I recorded fish overnight in isolation, in same-sex pairs, and in opposite-sex pairs using three species of knifefishes that vary in sociality: territorial *Apteronotus albifrons*, semi-social *Apteronotus leptorhynchus*, and gregarious *Adontosternarchus balaenops*. I collected blood samples before and after experimental housing to measure androgens, estradiol, and cortisol. *A. leptorhynchus* chirped significantly more than the other two species. Males chirped more than females in *A. albifrons* and *A. leptorhynchus*, but chirp rates were sexually monomorphic in *A. balaenops*. Relative gonad mass predicted chirp rates and/or gonadal steroid levels in *A. albifrons* and *A. balaenops*. In contrast, body size better explained variation in chirping and androgens in male *A. leptorhynchus*. Smaller fish and fish in worse reproductive condition also had higher cortisol levels in *A. albifrons*. These data suggest that species living in small groups with unstable dominance hierarchies, like *A. leptorhynchus*, may require more communication to resolve conflict. Body size and reproductive condition also coregulate signaling and steroid profiles differentially across species in a way that maps onto species' social structure.

Does global ocean change affect vulnerability of invertebrate prey to Gray Triggerfish predation?

Alanna Frick, Amanda Kirkland, T. Erin Cox

Ocean acidification and warming can impact the physiology and defenses of organisms and these effects in turn can scale to impact predator-prey dynamics. Gray Triggerfish (*Balistes capriscus*) are an economically and commercially relevant fish species in the northern Gulf of Mexico that feed on benthic invertebrates at artificial reefs. Understanding how ocean warming and acidification will impact both the vulnerability of their prey and fish-feeding behavior is essential to inform effective management strategies. As a first step to investigate future predator-prey dynamics, we exposed two prey species, yellowline arrow crab (*Stenorhynchus seticornis*) and titan acorn barnacle (*Megabalanus coccopoma*) to ambient or combined warming and acidification conditions in a highly controlled laboratory experiment. We hypothesized that exposure to stressors would result in weakened structures and altered

physiological states in prey causing them to be more vulnerable to predation. Following 30 days of exposure to stressor conditions, invertebrate species were placed in a mesocosm with a Gray Triggerfish. The time until first interaction between predator and prey species, total time until consumption, and number of overall interactions between species were recorded. Preliminary results indicate that the yellowline arrow crabs were more susceptible to predation than the titan acorn barnacles regardless of condition. Understanding how Gray Triggerfish feeding habits will be impacted by global ocean change is crucial for continued rebuilding and management of the fishery population.

Feeding innovations during the first actinopterygian adaptive radiation

Matt Friedman, Sam Giles, Rodrigo Tinoco-Figueroa

Living actinopterygian groups like cichlids, wrasses, and icefishes are celebrated examples of adaptive radiation, often showing substantial divergences in trophic ecology. However, all of these lineages have relatively shallow evolutionary roots, likely originating in the Late Cretaceous or early Cenozoic. The first major episode of morphological—and by extension ecological—diversification among actinopterygians took place hundreds of millions of years earlier, deep within the Paleozoic. Past work has hinted at a diversity of feeding modes within Carboniferous actinopterygians, but a lack of detailed anatomical information on jaws, dentitions, and other features has obscured the full range of innovations that arose during or before this interval. We use micro-CT scanning to investigate structures related to prey capture in a range of early actinopterygians. Key results include: evidence for the evolution of consolidated toothplates and precise occlusion associated with durophagy, fang-like dentary and coronoid teeth and novel strategies for accommodating them, elaborate basihyal dentitions suggestive of a “tongue-bite” apparatus, combs of peg-like teeth, and others. These differences are amplified by substantial variation in overall mandibular geometry, including size and shape of the adductor fossa and positioning of the jaw joint. Beyond providing clues about feeding in Paleozoic actinopterygians, these features represent substantial new character data bearing on the perennially challenging question of early ray-finned fish interrelationships.

A latitudinal gradient of deep-sea invasions for marine fishes

Sarah Friedman, Martha Munoz

Although the tropics harbor the greatest species richness globally, recent work has demonstrated that, for many taxa, speciation rates are faster at higher latitudes. Here, we explore lability in oceanic depth as a potential mechanism for this pattern in the most biodiverse vertebrates – fishes. We demonstrate that clades with the highest speciation rates also diversify more rapidly along the depth gradient, drawing a fundamental link between evolutionary and ecological processes on a global scale. Crucially, these same clades also inhabit higher latitudes, creating a prevailing latitudinal gradient of deep-sea invasions concentrated in poleward regions. We interpret these findings in the light of classic ecological theory, unifying the latitudinal variation of oceanic features and the physiological tolerances of the species living there. This work advances the understanding of how niche lability sculpts global patterns of species distributions and underscores the vulnerability of polar ecosystems to changing environmental conditions.

Illuminating the effects of artificial light at night: vocal phenology of avian cavity nesters

Alaina Friedrich, Donald Miles, Brett Seymoure

The predictability of natural light and dark cycles has had a paramount effect on the organization of Earth's biota. A growing body of literature suggests that anthropogenic light at night (ALAN) and noise have elicited a diverse response to the alteration of natural light regimes with a complex network of ecological consequences. The full extent of ALAN's impact is unknown; field studies on wild populations are lacking and many avian studies are limited to diurnal activity during the breeding season. In songbirds, there is evidence that ALAN impacts several biological processes and life history events in wild populations. ALAN's impact on the cavity-nesting guild has received limited attention. Woodpeckers are considered to be predictors of bird diversity in

forests as ecological engineers that provide critical nesting habitat to other species. This study aims to evaluate the seasonal impact of ALAN at a community level and to investigate abnormal nocturnal behavior. ALAN-induced differences in woodpecker occupancy, abundance, and behavior may have cascading effects on habitat availability for secondary cavity nesters. We used bioacoustic methods to evaluate differences in vocal phenology and daily activity of nine cavity-nesting species in southeastern Ohio at both dark and light-polluted sites over the course of three meteorological seasons. Preliminary results have demonstrated potential shifts in species' activity patterns at ALAN-impacted sites, providing grounds for further investigation within this guild.

The expression of Hox genes in the flour beetle *Tribolium castaneum*

Claire Fu, Gabrielle Jerz, Sophia Kelly, Nipam Patel

Homeotic (Hox) genes establish regional identity along the anterior and posterior axis of the body. They are well conserved across animal evolution, from insects, to worms, to humans, and in most species the genes are arranged into an organized complex, and their expression during development exhibits temporal and spatial collinearity that reflects their position on the chromosome. There are, however, some notable exceptions to these rules. In the fruit fly, *Drosophila melanogaster*, Hox genes exhibit spatial collinearity, but not temporal collinearity. This is consistent with their long germ mode of development where all segments form nearly simultaneously during embryogenesis. In contrast, embryos of the flour beetle, *Tribolium castaneum*, exhibit short germ development, in which the head is patterned initially, but the rest of the body segments are added sequentially during subsequent developmental stages. Thus, we hypothesized that *Tribolium* might display both spatial and temporal collinearity. We analyzed *Tribolium* Hox genes across multiple embryonic stages using five-color in situ HCR and confocal microscopy. We directly compared *Tribolium* Hox gene expression patterns to that of *Drosophila* and demonstrate that *Tribolium* Hox genes exhibits both spatial and temporal collinearity. These results demonstrate the developmental diversity within insects and provides further opportunities to understand the mechanisms of animal evolution.

The effect of anthropogenic sound on embryonic *Scyliorhinus retifer* respiration

Maryann Fuentes, Micaela Rivera, Allen Mensinger

The effects of anthropogenic sound on marine animals has recently emerged as an environmental concern. The increase in ship traffic and coastal development has impacted the soundscape of coastal waters. This study examined the effect of sound on the respiration rates of embryonic chain catsharks (*Scyliorhinus retifer*). Chain catsharks produce egg cases that are attached to hard substrate during extended (6 to 9 months) development. The static position of the eggs can potentially make the sharks more vulnerable to anthropogenic sounds than free-swimming animals that can escape sound fields. Respiration rates were monitored by backlighting the egg cases and filming the embryos with a GoPro camera and gilling rates were recorded before, during, and after sound presentation. The embryos were exposed to five consecutive 15 or 60 seconds playback of boat motor sound (151 dB) with each presentation separated by 60 seconds of no sound. The initial short durations presentation caused complete gilling cessation with subsequent stimuli also reducing gilling rates. The longer duration exposure also reduced gilling rates however it often resumed before sound extirpation but remained below control levels. These results indicate that repeated sound exposure reduces respiration rates in embryonic sharks, potentially leading to extended development times and/or insufficient oxygenation of the tissues.

Facultatively symbiotic species as models for exploring immune-symbiosis interplay

Lauren Fuess

Symbiotic associations are ubiquitous throughout nature and have immense fitness consequences for both host and symbiont. In the case of endosymbiosis,

these relationships are often intimately connected to host immune function. Symbionts and host immunity are tightly linked via bidirectional feedback loops; host immunity often regulates symbiotic interactions and symbiotic associations may shape development and function of the immune system. As a consequence of this tight association, the maintenance of symbiotic relationships is hypothesized to exert significant selective pressure on host immunity, contributing to diversification of immune systems. Still the nuances of immune-symbiosis interplay, and broader consequences of this relationship, remain understudied in a diversity of systems. Facultatively symbiotic organisms, which display immense natural variation in host-symbiont relationship, provide excellent model systems for studying host-symbiont relationships drive diversification of immunity. Here I will discuss ongoing work applying integrative 'omic analyses to study symbiosis-immune interplay in two facultatively symbiotic cndiarans, *Exaiptasia diaphana* and *Astrangia poculata*. Continued work in these powerful systems will provide critical new insight regarding how symbiotic interactions drive diversification of immunity.

Round table discussion: The future of immune 'omics

Lauren Fuess

Join the presenters from our symposium (Immunity in the 'omics age: what can 'omics approaches tell us about immunity in natural systems?) for an interactive panel discussion on the future of non-model immunology in the age of 'omics approaches. We will discuss commonalities from our presentations throughout the day, pressing questions moving forward, and best practices for applying emergent 'omic technologies to studying immunity in diverse systems.

Neural mechanisms of rapid, precise, and adaptive motor control

Terufumi Fujiwara

We have a remarkable capacity to rapidly and precisely control body movement. We can further adaptively adjust movements depending on environmental and body states. Because motor functions are broadly distributed across the brain, and so many neural populations are recruited, it is challenging to link neural activity to body movement causally. We leverage a convenient model organism, the fruit fly, *Drosophila*, to motor control research. Thanks to the advanced genetic toolkit and electron microscopy reconstruction dataset, we can flexibly record and manipulate the activity of specific neural populations in the almost entirely elucidated neural circuit. Recently, we have shown that the fly brain monitors and modulates the movement of individual leg strides during walking, suggesting an unexpectedly sophisticated motor control computation in its tiny brain. Including my previous work, most of the motor control research in flies has focused on spontaneous locomotion, and it was unclear how the fly actively controls body movement for a specific goal. My lab has been developing novel behavioral paradigms where the fly rapidly, precisely, and adaptively controls movement. These tasks can be performed under a head-fixed condition, allowing simultaneous neural recording for circuit dissection. Because the neural circuit structure is much simpler in flies, computational principles could be directly observed in the activity of single neurons. Therefore, findings in flies could provide intuitive insights into how the brain controls movement, one of the most fundamental yet unsolved questions in system neuroscience.

The Effects of beta-estradiol on Crayfish Behavior and Gonadal Structure

Alyson Furstenau, Daniel Bergman, Megan Gasparaitis, Kayla Shields, Hannah Shull, Lauren Wilmore

An essential aspect of crayfish survival is the ability to find a mate and reproduce. If these abilities are negatively impacted, then a decrease in crayfish populations is likely to occur. Although estrogen is found in both male and female crayfish, an increased concentration has the potential to feminize the male sex organs and lead to reduced fertility. β (beta)-estradiol, an estrogen steroid hormone found in most birth control medications, has entered many of our local bodies of water via sewage effluent. In this experiment, we will expose male and female crayfish to acute and chronic levels of β (beta)-estradiol, and

then observe whether they can distinguish male and female odors, as well as the attractiveness of each. Gonads from chronically exposed crayfish will be analyzed to detect alterations in structure and size. We hypothesize that increased exposure to β (beta)-estradiol will negatively impact crayfish when attempting to find a mate and potentially change their reproductive organs.

Using microCT imaging to explore woody plant structure-function

Morgan Furze

In long-lived woody plants, nonstructural carbohydrates (i.e., sugars and starch) are produced through photosynthesis and can be stored for later use. This energy reserve serves as a potential resiliency mechanism to stress, as plants can draw on existing reserves to survive and persist during unfavorable conditions when photosynthesis is limited. Traditionally, methods to quantify nonstructural carbohydrates have relied on destructive tissue sampling in which information about the spatial and temporal dynamics of carbon storage is lost. MicroCT imaging has emerged as a powerful tool for investigating structure-function relationships in plants and has more recently been used to quantify starch dynamics in intact plants. The non-destructive nature of microCT imaging which allows plant physiology to be monitored over time combined with 3D visualization holds promise for advancing our understanding of plant carbon storage and allocation. Here, we highlight examples of how microCT imaging of woody plants from diverse environments, ranging from forests to agroecosystems, can provide insight into their carbon physiology.

Physiological adaptations for migration influence how birds to respond to environmental changes

Leonida Fusani, Andrea Ferretti, Ivan Maggini, Valeria Marasco

Prior and during migration, birds undergo major physiological transitions: increase in food intake, deposition of large amounts of subcutaneous fat, shifts in the rhythms of activity, and changes in the digestive tracts to mention a few. In this talk I will present a series of experiments that our groups has conducted in the field and in the laboratory to understand better how such adaptations respond to environmental factors. We showed that food availability, changes in average temperatures, exposure to predation and interaction with other species all can affect migratory behavior, resulting in changes of the stopover duration, time of migration, and association with other species. We also found that in migratory Passerines the well-conserved hormonal systems that control food intake and fat metabolism in vertebrates has been remodeled, probably to allow a larger flexibility in body mass regulation to support the increased energy needs during migration. Prior and during migration, birds undergo major physiological transitions: increase in food intake, deposition of large amounts of subcutaneous fat, shifts in the rhythms of activity, and changes in the digestive tracts to mention a few. In this talk I will present a series of experiments that our groups has conducted in the field and in the laboratory to understand better how such adaptations respond to environmental factors. We showed that food availability, changes in average temperatures, exposure to predation and interaction with other species all can affect migratory behavior, resulting in changes of the stopover duration, time of migration, and association with other species. We also found that in migratory Passerines the well-conserved hormonal systems that control food intake and fat metabolism in vertebrates has been remodeled, probably to allow a larger flexibility in body

Light pollution disrupts the circadian clock in two mosquito species in their overwintering dormancy

Lydia Fyie, Katie Westby, Megan Meuti

Increasing light pollution caused by artificial light at night (ALAN) is a growing threat to organisms as it alters the light signals that regulate their daily and seasonal biology. To survive unfavorable conditions in winter temperate insects such as the Northern house mosquito (*Culex pipiens*) and the tiger mosquito (*Aedes albopictus*) enter a programmed dormancy known as diapause that is primarily cued by short daylengths. Additionally, diapause is stage-specific and varies between insects: *Cx. pipiens* enter diapause as adult females and *Ae. albopictus* enter a maternally-programmed, egg diapause.

Our previous studies found that exposure to ALAN inhibited diapause initiation in both species, but the mechanism is unknown. The circadian clock is involved in the regulation of diapause in several insect species. Therefore, we examined whether exposure to ALAN interfered with the daily expression of core circadian clock gene transcripts (i.e. cycle, Clock, timeless, and period) in the heads of females of *Cx. pipiens* and *Ae. albopictus* that were exposed to short-day, diapause-inducing conditions. We found that although rhythmicity was maintained for all genes we measured, exposure to ALAN altered the abundance of these genes in both species. Our results indicate that ALAN may inhibit diapause initiation by perturbing circadian clock gene expression. As the circadian clock is relatively conserved among insects, this implies that impacts to overwintering dormancy may be widespread through insect taxa.

Investigating the Occurrence of Plastic in Historic Fishes from Galveston Bay Estuary System

Bryan Gahn, Karl Kaiser, Kevin Conway, Christopher Marshall

Marine ecosystems are facing increasing threats from plastic pollution, with plastic accumulating in fish tissues decreasing behavioral performance, overall fitness, and posing health concerns regarding consumption of these fishes. To efficiently manage ecosystems in the context of plastic pollution, new quantitative methods are required to assess the magnitude, pattern of accumulation, and future potential body loads. Here we focus on the microplastic body burden of three economical and environmental important fish Orders, Clupeiformes (gulf menhaden-*Brevoortia patronus* and anchovies-*Anchoa mitchilli*), Mugiliformes (stiped mullet-*Mugil cephalus*), and Acanthuriformes (spotted sea trout-*Cynoscion nebulosus*) in the Galveston Bay Estuary System (GBES). To fully understand the issue of microplastics in these fishes, it is important to understand and quantify historical accumulation as well as current abundance. Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) allowed for the accurate quantification of 12 unique plastics in fish muscle tissue. Processed archival fish tissue samples collected over the past 50 years in the GBES resolved the historical trajectory of plastic accumulation and relative abundance in these fishes. Total plastic body burden increase over time in the GBES is strongly correlated with global plastic production. Moreover, relative proportions of unique plastics appear independent of trophic level and feeding mode in GBES fishes. This comprehensive temporal analysis sheds light on the evolving plastic pollution landscape and offers insights into plastic's persistent nature within the GBES, and likely globally.

Vestigial organs alter fossil placements in an ancient group of terrestrial chelicerates

Guilherme Gainett, Benjamin Klementz, Pola Blaszczyk, Emily Setton, Rodrigo Willemart, Efrat Gavish-Regev, Prashant Sharma

Vestigial organs provide a link between ancestral and derived morphologies, and have potential to resolve the phylogeny of contentious fossils that bear features unseen in extant taxa. Nonetheless, the empirical importance of vestigial organs in phylogeny remains poorly documented. Here we show that extant daddy-longlegs, a group once thought to possess only one pair of median eyes, have a pair of both vestigial median and vestigial lateral eyes. Gene expression surveys of eye-patterning transcription factors, opsins, and structural proteins in the daddy-longlegs *Phalangium opilio* show that the vestigial median and lateral eyes are innervated by brain regions positionally homologous to the median and lateral eye neuropils, respectively, of chelicerate groups like spiders and horseshoe crabs. Gene silencing of eyes absent shows that the vestigial eyes are controlled by the retinal determination network. The incidence of lateral eyes in extant taxa bears upon the placement of the oldest harvestmen fossils, a putative stem group that possessed both median eyes and lateral eyes. Phylogenetic analysis of harvestman relationships with an updated understanding of lateral eye incidence resolved the four-eyed fossil group as a member of an extant daddy-longlegs suborder, which in turn resulted in older estimated ages of harvestman diversification under total evidence dating. This work underscores that developmental vestiges can influence our understanding of character evolution, placement of fossils, and inference of divergence times.

Does experimentally increased testosterone change metabolic rate via mitochondria in male songbirds?

Holland Galante, Timothy Greives, Britt Heidinger, Jeffery Kittilson, Samuel Lane, Emily Elderbrock, Kendra Greenlee

Transitions between life history stages consist of changes in behavior and physiology which are underlined by changes in metabolism and energy production, occurring primarily in the mitochondria. Previous work in seasonal breeding songbirds observed that increased levels of sex steroids, specifically testosterone, are accompanied with increases in basal metabolic rate (BMR). In humans, testosterone modulates mitochondrial function through activation of androgen receptors binding to transcription elements present within the mitochondria, however, the mechanism which testosterone affects BMR remains unclear. One possibility is that testosterone is altering mitochondrial abundance. Mitochondrial abundance can be quantified by assessing mitochondrial DNA copy number (mtDNAcn). However, to what degree mtDNAcn is predictive of BMR as well as the effects of sex steroids on mtDNAcn in non-human models is unknown. Here we investigate whether increased testosterone levels increase both BMR and mtDNAcn in captive adult male house sparrows (*Passer domesticus*). We first experimentally elevated testosterone under short-day photoperiods with subcutaneous implants and then induced a natural endogenous rise in testosterone by transitioning the birds to a long-day photoperiod. We measured BMR using flow-through respirometry to measure O₂ consumed and collected blood samples to quantify mtDNAcn via qPCR. We predicted that elevated testosterone would result in an increase BMR and mtDNAcn. These data will enhance our understanding of the physiological mechanisms that underpin seasonal reproduction as well as other life history events.

Noise effects on black-capped chickadee, tufted titmouse, and white-breasted nuthatch audition

Megan Gall, Trina Chou

Anthropogenic noise has been shown to affect communication in a large number of species. Songbirds, in particular, have been shown to alter the structure and timing of communication in response to noise. However, the effects of noise on auditory processing have been less well explored. Here, we investigated the effect of noise on the auditory thresholds of black-capped chickadees (*Parus atricapillus*), tufted titmice (*Parus bicolor*) and white-breasted nuthatches (*Sitta carolinensis*). We found that nuthatches had the lowest thresholds in quiet, but the highest thresholds in noise and the highest critical ratios. Titmice had intermediate thresholds in quiet, and the lowest thresholds in noise, resulting in the lowest critical ratios. Chickadees were intermediate in their thresholds in quiet and in noise, as well as in their critical ratios. These results suggest that species may differ in the effects of noise on auditory processing. We suggest that this may result in species-level differences in the effects of noise on communication and other acoustically-mediated behaviors, such as the detection of predators.

Wall obstacles impact escape response kinematics in Pacific staghorn sculpin (*Leptocottus armatus*)

Jacquelyn Galvez, Paolo Domenici

Effective escape maneuvers are critical for prey fishes, as fast-start performance has a direct impact on survival in predator-prey interactions. For fish living in structurally-complex environments, the presence of physical obstacles—such as rocks for bottom-dwelling fishes—may impact fast-start behaviors and overall performance. Incorporating proxies for such obstacles in escape response studies will improve their ecological relevance and offer better insight into the predator evasion tactics employed by these fishes. In this study, we investigated the effects of wall obstacles on escape response in Pacific staghorn sculpin (*Leptocottus armatus*). We used a mechano-acoustic stimulus to trigger fast-start behaviors, and used a high speed camera to film fish with and without a wall barrier located parallel to the body. We analyzed various kinematic and performance metrics, including responsiveness, escape directionality, turning angle, turning rate, and distance traveled. Preliminary results indicate that fish were less responsive in trials with the obstacle compared to those without. Additionally, escape directionality toward the stimulus was

higher in obstacle trials, a trend also observed in other studies. Further investigating the impact of fish size, obstacle experience, and obstacle positionality (left versus right) will provide a more holistic understanding of predator evasion for these bottom-dwelling fish.

Testing hypotheses for the origin of the tardigrade stylets using an evo-devo approach

Mandy Game, Frank Smith

The stylets of tardigrades function as a key component of their feeding apparatus. Despite their significance, the origins of the tardigrade stylets remain mysterious. The stylets are situated in the tardigrade head, sparking questions regarding their homology to anterior structures of other panarthropods. In one hypothesis, the tardigrade stylets are homologous to the frontal appendages of onychophorans and the labrum of arthropods. In a second non-mutually exclusive hypothesis, tardigrade stylets are serially homologous to legs. In order to understand whether tardigrade stylets are homologous to anterior appendages of other panarthropods, we investigated the expression patterns of *six3*, a marker of the labrum and frontal appendages, and *forkhead (fkh)*, a marker of the gut but not appendages, in the tardigrade *Hypsibius exemplaris*. In order to understand whether tardigrade stylets are serially homologous to tardigrade legs, we investigated expression patterns of *Distal-less* and other leg patterning genes. We recovered weak or conflicting support for both hypotheses. We discuss our results in terms of the challenges of testing hypotheses of homology.

Fish out of Water: Kinetics of Amphibious Transition

Kaelyn Gamel, Henry Astley, Brooke Flammang

Underwater walking was an evolutionary predecessor to terrestrial walking. The evolutionary transition from buoyancy-supported benthic substrate interactions to gravity-mediated forces in littoral and terrestrial habitat use varies significantly with regards to the mechanical demands of the locomotor. These mechanical demands have been difficult to measure, especially for extinct transitional taxa, but recent measurement technology advancements allow acquisition of substrate reaction forces from aquatic walkers. Using our custom designed underwater force plate, we quantified the forces generated by a bioinspired robot across varying the submergence depths. These data explore the fundamental physical requirements for weight-supported walking on land and expand the knowledge on how submergence level may mitigate physiological adaptations for terrestrial locomotion.

Wnt signaling negatively regulates cardiac progenitor division during *Ciona robusta* heart growth

ZIXI GAO, Richard Garcia, Hannah Gruner, Bradley Davidson

Wnt signaling plays a pivotal role in coordinating cell development and organ growth, while its contributions to heart development remain unclear. We're studying Wnt signaling in *Ciona robusta*, a basal chordate tunicate. *Ciona*'s heart growth is driven by progenitors known as the undifferentiated lines (UL). Recent scRNA sequencing studies indicate Wnt receptors (similar to *Frizzled4*) and target genes (similar to *Axin2* and *LGR5*) expression in the ULs. However, the exact role of Wnt in *Ciona*'s heart development remains undiscovered. Here we show that inhibiting Wnt in early juveniles promotes UL proliferation. To assay Wnt's effect on *Ciona* heart growth, *Ciona* juveniles were treated with IWR-1-Endo (Wnt antagonist) or vehicle (DMSO) starting at 3 days post fertilization (dpf) for 48 hours, fixed and stained with DAPI to label cell nuclei. Our preliminary result indicates that Wnt stimulates UL proliferation as we observed one less UL when inhibiting Wnt. However, due to juvenile growth arrest in Petri dishes and potential issues with assaying UL proliferation by scoring its numbers, we conducted a more rigorous assay by putting juveniles into a tank at 2dpf for proper growth and by Edu staining to label proliferating cells. As detailed in my labmate Richard Garcia's abstract, the data contradicted our preliminary results, indicating that Wnt suppresses UL division, with other factors influencing division symmetry. These experiments start to map Wnt signaling in coordinating progenitor division for *Ciona* proportional heart growth.

Wnt signaling regulates stem cell proliferation during *Ciona robusta* heart growth

Richard Garcia, ZIXI GAO, Hannah Gruner, Bradley Davidson

Wnt signaling is a conserved pathway with numerous developmental roles including regulating heart formation and growth. Its contributions have not been fully characterized, so we investigated how Wnt signaling affected heart growth in *Ciona robusta* (a chordate of the tunicate phylum). In *Ciona* juveniles, heart growth is driven by a distinctive group of cardiac progenitors known as the undifferentiated line (UL). Recent scRNA sequencing studies indicate that Wnt receptors and target genes are expressed in the UL. To determine any contribution of Wnt signaling to UL division, a Wnt inhibitor (IWR-1-endo) was applied to developing juveniles on day 7 of development for 24 hours. Animals were then exposed to an EdU pulse (to visualize cell division) for 30 minutes followed by rinsing. A pulse set of control and experimental animals was then fixed. Another set of animals was allowed to grow for a 36-hour chase period followed by fixation. Then, all samples were stained with DAPI to label nuclei. The pulse and chase experiments showed a significant increase in EdU-labeled progenitor cells without a significant increase in total cells along the UL. These results indicate that the Wnt signaling pathway suppresses cardiac progenitor proliferation, while a Wnt-independent mechanism maintains UL length. This experiment has begun to outline the role of Wnt signaling in coordinating the dynamics of progenitor division for proper organ growth.

Towards a general model for the evolution of reptile viviparity

Guillermo Garcia-Costoya, Akhila Gopal, Noa Ratia, Karla Alujevic, Madison Glenwinkel, Shea McKendree, Kaitlyn Napier, Cody Chapman, Allison Dorny, Michael Logan

Viviparity, or live birth, is a common reproductive strategy in squamate reptiles, with more than 100 independent evolutionary origins. Viviparity is often associated with the presence of cool climates and thus is frequently observed in species that occur at high latitudes and/or elevations (i.e., the "cold climate hypothesis"). While this hypothesis has been supported by phylogenetic and phylogeographic studies, these types of studies have an important limitation in that they must infer microevolutionary processes from macroevolutionary patterns. To address these limitations, here, we directly explore the processes that might have given rise to the evolution of viviparity using a mathematical framework. First, we develop a model to predict the optimal parity strategy for any ectotherm based on the relationship between the mother and embryos' thermal physiology and the thermal characteristics of the environment they inhabit. We then compare the model's prediction with 1) data from the literature on reptile thermal physiology and reproduction, and 2) a case study of western fence lizard (*Sceloporus occidentalis*) populations distributed across an elevational thermal gradient. In addition to formalizing the processes that may have led to the evolution of viviparity, our model broadly illustrates how ectotherm reproductive strategies are mediated by thermal environments and thus may serve as a predictive tool to forecast their responses to climate change.

Olfactory Morphology in Spotted Ratfish (*Hydrolagus coliei*)

Valentina Garcia-Nunes, Lauren Simonitis

Hydrolagus coliei is a cartilaginous fish in the chimaeridae family inhabiting the depths of the Northeastern Pacific ocean. Knowledge of chimaera sensory morphology is currently limited. Though it is known that some chimaera species exhibit ontogenic and sex-specific differences in diet, location, and migration pattern (Di Giacomo and Perier 1996; Last and Stevens 2009; Mauchline and Gordon 1983; Moura et al. 2005; Quinn et al. 1980), sensory morphology has not been compared between sexes or size classes. 49 *H. coliei* varying in size and sex were collected off of San Juan Island, WA, USA using deep water trawls, preserved, and CT scanned.

The olfactory nerve, nostrils, rosettes, lamellae, and raphe of each specimen were measured using 3DSlicer and ImageJ, and the data was analyzed in R. Preliminary results show a positive linear relationship between rosette diameter and total length of the specimen, as well as rosette surface area and total length. Though the distance between nostrils increases with total length,

nostril diameter does not scale with the total length, suggesting that the olfactory system is important from an early age. There is, however, a negative linear relationship between the number of lamellae per rosette and total length of the specimen. This is notable since lamellae add surface area, meaning that in theory more lamellae means more surface area resulting in a better sense of olfaction.

Patterns of diversification and distribution in *Raorchestes* frogs on the Indian subcontinent

Sonali Garg, James Hanken, S. D. Biju

The Indian subcontinent is a major centre of diversity for the genus *Raorchestes*, one of the largest radiations of the Old-World tree frog family Rhacophoridae. The Western Ghats in Peninsular India has particularly been the focus of extensive research, while the geographically distant Northeast of India has remained relatively neglected. This largely disjunct distribution of the genus and gaps in basic knowledge on its existing diversity have propagated taxonomic and distribution range ambiguities for several species, consequently excluding them from systematic and phylogeographical investigations. We reviewed the diversity and distribution of *Raorchestes* frogs with near-complete representation, combining both previous and our extensive new data, resulting in the discovery of over a dozen new species and resolution of long-standing nomenclatural confusions. Evidence from multiple lines including external morphology, skeletal features, acoustic properties, molecular phylogeny, and geography, enabled us to better characterise the species themselves, their systematic affinities, and understand patterns of diversification and distribution of this large radiation across Asia. We confirm that the genus originated in southwestern India, rapidly diversified, and expanded ranges in northern Western Ghats during the Miocene. We also find that two species-groups exploited dispersal routes between the Western and Eastern Ghats closer to Pliocene, and *Raorchestes* eventually colonised Eastern Himalayas and Indo-Burma through Northeast India with further diversification of a single lineage that dispersed out-of-India into the rest of Asia.

Teaching about trade-offs and constraints

Theodore Garland

Trade-offs in economics and daily life are commonly understood (e.g., living near a city center usually offers more entertainment opportunities, but rents are also more expensive than living in the suburbs). In biology, trade-offs exist for various reasons, related to allocation constraints (the classic Y-model for resource allocation), functional conflicts, shared biochemical pathways, antagonistic pleiotropy, and ecological circumstances. These categories sometime overlap and/or represent different ways of looking at a given phenomenon. Appealing to trade-offs can be an approachable way to aid in teaching a range of subjects in both organismal and evolutionary biology. This talk will provide a number of examples and case studies, drawn from both my own research in evolutionary exercise physiology and from the literature.

Do repetitive locomotor trials lead to reduced muscle strain in snakes?

Kelsey Garner, Jessica Tingle, Henry Astley

In the lab and in the wild, animals perform a variety of tasks that require locomotion, often in quick succession. These behaviors can cause substantial fatigue, especially if energy intensive and executed repeatedly, which in turn can reduce muscle performance. While most terrestrial species must maintain static or dynamic stability, limbless taxa do not face this constraint, and may be able to more freely adjust their posture and consequent muscle strain trajectories to accommodate reduced performance. We used a series of fluoromicrometry trials to opportunistically test the prediction that with repeated locomotor tasks, muscles may not contract as much or as quickly, and snakes may take longer or more frequent pauses indicative of fatigue. We implanted radio-opaque beads on opposing ends of three muscles in four corn snakes (*Pantherophis guttatus*), and then recorded the snakes performing multiple modes of locomotion. For three consecutive days, each snake underwent multiple trials per locomotor mode and was rested between modes before being tested again. Preliminary results show changes in muscle strain

suggestive of fatigue onset over the course of the trial period. Our results can help future lab studies on snakes better prevent or account for the effects of fatigue in repeated trials, and our results also highlight potential consequences of physiological limits on behavioral demands that ectotherms must meet in nature.

Effects Of Cold Temperatures on Avian Immune Responses to A Bacterial Pathogen

Jesse Garrett-Larsen, Alexa Jordan, Annabel Coyle, Kate Langwig, Dana Hawley

The host immune response to a pathogen can be highly variable among individuals, resulting in heterogeneity in both infection and disease severity. Understanding such heterogeneity is key as it can influence the likelihood of disease outbreaks. Environmental factors such as low winter temperatures may exacerbate immune heterogeneity, altering seasonal disease outbreaks. For endotherms, energetic constraints at subthermoneutral temperatures may alter both the magnitude and variability of immune responses, but the effect of ambient temperature on immune variability has not been directly tested. We used temperature-controlled chambers housing wild-caught house finches at sub-thermoneutral (4-9°C night-day) or thermoneutral (22-27°C night-day) ambient temperatures to test effects of temperature on host immune responses. Half of the birds at each temperature were inoculated with the bacterial pathogen *Mycoplasma gallisepticum*, and several host immune measures were quantified over the course of infection. To diversely quantify immune responses, we measured whole-body responses and aspects of cellular and humoral immunity, including conjunctivitis severity, mass, fever, phagocytic activity, haptoglobin, and anti-MG antibodies. We present analyses testing how ambient temperature, MG infection, and their interaction influence the mean and degree of variation in host immune responses. We predict that mean responses will be depressed, and overall responses will be more variable in hosts housed at subthermoneutral versus thermoneutral temperatures and that temperature effects will be strongest for pathogen-infected hosts, with consequences for downstream disease dynamics.

How spider bodies set the communication frequency

Reese Gartly, Senthurran Sivalinghem, Lachlan Fisher, Mouad Elganga, Benjamin Rubin, Andrew Mason, Natasha Mhatre

Spider leg joints are multi-functional and are important both in vibration sensing and locomotion. We expect that the biomechanics of leg joints are tuned to enable them to support the spider's mass will also set the vibration frequency range. We studied 11 spider species and measured the rotational stiffness of two joints on two legs. Using mixed effects models we examined the scaling relationships between joint stiffness and spider mass, and whether the joint and species identity affect this relationship. We found that heavier spiders had stiffer joints, and the relationship between mass and stiffness was nearly allometric. These data when incorporated into experimentally validated multibody models of spiders, suggests that spiders of different body masses will nonetheless be mechanically stimulated by very similar vibration frequency ranges. Thus, our data suggest that using the whole body as a sensor prevents spiders from niche partitioning the vibrational signal space.

Comparative morphometrics of the postorbital ligament in birds (Class: Aves)

Samantha Gartner

Ligaments connect bone to bone in the musculoskeletal system and have different mechanical properties (break stress and strain, young's modulus) based on their structural properties (length, cross section area (CSA)). The post-orbital ligament in birds connects the neurocranium to the lower jaw in most species, coupling the movement of the two bones. When loaded, this ligament is predicted to cause fast jaw closing and force transfer through the quadrate, pterygoid, and jugal bones to the upper jaw. In this study, we collected morphometrics (length, mass, width) of the postorbital ligament in 10 species of birds (Class: Aves). We also measured different characteristics of the skull to standardize for size of the birds. The structural properties of the postorbital ligament are found to differ across species indicating potential differences in

mechanical properties within this ligament. This indicates different species of birds could be using the postorbital ligament differently; some could be using it for force transfer while others could use it to store energy. Future investigations into this ligament should test mechanical properties to further understand the function of postorbital ligament.

Keystone molecules disrupt estuarine food webs: who consumes defended grazer tissue?

Anahy Garza

Many gastropods can avoid predation due to chemical defenses. Chemical defenses may function as keystone molecules if impacts on food webs and community composition are disproportionate to their concentration. In Northern Hemisphere estuaries, grazing sea slugs (*Alderia* spp.) reach high densities (>1,000/m²) where the alga *Vaucheria* forms thick mats. Slugs produce polyketide compounds that protect them from small predators and also reduce the abundance of mudflat infauna. The fate of slug biomass remains unclear, however, with important implications for understanding energy flow in this key nursery habitat. Here, we test whether gastropod scavengers or mudflat detritivores consume live or dead *Alderia*, and could represent an alternative path for energy to reach higher trophic levels. Evidence indicates that four detritivore species feed on dead slug tissue that is rejected by predators, but live slugs were unpalatable to detritivores and predators alike; active release of polyketides in a mucus secretion may protect slugs from detritivores. Several gastropod species fed on live slugs which were always rejected by non-molluscan predators, indicating gastropod consumers are not deterred by the chemical defense of *Alderia*. Identifying the fate of carbon fixed by *Vaucheria* will help model energy flow in estuarine habitats. Moreover, this work highlights the unexpected impacts climate change is likely to have on estuarine communities by altering the distribution and abundance of *Alderia* and the compounds that slugs release.

Evolution of odorant receptor repertoires across Hymenoptera

Shubham Gautam, Sean McKenzie, Julian Katzke, Francisco Garcia, Shûhei Yamamoto, Evan Economo

Olfaction facilitates recognition and communication between nestmates of eusocial insects. Olfactory discrimination of chemical cues is achieved through a one neuron-one receptor strategy, with each receptor type associated with a single glomerulus in the antennal lobe of the brain. Recent studies suggest that independent evolution of eusociality may be associated with expansion in odorant receptor (OR) gene repertoires, specifically within the 9-exon subfamily, ascribing an important role to these genes in the evolution of eusociality. In particular, ants and *Polistes* wasps are thought to have expanded 9-exon ORs. This putative expansion at molecular level should correspond with the expansion of the related T6/Tb cluster of glomeruli in the antennal lobes of the brain. However, this hypothesis has not been tested with a broad comparative analysis. Here, we compiled existing genomic data on OR gene repertoires and added new neuroanatomical data from a variety of hymenopteran lineages including a remarkable ~100my old fossil ant brain, to shed light on the evolution of odorant receptor repertoires across the group. In contrast to the widely held hypothesis, our results suggest that the expansions in OR repertoires do not correlate with the evolution of eusociality. The pattern of evolution is complex, but post-hoc exploration of our data hinted at loss of flight as a possible factor shaping some of the variation in OR repertoires in Hymenoptera.

Resolving the embryonic origin of hindgut tissue in the tardigrade *Hypsibius exemplaris*

Elizabeth Gavrilo, Mandy Game, Frank Smith

Like many tardigrade species, the body axis of the tardigrade *Hypsibius exemplaris* terminates with a pair of legs. However, during embryogenesis, a cap of cells extends beyond the posteriormost leg pair. A previous study found that caudal, a marker of hindgut in other animals, and the posterior Hox gene *Abdominal-B* were expressed in the posterior cap. We tested whether the

posterior cap gives rise to the hindgut in *H. exemplaris*. We found that the expression of caudal and the posterior Hox gene *Abdominal-B* overlapped with expression of the gut marker forkhead in the posterior cap during the early limb bud stage. This result indicates that the posterior cap is composed of presumptive gut cells. Later in embryogenesis, coexpression of caudal, *Abdominal-B*, and forkhead was restricted to internalized cells, anterior to the hind legs. At the hatchling stage, expression of caudal, *Abdominal-B*, and forkhead overlapped in a region of the hindgut immediately posterior to the midgut. *Abdominal-B* was strongly expressed in the malpighian tubules, a component of the hindgut. Both caudal and *Abdominal-B* were expressed in the cloaca, the posterior opening into the hindgut. Additionally, expression of the endodermal marker *gata4/5/6* was restricted to the midgut. Based on our results, we conclude that the posterior cap of cells ingresses during embryogenesis to give rise to the hindgut and that the hindgut is ectodermal in origin.

Holey-Head-Fish-Friend: Inside the Bizarre Cranial pit of the Rockhead Poacher, *Bothragonus swanii*

Daniel Geldof

The Rockhead Poacher, *Bothragonus swanii*, is a small armored subtidal fish found in the northwest United States and Canada. The top of its skull features a deep depression with intricate internal morphology. This feature, known as the cranial pit, is unique among fishes. No complete explanation of the pit's purpose currently exists, but prior research suggests an acoustic function. I examined *B. swanii* with contemporary biovisualization techniques: CT/diceCT; alizarin staining; and histology. The pit's close physical linkage to the otoliths and nerve cord support the acoustic hypothesis. I present new high-resolution images, provide my interpretations, and outline future live experiments on *B. swanii*.

Modeling Hyolingual Musculature of *Anolis* using Avizo XFiber

Jacob George, Christopher Zobek, Susan Williams, Haley O'Brien, Casey Holliday

Muscle architecture has significant implications for the function and performance of musculoskeletal systems. Characterization of muscle architecture is difficult with classical dissection methods, particularly for structures and organisms of smaller sizes. Developments in digital imaging have opened new ways to analyze muscle architecture, such as digital fiber tracing. Our study employed digital fiber tracing to quantify the small and delicate musculature associated with the hyolingual apparatus of the squamate *Anolis*. This structure plays a critical role in their life history and ecology due to its key role in the extension of the dewlap, a cervical flap displayed during intra- and interspecific interactions. We analyzed the hyolingual musculature of *Anolis carolinensis* using a combination of Diffusible Iodine-Based Contrast-Enhanced Computed Tomography (diceCT) and digital fiber tracing using Avizo XFiber software. We modelled fascicle lengths, fascicle orientations, and muscle volumes in three-dimensional space, including the *M. omohyoideus*, the *M. sternohyoideus*, the *M. hyoglossus*, the *M. verticalis*, and the *M. branchiohyoideus*, a muscle that is hypothesized to be directly involved in the mechanisms that lead to the protrusion of the dewlap fold during display behaviors. Through this initial application of digital fiber tracing to squamate hyolingual musculature, this study also showcases the benefits of digital methods to analyze complex anatomical features in small vertebrates.

The International Union for Conservation of Nature (IUCN) SSC Marine Star Specialist Group

Sophie George, Monika Böhm, Amanda Vincent

The Marine Star Specialist Group (MSSG) was recently added to a growing list of International Union for Conservation of Nature (IUCN) Species Survival Commission (SSC) specialist groups. This new group focuses on the asteroids (sea stars), ophiuroids (brittle stars), and crinoids (feather stars and sea lilies). The group will strengthen the SSC's vision - to prevent the loss and aid the recovery of species belonging to these three classes. Among the sea stars, only the sunflower sea star (*Pycnopodia helianthoides*) has been

assessed for the IUCN Red List of Threatened Species to date, as Critically Endangered. This sea star, like many others in the Northeast Pacific, is threatened by sea star wasting disease. Sea stars living in other parts of the world also face similar threats. For example, *Marginaster littoralis* is listed in Australia as critically endangered and according to experts may even be extinct. The Antarctic sea star (*Odontaster validus*), has seen declines in their population because of recent disease outbreaks. The conservation status of many cave-dwelling sea stars and brittle stars is unknown. Other brittle stars, feather stars, and sea lilies are found in the mesophotic zone; threats to coral reefs such as deep-sea mining and fishing activities could be quite damaging to these species. Experts all over the world have joined the group and new members are welcome to join this conservation effort.

The effect of temperature on kinematics, aerodynamics and aeroacoustic communication in mosquitoes

Marcos Georgiades, Rachel Tran, Joerg Albert, Simon Walker, Richard Bompfrey

Male *Anopheles gambiae* mosquitoes locate females in swarms using the sounds generated by their wings. This interaction of the flight motor used for locomotion and the sensory physiology of communication can be a low-cost mechanism for mate location but is inherently dependent on key biomechanical parameters such as the wing beat frequency. One example of how this coupling impacts upon courtship is the observation that wing beat frequency is correlated with ambient temperature. Here, we investigate the relationship between varying male and female wing kinematics, their aerodynamics, and the acoustic cues that are relied upon for successful mating in the context of mosquito hearing: specifically, the antennal frequency response of the Johnston's organ.

After acquiring raw footage of flight with a synchronised ten-camera array operating at 12,000 frames per second, we used a voxel carving method to extract the geometry and kinematics of mosquitoes across a range of temperatures. As the temperature increases, so does the wing beat frequency. Concomitantly, the wing stroke amplitude decreases until the wing sweeps through the air for approximately just one chord length before flipping and reversing direction for the next half stroke. With these kinematics, estimates of lift and thrust from traditional aerodynamic theory that has previously been used to assess insect flight, such as quasi-steady blade element theory, deteriorates. Direct numerical simulations show that a greater proportion of the forces required for weight support are generated in the rapid rotations at stroke reversal.

Evolution of Infant Feeding in Mammals

Rebecca German, Christopher Mayerl, Devon Stuart

The origin of the mother/infant dyad is one of the hallmark features of mammalian evolution. The subsequent variation amongst mammalian taxa is well documented on the maternal side, but much less is known about variation among infants. In particular the relationships between oropharyngeal anatomy and feeding function and biomechanics is known only for a few species. We tested the hypothesis that while there are many similarities across infants, there will be specific differences in function that vary with anatomy, rather than taxonomic group. We compared five species for which data exist: opossums, non-human primates, cats, dogs, and pigs, using cine-fluoroscopy and video-fluoroscopy to measure tongue movement and bolus size. We found that the biomechanics of suckling in the two short-faced species, cats and macaques differed from those of the long-faced species, dogs, pigs, and opossums. While broad aspects of suckling are consistent across all species, subtle differences in oral anatomy are correlated with suckling function.

Otoliths of the deepest-living fishes

Mackenzie Gerringer, Werner Schwarzhans

Bony fishes have a well-developed acoustic and vestibular sense, which is registered in the highly specialized otolithic end-organ containing a pair of three aragonitic otoliths. Usually, the largest otolith is the sagitta, which is considered to be primarily responsible for sound detection and is known

to be morphologically diverse and specific across species and higher taxonomic levels. We investigated sagittal otoliths of abyssal and hadal fishes of three families containing taxa adapted to these deep habitats: the Liparidae (snailfishes), Macrouridae (rattails), and Ophidiidae (cusk eels). The purpose of our study was to ascertain whether specific depth-dependent effects on otolith size or morphology could be observed in comparison to the shallower-living counterparts in these groups. We were able to identify a trend toward size reduction in otoliths with depth and certain "simplifications" in otolith morphology. However, we also observed that such trends would only become detectable when studying otoliths within well-defined clades because of the many complexities that occur in otolith morphologies that are unrelated to habitat depth. We propose future work to study freshly caught hadal liparids for the physiology of the otolith end-organ and macula acustica to learn more about the functioning of the organ in fishes living at great depths. Together these findings provide new insights into the drivers of otolith diversity and the evolution of fishes into deep-sea environments.

Introduction to the Symposium

Alexander Gerson, Maria Stager, Cory Elowe

In recent years, a suite of novel and established tools have become widely available or more accessible and have allowed new insights into the physiological mechanisms that allow birds to live and thrive in challenging environments. Genomic tools, new approaches to measure performance at multiple levels of organization, innovative statistical approaches, easier access to metabolite assays or hormone detection, and many others, have spurred rapid advances in our understanding of avian physiology. These new tools have been leveraged to investigate important questions regarding avian responses to our rapidly changing climate in an attempt to understand species resilience and limits. It is the goal of this symposium to highlight recent advances in this field while also synthesizing new discoveries with existing knowledge and to discuss new directions and identify new areas of interest

Natural Habitats on Assateague Island Support a Diverse Plant-Pollinator Network

Amanda Getz, John Hranitz, Victor Gonzalez

Pollination services by insect pollinators are no longer presumed adequate in natural ecosystems or agriculture. Plants and their pollinators form two-level food webs whose species richness and structure may be impacted by climate and agriculture. In 2019 (July-Oct) and 2023 (June-Oct), we surveyed plant-pollinator (p-p) interactions in a naturally dynamic ecosystem on Assateague Island, a protected mid-Atlantic coast barrier island. Our surveys detected p-p interactions by bees (43.3%), dipterans (26.7%), wasps (13.3%), lepidopterans (10%), and coleopterans (6.7%). Most bees were secure, but four species rated elevated conservation status. The p-p network was diverse (Shannon Diversity Index = 3.8). Network-level analysis revealed relatively low level of specialization in community. The linkage density was 3.13 but the realized proportion of links (connectance = 0.112) was relatively low. Niche overlap was lower among plants ($O = 0.053$) than insect pollinators ($O = 0.109$), and overall appears low. High diversity combined with the low degree of niche overlap yielded a network with moderate modularity ($Q=0.62$, 10 modules), wherein some plants and pollinators have stronger interrelationships than in the community as a whole. Nestedness was relatively low (BINMATNEST $T = 18.96$), indicating the community lacked highly structured subsets of species. The natural pollination network on Assateague Island appears robust, supporting four bees ranked at elevated (vulnerable to imperiled) global or regional conservation status.

The Enrichment Act: The Base Level of Enrichment Needed for Captive Marine Mammals

Sara Ghandour

It is not unknown that the main attraction to many aquariums is their beloved marine mammals. These intelligent creatures-- such as otters, seals, sea lions, and more-- are generally known to receive the utmost level of care and commitment. Yet, what many people disregard as they ogle these animals is their lack of enrichment and their extremely minimalistic habitats. I believe that the level of enrichment that common captive marine mammals are receiving

is well below their cognitive potential. Using peer-reviewed scientific papers and personal experiences, I want to elaborate on the base level of enrichment that I believe is needed for each species of captive marine mammal. Rather than spending hours in a giant “bathtub” with only a bucket and a car tire to entertain them, I propose “The Enrichment Act.” This suggests that the base-level requirement of captive marine mammal habitats resembles that of their natural environment, and the enrichment they receive matches their cognitive abilities.

Anatomy and Evolution of Bioluminescence in the Gulper Eels (Anguilliformes)

Michael Ghedotti, Kandice Agudo, Flor Gonzalez, Kurt Riggin, Matthew Davis, W. Leo Smith, Benjamin Frable

Gulper eels in the genera *Eurypharynx* (Pelican Eel) and *Saccopharynx* (the Swallower Eels) are deep-water fishes with extremely enlarged jaws producing enormous oral cavities, a pronounced black coloration, a caudal light organ that has been suggested to be used in prey luring, and dorso-lateral “white lines” identified as putatively bioluminescent. In this study we seek to provide a first description of the histology of the caudal bioluminescent organ in *Eurypharynx*, histologically describe the “white lines”, infer if the “white lines” in both genera are bioluminescent, and place these structures into an evolutionary context. We used histological sectioning and gross examination to explore the anatomy of museum specimens and a DNA-sequence based phylogeny for the anguillid eels to explore character evolution. The bioluminescent organs in *Eurypharynx* are epidermal and intrinsic using partial screening via ultra-black melanosome anatomy within the caudal bioluminescent organ and the white lines in both genera likely are bioluminescent. The presence of skeletal muscle associated with both structures suggest a more dynamic ability to reveal light. The anatomy in the context of the phylogeny suggests that intrinsic caudal and “white line” bioluminescent organs evolved once in the most recent common ancestor of *Eurypharynx* and *Saccopharynx*.

What drives heat and cold tolerances in Galápagos Pocillopora communities?

Katrina Giambertone, Cheryl Logan, Margarita Brandt

Mass coral bleaching and mortality events jeopardize the persistence of coral reefs worldwide. Galápagos corals experience a wide range of temperatures and inhabit marginal conditions compared to other tropical reefs. The stress tolerance of corals remaining after recent warm- and cold-water bleaching events in the archipelago may be the product of adaptation and acclimatization. In the spring of 2019, we investigated thermal tolerance differences in *Pocillopora* sp., a widespread reef-building coral in the Indo-Pacific found throughout the Galápagos. We unexpectedly found that corals from northern sites—which experience warmer and less variable annual average temperatures—were more resilient than those from central and southern sites, having both greater heat and cold tolerances. In this study, we sequenced the mitochondrial open reading frame (ORF) to determine *Pocillopora* haplotype, amplified actin using quantitative PCR to determine symbiont type, and used Tag-Seq to explore gene expression patterns in our samples. We aim to understand how these variables correlate with heat and cold tolerance. Preliminary results suggest that the thermally tolerant symbiont *Durisdinium* is absent from most central and southern sites, and a thermally tolerant *Pocillopora* ORF haplotype (type 1) exists in far northern and most central southeastern communities, possibly explaining differences in thermal tolerance across regions. Our results can provide insight into the adaptive potential of reefs worldwide as they endure warm and cold stress events.

Fear and peripheral sensory processing: The effect of an alarm cue on lateral line sensitivity

Brendan Gibbs, James Strother, James Liao

The physiological state of fear profoundly changes animal behavior and underlying neuronal activity. Considerable attention has focused on fear processing in the central brain regions such as the amygdala and habenula, while the effect of fear on the peripheral nervous system is less understood. Here,

we used larval zebrafish as a model to understand how a fearful state may influence the activity of the lateral line, a peripheral sense organ composed of superficial hair cell bundles called neuromasts. Fish were induced into a fearful state using the alarm cue chondroitin sulfate (CS) which is detected by the olfactory system. Behavioral experiments showed that 5-7dpf zebrafish detect trace amounts of CS (0.5-1.0 μ M CS-E3 solution) to illicit stereotypical fear responses such as darting and freezing behaviors. Activity of the lateral line afferent ganglia was then recorded prior to- and after- exposure to CS (0.5-1.0 μ M CS-E3 solution). Single unit recordings of ganglia neurons showed that that CS exposure increased spontaneous spiking rates ($n=4$ individuals; control= 9.4 ± 1.4 ; CS= 22.3 ± 3.5 Hz). To determine the effect of CS on evoked spiking rates, a piezo stimulator was used to stimulate the D1 neuromast (5-30 Hz stimulus) while multiunit activity was recorded. Exposure to CS caused a phase-locked sensitivity increase to mechanical stimuli ($n=16$ individuals). Since dopaminergic efferents from the telencephalon have been shown to alter hair cell sensitivity, afferent firing patterns were recorded after exposure to a dopamine antagonist (SCH-23390) and CS. Exposure to CS in the presence of SCH (5 μ M-E3 solution) did not increase spontaneous spike rates relative to the control ($n=4$ individuals; control= 8.2 ± 0.7 ; SCH= 4.9 ± 0.6 ; SCH+CS= 4.1 ± 1.1 Hz) suggesting dopamine modulates hair cell sensitivity during fearful states.

Intraspecific interactions and refuge availability determine thermoregulatory costs in ectotherms

Matthew Gifford, David Adams

Ectotherms rely on the external environment to regulate their body temperature and maximize performance of physiological processes. Maintaining a constant body temperature through behavioral thermoregulation is vital for ectotherm growth, reproduction, and survival. Recently, individual based models have been used to demonstrate how the spatial distribution of operative environmental temperature (i.e., thermal heterogeneity) plays a role equal in importance to the frequency distribution of such temperatures. Such models have advanced our understanding of the costs and benefits of thermoregulation but exclude important complexities that exist in natural environments. For example, intraspecific interactions can modify the availability of microhabitats for certain individuals in a population, effectively changing the spatial heterogeneity experienced among individuals. We developed an individual based model of lizard thermoregulation that incorporates variation in the availability of thermal refuges and size-based intraspecific interactions. Intraspecific interactions influenced predicted variation in lizard body temperature, but the magnitude of the effect was dependent on refuge availability. In addition, the main costs of thermoregulation were not realized as energetic costs, but instead as either direct mortality via thermal stress or potentially increased exposure to predation. Our modeling results suggest that demographic consequences might emerge as a result of restricted access to thermal heterogeneity mediated by both habitat structure and intraspecific interactions.

Temperature Stress as a Maternal Effect on Lizard Reproduction

Anthony Gilbert, Sydney Wayne, Mike Norris, John Rodgers, Daniel Warner

Temperature stress has become commonplace for ectothermic organisms persisting in habitats affected by rising global temperatures. While argued to be an environmental stressor that can reduce population growth rates and hinder long-term persistence, mechanistic studies describing how temperature stress impacts reproductive physiology across generations are limited. Many oviparous species deposit eggs in nests that are at the mercy of the environment, but a critical component often missing from this consideration is females are likely experiencing temperature stress during vitellogenesis, which might alter maternal investment into offspring, and subsequent offspring growth and survival. Here, we used a 2x2 factorial design to describe the effects of temperature stress on the reproductive physiology of brown anoles (*Anolis sagrei*) when temperature stress is experienced both by pregnant mothers and by eggs in the nest. Thermally stressed females reduced investment into individual eggs, and these effects may persist to have potentially deleterious effects on offspring phenotypes and survival to reproductive maturity. The

effects of warming climates are likely to include transgenerational responses that can ultimately determine the mechanisms underlying population-level responses to novel- and hazardous- conditions.

Neuropeptide specification of Cutter and Nurse behavior in Leafcutter Ants

Michael Gilbert

Social insects offer powerful models to investigate the link between epigenetic regulation of genes and behavior because distinct tasks are segregated among nearly genetically identical females. Workers of the leafcutter ant genus *Atta* exhibit an extreme example of this task partitioning, wherein behaviors are typically segregated among highly phenotypically distinct individual castes. Here, we identify specific neuropeptides as mediators of caste division of labor in *A. cephalotes* and manipulate two of these neuropeptides to profoundly shift Major behavior. First, we found high levels of the neuropeptide CCAP specifically in the cutter caste, and CCAP peptide injection into Major led to leaf movement behavior and transcriptomic shift towards that of leaf cutter. Second, we investigated neuroparsin-A (NPA) in brood caretaking behavior. NPA showed elevated expression in forager castes relative to caretaking castes. RNAi-mediated reduction of NPA in the brain of Majors resulted in acquisition of caretaking behavior. In contrast, injection of NPA peptide into the brain of Minors suppressed caretaking behavior. We compared *A. cephalotes* RNAseq data with transcriptomes of a eusocial mammal (Naked Mole Rat) revealing global similarities in specific gene expression between foraging and caretaking castes suggesting evolutionary conservation of broad determinants of caste behavior. We propose that neuroparsin-A governs caretaking behavior in leafcutter ants by controlling conserved mechanisms of behavioral regulation shared between insects and mammals.

Feeding affects fins: plasticity in the pectoral fin skeleton of *Satanoperca daemon* reflects foraging

Michelle Gilbert, Alexandra Kwiatkowski, Yara Haridy, Sofia Piggott, Brett Aiello, Craig Albertson, Thomas Stewart

The fins of fishes are remarkably diverse, and this variation is tied to the ecology and locomotor mode of a taxon. While numerous genetic factors are known to pattern fins in development, it is unclear how developmental plasticity shapes the fin skeleton. Here, we analyze the cichlid *Satanoperca daemon*, raising juveniles under three distinct feeding regimes, and assess how foraging mode impacts skeletal pattern of the pectoral fin. Using a combination of geometric morphometrics, linear measures and μ CT scanning, we interrogate numerous dimensions of pectoral fin anatomy, including the anatomy of the endoskeleton, dermal fin rays, and girdle. While pectoral girdle shape is consistent between treatments, plasticity is pervasive across the pectoral fin skeleton. Anteroposterior patterning of both the radials and fin rays are affected by feeding regime. Notably, fins of fishes from different treatments show distinct patterns of fin ray branching, suggesting altered fin stiffness. We argue that observed changes in the fin likely reflect developmental plasticity resultant from altered swimming behaviors when fishes are forced to forage in different ways. These data reveal that non-genetic mechanisms can shape both the endoskeleton and dermal skeleton of fins, and foraging mode can induce plastic changes in elements that do not directly interface with food items.

Phylogenetic affinities of the earliest large-bodied actinopterygians and evolutionary implications

Sam Giles, Rodrigo Tinoco-Figueroa, Matt Friedman

Phylogenetic affinities of the earliest large-bodied actinopterygians and evolutionary implications

Ray-finned fishes originated before the Devonian, but their early history is dominated by small, fusiform taxa. The first appearance of a large-bodied predatory form is *Tegeolepis clarki*, from the Famennian Cleveland Shale, at least 60 million years after the group's origin. The relationships of *Tegeolepis* to other early actinopterygians are highly uncertain due to a combination of limited anatomical information and peculiar morphological attributes, including

large fangs, small scales and a pointed snout. Here, on the basis of new material, we reinvestigate the anatomy of *Tegeolepis* and other early actinopterygians. In contrast to prevailing hypotheses that place *Tegeolepis* as a very early diverging branch in actinopterygian phylogeny, we instead find that it shows many specialised features that ally it more closely with post-Devonian taxa. More specifically, these features suggest a close relationship with some—but not all—large-bodied predatory actinopterygians known from Carboniferous deposits. In addition to resolving a long-standing puzzle in early actinopterygian phylogeny, this has implications for patterns of survivorship across the end-Devonian extinction, indicating that the lineage containing the largest early actinopterygians crossed this boundary. This builds on the emerging picture of a more complicated pattern of extinction and recovery than previously supposed for ray-finned fishes.

Mechanisms of Freeze Tolerance in the Intertidal Mollusc *Mytilus trossulus*

Lauren Gill, Jessica Kennedy, Katie Marshall

The bay mussel, *Mytilus trossulus*, can survive internal ice formation. Depending on air and ocean temperatures, freeze tolerant intertidal organisms, like *M. trossulus*, endure repeated freezing and thawing based on air and ocean temperatures. This freezing process can lead to protein denaturation, prompting the activation of the heat shock response which involves the production of chaperone proteins (like HSP70) and an increase in ubiquitin-conjugated proteins. Additionally, preventing cellular damage through the freezing process necessitates the efficient removal of water from cells, potentially making aquaporin proteins important during freezing. As vital ecosystem engineers and habitat providers, *Mytilus* spp. play a key role in shoreline health. However, our current understanding of the mechanisms underlying freeze tolerance in intertidal species remains limited. To investigate their freeze tolerance, we froze mussels from Vancouver, Canada either in single or repeated exposures (1×8 h, 2×4 h, or 4×2 h). We found that mussels exposed to freeze-thaw cycles (rather than a single freeze) showed greater survival and an upregulation of HSP70 and ubiquitinated proteins. Two previously unidentified aquaporins (AQP1 and AQP4) were classified in *M. trossulus* but were not upregulated by freeze-thaw. Overall, the study highlights the significance of natural freeze-thaw cycles in aiding *M. trossulus* survival in extreme cold conditions by providing a period of damage repair between freezes.

Toward an Energetic Definition of Stress

James Gillooly

The long-term costs of physiological stress are notoriously difficult to quantify in part because the concept of stress itself remains opaque. It remains unclear how measures of GC hormone levels capture the multi-faceted stress response, how elevated GC levels negatively impact the structure or function of organisms, and how one quantifies any cumulative effects of elevated GC levels through time. Here I consider if physiological stress is better viewed in terms of individual energetics. I first examine how measures of physiological stress using GC hormones are related to rates of energy use using data from a diverse assortment of vertebrates. I next examine if/how standard measures of physiological stress relate to lifespan across species. Based on these analyses, I conclude that physiological stress is best viewed as an energetic rate process. Redefining physiological stress in this way may allow one to more easily quantify the effects of chronic or acute stress on health and longevity.

Keep the Pace: Lifelong Effects of Torpor Use During Development in a Small Hibernator

Sylvain Giroud, Barbara Fux, Sebastian Vetter, Hanna Rauch-Schmücking, Johanna Painer, Steve Smith, Sabine Lagger, Gerda Egger, Caroline Gilbert

Torpor, or heterothermy, allows individuals to save energy via metabolic depression associated with hypothermia. Social thermoregulation can be combined with torpor, enabling individuals to minimize energy needs while maintaining relatively high temperature. To date, little is known about developmental flexibility of heterothermy and how it relates to its expression later

in life. We assessed torpor patterns of juvenile garden dormice (*Eliomys quercinus*) subjected to four distinct conditions, i.e., housed singly or grouped by four individuals, and provided with food ad-libitum or intermittently-fasted. We determined epigenetic mechanisms and followed heterothermy expression in adult individuals expressing different levels of developmental torpor; the highest (top third) or the lowest (bottom third) frequency, namely 'high torpor' ('HT') and 'low torpor' ('LT') respectively. Juvenile dormice use more frequent and longer torpor bouts when housed individually than in groups, and torpor was stimulated by reduced food availability during fall. We observed significant differences in mi-RNA levels regulating various metabolic pathways in individuals according to their developmental torpor phenotype. HT dormice still displayed higher torpor frequency and longer torpor duration compared to LT individuals one year later irrespective of food availability. This significant difference in torpor phenotype remained visible among two and three-year-old dormice fed ad-libitum, but not when intermittently-fasted during fall. We conclude that developmental torpor use in the garden dormouse determines, via epigenetic regulations, individual heterothermy expression later in life.

Comparing superficial neuromast distribution in four morphs of *Astyanax mexicanus*

Beth Giuffrida, Allen Mensinger, Maya Enriquez

Astyanax mexicanus, the Mexican tetra fish, has evolved to include sighted, surface dwelling morphs and blind cave dwelling morphs. Other differences exist between the two morphs to include a loss of pigmentation, larger jaws and a greater number of taste buds in the cavefish. It is also known that differences in neuromast distribution and morphology exist between surface and cavefish; for example, cavefish have a greater number of cranial superficial neuromasts. This study considers whether there is a difference in superficial neuromast distribution between three different cave populations: Pachon, Tinaja, and Molino. Fixed laboratory raised larvae (6dpf) from each cave as well as a surface population were obtained from the Stowers Institute and examined using scanning electron microscopy. Preliminary data suggests that there is little to no difference in anterior superficial neuromast distribution between the Pachon and Tinaja populations. Fish from the Molino cave may show a difference with specific regard to the cheek region. General morphology appears to be similar between the three caves; all three show superficial neuromasts located on raised structures that are thus far only seen on the dorsal side of the surface larvae. Future plans include DASPEI staining to further explore comparisons between the cave populations to better understand the scope of *Astyanax* evolution.

Hot bees adjust wing kinematics to conserve water and improve heat tolerance when lifting loads

Jordan Glass, Nicholas Burnett, Stacey Combes, Ethan Weisman, Alina Helbling, Jon Harrison

Heatwaves are becoming increasingly common due to climate change, making it crucial to identify and understand the capacities for insect pollinators, such as honey bees, to avoid overheating. We examined the effects of hot, dry air temperatures on the physiological and behavioral mechanisms that honey bees use to fly when carrying nectar loads, to assess how foraging is limited by overheating or desiccation. We found that metabolic rates and flight muscle temperatures increased linearly with load mass at air temperatures of 20 or 30°C, but, remarkably, there was no change in flight muscle temperature or metabolic rate with increasing nectar loads at an air temperature of 40°C. At high body temperatures, bees apparently increase flight efficiency by lowering their wingbeat frequency and increasing stroke amplitude to compensate, reducing the need for evaporative cooling. Even with reductions in metabolic heat production, desiccation likely limits foraging at temperatures well below their critical thermal maxima in hot, dry conditions. While critical thermal maxima are commonly used to assess ectotherm upper thermal limits, these likely overestimate the temperatures at which flying insects can continue functioning as effective pollinators. This research was partially supported by USDA 2017-68004-26322.

Sexual Dimorphism in a Vocalization of Anna's Hummingbirds

Sierra Glassman, Adi Domer, Robert Dudley

Anna's Hummingbird (*Calypte anna*) exhibits pronounced sexual dimorphism, with males being larger and having more vibrant coloration compared to females. Whereas non-vocal dimorphism (such as dive sounds during male courtship) is well-documented, our understanding of vocal dimorphism within hummingbirds remains limited. Additionally, aggression is an under-explored aspect of hummingbird sexual dimorphism, as territoriality research has mainly been conducted on males. One common vocalization of Anna's Hummingbirds is the "chip note," which is produced by both sexes in a wide array of contexts (in contrast to dive sounds). Here, we correlated temporal parameters of chip notes with demographic and behavioral contexts gathered from field observations and audio recordings. The rate of chip note production differed between male and female hummingbirds but did not vary with behavioral context. Male Anna's Hummingbirds produced chips faster than females. This distinction also remained consistent when comparing chip note production only for those males and females displaying territorial behavior. Although Anna's Hummingbirds produce chips across a broad spectrum of behaviors, chips are likely important in territorial defense and represent an additional feature of sexual dimorphism within this species. Although our study did not elucidate the precise cause of chip dimorphism, our findings enrich our understanding of sexual dimorphism in hummingbird vocal and territorial behavior.

Parasitism and pace-of-life in western fence lizards (*Sceloporus occidentalis*)

Madison Glenwinkel, Guillermo Garcia-Costoya, Akhila Gopal, Noa Ratia, Karla Alujevic, Shea McKendree, Kaitlyn Napier, Cody Chapman, Allison Dorny, Michael Logan

Mounting evidence suggests that in animals, key physiological, life history, and behavioral traits covary with each other in what is known as the pace-of-life syndrome (POLS). According to POLS, individuals are placed along a fast-slow continuum in which a "fast" individual grows and reproduces earlier in life with high fecundity, a high metabolic rate, and a reduced ability to withstand infections and parasites. POLS can be apparent both between and within populations. We investigate the relationship between POLS traits (boldness and resting metabolic rate), thermal preference, and ectoparasite load while considering the mediating effects of elevation, sex, age, and body size. Using two populations of western fence lizards (*Sceloporus occidentalis*) living along an elevational gradient in the Great Basin desert, we tested two main hypotheses: 1) lizards on the "fast" side of the POLS spectrum will have a higher ectoparasite load, and 2) that this relationship is mediated by both endogenous features of lizards and exogenous features of the environment. This study sheds light on the complex interplay between an individual's life history strategy, immunological investment, and environmental conditions with potential implications for understanding how populations will adapt to future changes in their environments.

Anthropogenic sound increases zooplankton susceptibility to fish predation beneath lake ice

Leah Glimsdal, Allen Mensinger

The effects of anthropogenic sound in marine environments has attracted considerable attention in the last decade, however little is known on how freshwater organisms may be affected, especially during the winter months. Ice cover on northern lakes blocks meteorological (i.e. wind, rain) contributions to the soundscape resulting in lower ambient sound intensities during the winter. However, recreational activities such as snowmobiling and ice fishing may negatively impact under ice aquatic communities. This study examined the effects of motorized ice auger drilling on the behavior of fish and zooplankton on Pike Lake in northern Minnesota. Fish activity was monitored with underwater cameras that were deployed directly below, two meters, and four meters below the ice. Videos were analyzed for fish presence two

minutes before, during, and after drilling. Zooplankton were collected from directly below or three meters below the ice five minutes before and after ice auger drilling.

Yellow perch (*Perca flavescens*) and golden shiner (*Notropis hudsonius*) observations increased after ice drilling compared to before drilling ($p = 0.02$). The drilling caused a zooplankton migration from the ice surface to three meters ($p < 0.0001$). This movement led to fish migration from the depths to prey on the displaced zooplankton. This is one of the first studies to demonstrate that zooplankton can be negatively affected by anthropogenic sound.

Brookesia tuberculata population characteristics along an altitudinal gradient on Montagne d'Ambre

Gene Glover, Mark Scherz, Andolalao Rakotoarison, Fandresena Rakotoarimalala

While discovery of new species has been at the forefront of biological research in Madagascar, basic data crucial to their conservation such as abundance and distribution has continued to lag. This study looked to further understand the population densities and behaviors of one such species, *Brookesia* (*Evoloticauda*) *tuberculata*, as they change across an altitudinal gradient. Data was obtained through a combination of nocturnal transects, diurnal leaf litter analysis, and 3 pointed behavioral studies conducted from mid-April through early May, 2022. This study found that *Brookesia* (*Evoloticauda*) *tuberculata* are most abundant on Montagne d'Ambre's western face in the 1000-1100 meter elevation range within primary humid forest but can be found as low as 950 meters in dry forest and as high as 1475 meters at the peak in high elevation primary humid forest. During nocturnal transects, perching characteristics such as height, orientation, and substrate were also quantified, showing distinct behavioral differences between high and low elevation populations. These findings support the previously published genetic results which found two distinct populations separated by elevation. The results from this research can help to provide a baseline for species distribution for the future, which is important for conservation threat assessments, especially in light of increasing pressure from illegal logging at the lower bounds of the range.

Drought conditions may influence body condition and fat deposition in an overwintering shorebird

Kenneth Glynn, Jamie Cornelius

Climate change, especially when coupled with human activity on the landscape, is expected to bring about environmental instability in ecosystems around the globe. The adaptive plasticity of species within these changing landscapes is of great interest to predict the long-term viability of populations and the sustainability of the habitats they utilize. Rice fields in the Central Valley, CA serve as vital wintering habitat for many shorebird species, such as Dunlin (*Calidris alpina*), that seek shallow water and invertebrate prey items. Yet the longevity of these habitats remains in question as water availability changes drastically under decades of drought in the region. How overwintering in these habitats might influence the health and migratory readiness of this species is unclear. Dunlin were captured in flooded rice fields over three years (2021-2023) in November - April to assess body condition as a consequence of utilizing these habitats over time. Fat deposits varied significantly by month, suggesting there may be periods of intense fattening during the coldest winter months and just prior to spring migration. Size-corrected mass also varied by month but did not fully align with fat deposits: mass, was highest in early and late winter months. Fat deposits and mass did align when compared between years, yet only fat varied significantly. Variable drought conditions, resource competition and specific foraging locations should be considered when interpreting these results.

Alliances between blood-feeding marine invertebrates and beneficial bacteria

Shana Goffredi, Ralph Appy

Persistent bacterial presence is believed to play an important role in animal adaptation to specific niches that would otherwise be unavailable, including the exclusive consumption of blood by invertebrate parasites. Nearly all

blood-feeding animals examined so far host internal bacteria that aid in some essential aspect of their nutrition. Obligate blood-feeding (OBF) invertebrates exist in the oceans, yet symbiotic associations have not yet been well explored. Here, we describe the microbiome of 8 phylogenetically-diverse marine OBF species, including leeches (fish and elasmobranch specialists; e.g., *Pterobdella*, *Ostreobdella*, and *Branchellion*), isopods (*Elthusa* and *Nerocila*), a copepod (e.g., *Lernanthropus*), a flatworm (*Hysteroecleitha*), and nematodes (ex. *Philometra* and *Vasorhabdochona*). Amplicon sequencing analysis revealed their microbiomes to be low diversity, compared to fish surfaces, seawater, and non-blood-feeding relatives, and dominated by only a few bacterial genera. Bacterial cells, notably *Vibrio*, were localized to the digestive lumen near the blood meal for all taxa examined via fluorescence microscopy. Additionally, *Vibrio* cultivated from the OBF taxa were able to lyse vertebrate blood cells. For two crustacean taxa, *Elthusa* and *Lernanthropus*, the microbial communities associated with brooded eggs were very similar to the adults, indicating possible direct transmission. Virtually nothing is known about the influence of internal bacteria on the success of marine blood-feeders, but this evidence suggests their regular presence in marine parasites from several prominent groups.

Body and Craniofacial Shape Divergence Among Lake Malawi Cichlid Fishes

Tyler Golbus, Leah DeLorenzo, Kara Powder

Body shape and craniofacial morphology are highly variable across vertebrates, and often associated with biomechanical adaptations to habitats. Lake Malawi cichlids exemplify this, with dramatic differences in shape across their adaptive radiation. To investigate patterns of variation, we performed a comprehensive phenotypic analysis examining 99 species from Lake Malawi, representing all major genera. We employed geometric morphometrics to assess how shape varies with habitat (deep benthic, shallow benthic, mbuna, utaka, diplotaxodon, and rhamphochromis). We discovered significant differences in mean body and head shape between all habitat pairs ($0.05 < p < 0.001$) except between diplotaxodon and rhamphochromis. The primary variation described the relative width and length of the body and head with trends seen previously in cichlids and the pelagic/benthic axis. These changes are associated with locomotion and feeding strategies. For example, open-water rhamphochromis was characterized by a long, narrow body, while deep benthic had a wider and shorter body and head. We further noted significant differences in disparity between shallow benthic and both mbuna and utaka ($0.0061 < p < 0.0065$). Future research will investigate the genomic origins of the observed phenotypic variation utilizing sequenced genomes. This exploration aims to reveal the link between genetics and environmental influences, enriching our understanding of the astounding diversity of cichlid fishes. This work is supported by NSF-IOS #1942178, NIH R15DE029945, and NIH P20GM121342.

Osteohistology of a tiny phytosaur femur: implications for the evolution of early archosaur growth

Erika Goldsmith, Daniel Barta, Sterling Nesbitt, Ben Kligman, Adam Marsh, William Parker, Michelle Stocker

Fossil embryos can provide invaluable insight into the evolution of prenatal morphologies, heterochronies, and allometric trajectories within extant Archosauria but are exceedingly rare in the Late Triassic fossil record, a critical time period for archosaur evolution. Fine-scale efforts to extensively sample Upper Triassic sediments in Petrified Forest National Park have yielded diminutive phytosaur (semiaquatic predators superficially resembling extant crocodylians) femora (PEFO 45199, PEFO 45274) from the same locality with midshaft circumferences of ~7 mm (~4% of that from the largest phytosaur), allowing the opportunity to assess the presence of hatchling phytosaurs in North America. We conducted an osteohistological analysis of PEFO 45274 to assess the growth dynamics. PEFO 45274 preserves low vascularity (~5 canals/mm²) parallel-fibered bone with primary osteons, no hatching line, nor any lines of arrested growth suggesting negligible differences between in ovo and post-hatching growth patterns. The presence and erosion of endosteal lamellar bone implies cortical drift occurred; therefore, we interpret PEFO 45274 as a slow growing post-hatching individual less than one year

old. Surprisingly, osteohistology from larger phytosaur femora implies faster growth rates in comparison to PEFO 45274, suggesting the ontogenetic shift from rapid-to-slow growth rates might not occur as expected in Phytosauria. This study highlights the importance of including osteohistology from multiple ontogenetic stages to investigate archosaurian ancestral growth rates given the phylogenetic position of phytosaurs near the base.

Turning Over a New Leaf: Increasing Plant Diversity to Support California Native Bee Diversity

Katie Goldstein, Luis Arredondo, Mariel Dawson, Briana Munoz, Lisbeth Nicolas-Lopez, Esveidy Rodriguez, Erin Krier, Alicia Fox

Native bee populations are being surveyed at Allan Hancock College, a coastal Californian community college, as part of an initiative to utilize the campus as a living laboratory and provide community college students with exposure to various field research techniques. In this ongoing study, native bees are surveyed at various locations on campus and at a nearby UC Natural Reserve, where specimens are collected in the field and later identified in the lab—to the genus if not the species. For each specimen collected, the corresponding plant is also documented. As of this time, 12 different bee genera have been identified on 14 different plant species. Comparisons in biodiversity across sites have facilitated a greater understanding of the relationship between plant diversity and native bee diversity, indicating that sites with the greatest amounts of plant diversity, plant density, and range of plant dormancies also display the greatest amount of native bee diversity. This study therefore aims to continue identifying strategies and practices that support the sustainable increase of native bee populations on campus, including habitat enrichment, informed campus landscaping protocols, and community education and outreach.

TBI-Induced Behavioral Change in Mangrove Rivulus Fish: Prevention and Rescue with Gardenin A

Marisol Gomez, Kathryn Leu, Reagan Brown, Trenton Hoaglin, Corinne Daise, Ryan Earley, Lukasz Ciesla

Brain inflammation caused by traumatic brain injury (TBI) promotes free radical production and is associated with significantly higher risk-taking behavior. Mangrove rivulus fish are an interesting model in which to explore TBI-related changes in behavior because they jettison from water, move about on land by tail-flip jumping, often land on their heads, and take significantly more risks post-TBI. We tested the hypothesis that behavioral changes can be either prevented or rescued by administration of the flavonoid Gardenin A, a “soft electrophile” with anti-inflammatory properties and the ability to scavenge reactive oxygen species (ROS). The experiment involved behavioral observations, injection of Gardenin A either prior to (prevention) or after (rescue) TBIs were delivered in a custom apparatus. Dose-response relationships between Gardenin A and risk-taking behavior are unknown so, we administered vehicle (canola oil) or one of five Gardenin A doses either before or after TBIs (N=20 fish per treatment). We predicted that Gardenin A would diminish TBI-related behavioral deficits in a dose-dependent (but not necessarily monotonic) fashion. While the project is still ongoing, we present an initial look at the data and discuss support for and against our hypothesis. Our results will provide a foundation for understanding whether radical scavenging compounds might help to alleviate debilitating behavioral changes and long-term cognitive impairments associated with TBIs sustained during contact sports and other activities.

Fine-scale morphological plasticity in staghorn coral as a function of flow, light and genotype

Maya Gomez, Jenna Dilworth, Daniel Olivares-Zambrano, Anthony Insinilla, Daniella Leon, Sophia Lee, Marissa Fine, Carly Kenkel

The complex branching morphology of staghorn coral, *Acropora cervicornis*, renders it a foundation species that contributes to reef rugosity and local hydrodynamics in the Caribbean. Morphological plasticity in staghorn varies as a function of genotype and the environment, yet underlying mechanisms giving rise to changes in skeletal deposition have not been defined. Wave

pressure may influence plastic responses and lead to differential investment in calcification. We tested the effects of perceived water flow and light on morphology, growth and fine-scale calcification in ten genotypes of *A. cervicornis* over seven weeks. Flow experience was manipulated by either suspending 5 cm coral fragments from nylon filaments, which allowed for passive movement in response to water flow, or preventing movement by fixed mounting. Flow treatments were fully crossed with light treatments of similar wavelengths but differing intensity, a high PAR (=250 $\mu\text{mol}/\text{m}^2/\text{sec}$) and low PAR (=100 $\mu\text{mol}/\text{m}^2/\text{sec}$). High light replicates exhibited greater linear growth and net photosynthesis compared to low light treatments, regardless of flow conditions. However, patterns of linear growth and net photosynthesis as a result of flow treatment were genotype specific, revealing a genotype $\times$ environment interaction. Subsequent analyses will test for fine-scale differences in morphology using microCT scan data. Uncovering drivers of morphological plasticity will allow for the optimization of reef restoration strategies that result in the greatest growth, structural integrity and complexity post outplanting.

Ephemeral River Islands are Home to Transient Communities of Boreal and Austral Migratory Land-Birds

Valentina Gómez-Bahamón

Migratory birds join different ecological communities throughout the year as they move from the breeding grounds to the overwintering areas. Those that breed in temperate areas, spend up to a third of the year elsewhere, migrating, stopping to rest and refuel, and wintering in localities where resources are available. Here, we document that ephemeral riverine islands of the Meta River in Eastern Colombia serve as important sites where multiple boreal and austral migratory land-birds coexist when they are overwintering or stopping over. These riverine islands are colonized by the fast-growing and densely packed plant *Tessaria integrifolia*, which is used by migratory birds as roosting site. The ephemeral dynamics of these islands in which emergence coincides with the arrival of migrating birds, and the increase in insect abundance, sets a stage for the formation of transient ecological communities that form every year. Attributes related to whether these islands are connected to the mainland, their land size, and age, likely influences the presence of predators such as mammals and snakes, likely affecting the probability of migratory land-birds establishing a roosting site. Likewise, these attributes of ephemeral islands are known to influence the presence of year-round resident birds that are specialists in Amazon River islands. Such occupancy can influence whether migratory species establish roosting sites in the Meta River, given that they are already occupied by year-round residents.

Role of surface chemistry on the toxicity of polystyrene nanoparticles on larval sea urchin

Helga Gonçalves, Kit Yu Karen Chan, Kathryn Riley

Plastic pollution is an emerging threat to aquatic ecosystems. In addition to those that are manufactured, microplastics (MPs) and nanoplastics (NPs) are formed when larger plastic materials degrade. Various adverse outcomes such as growth inhibition and developmental disruptions have been reported. Surface chemistry of these small particles can strongly alter their aggregation properties, bioavailability, and toxicity. Here, we compared the toxicity of polystyrene nanoparticles of different surface chemistry (terminated with carboxylate groups or amino groups) on larval sea urchins. Our first goals are to establish baseline concentration of no observed adverse effect level (NOAEL) and to test if the release of reactive oxygen species are the cause of observed deformity. Upon establishing the importance of changes in surface chemistry on toxicity, the next step is to investigate the role of eco-corona on the ecotoxicology of nanoparticle. Eco-corona form when bio- or environmental molecules adsorb onto nanoparticles, which in turn alter their surface chemistry. Our work will provide more environmentally relevant understanding of the adverse outcome pathway of nanoplastics.

Migration, aggregation, and philopatry of two nearshore elasmobranch species in Southern California

A-bel Gong, Andrew Nosal, Daniel Cartamil

Understanding the daily and seasonal movement patterns of elasmobranch species can inform when and where populations are most susceptible to threats, but these data are often lacking for nearshore species that are not actively managed. Two such species are the shovelnose guitarfish (*Pseudobatos productus*) and California bat ray (*Myliobatis californica*); this study quantified the broad- and fine-scale movement patterns of these species using passive acoustic telemetry. Twelve guitarfish (10 female, 2 male) were surgically implanted with coded acoustic transmitters at an aggregation site off La Jolla (San Diego County), California, USA, and tracked for 849.5 ± 548.9 (mean $\pm$ SD) days. Six bat rays (all female) were also implanted with transmitters and tracked for 1143.8 ± 830.9 days. These animals were detected at 187 acoustic receiver stations between Point Conception, California, and San Quintín, Baja California, México. Both species exhibited annual philopatry to La Jolla, especially in July, after traveling as far north as Santa Barbara (guitarfish) and San Miguel Island, California (bat rays). Based on their movement patterns and known reproductive phenology, we hypothesize that both species utilize the La Jolla aggregation site as a gestating ground, and possibly also a mating, pupping, and nursery ground. This site is within a no-take reserve, and we recommend that similar sites also be protected, given the increased susceptibility to anthropogenic stressors when aggregating.

Reacclimation to normal temperature failed to restore gamete performance in heatwave-exposed urchins

Jazczenya Gonzalez, Gretchen Hofmann, Kit Yu Karen Chan

Anthropogenic climate change is increasing the frequency and severity of marine heatwaves (MHWs), which are prolonged periods of elevated sea surface temperatures. While studies have linked MHWs and elevated adult mortality, along with shifts in community structures, few have explored its impacts on key early life history events and test whether organisms can quickly recuperate from MHW stress. We studied the purple sea urchin *Strongylocentrotus purpuratus* that plays a vital regulatory role in kelp forests through grazing. By quantifying male gamete performance, fertilization, and developmental success, we aimed to examine the legacy effect of MHW exposure. Adult urchins were exposed to a MHW (20°C) for 7 weeks, followed by a reacclimation phase at ambient temperature (14°C) for 14 days. While sperm concentrations were similar between MHW exposed and control, the MHW exposed individuals had relatively less cAMP after activation. Further, computer analysis would reveal if MHW sperm swam at lower velocities or if a smaller proportion of sperm was active. Additionally, when fertilized at optimal sperm concentration (>103 sperm ml⁻¹), fertilization success was comparable between MHW and control parents. However, none of the MHW offspring cleaved. Our result implies that the MHW negatively impacted sperm performance, even when returning to normal conditions after exposure. This developmental failure suggests that MHW events are detrimental to urchin populations, and more broadly, the health of kelp ecosystems.

Tyrosine hydroxylase mediates hormonally-dependent plasticity in the melanization of *Manduca sexta*

Paula Gonzalez, Yuichiro Suzuki

Developmental plasticity is the ability of a single genotype to express a variety of phenotypes depending on environmental conditions. Here, we focused on finding the link between the environment and phenotype by studying melanization in the genetically accommodated strains of the tobacco hornworm, *Manduca sexta*. We compared gene expression in the temperature-sensitive polyphenic and temperature-insensitive monophenic larvae and found that the melanin synthesis gene, tyrosine hydroxylase (TH), was differentially expressed. black mutant larvae treated with the juvenile hormone (JH) analog, methoprene, showed reduced melanization and expressed decreased levels of TH. We will present additional evidence linking hormonal changes to phenotypic changes via TH. Our study offers an example of how the environment can cause changes in the hormones that impact biochemical pathways that underlie distinct phenotypes within one population.

A Double-Edged Sword: Type VI secretion system (T6SS) competition in the *Euprymna-Vibrio* Symbiosis

Perla Gonzalez-Moreno, Michele Nishiguchi, Shelby Matsumoto

Symbioses between sepiolid squids and the bioluminescent bacterium *Vibrio fischeri* serve as a valuable model system to examine host colonization dynamics. Although multiple strains of *V. fischeri* are present in seawater, only a few successfully colonize the squid light organ (LO). Since the LO represents a haven for bacterial replication, multiple factors have led to competition for this niche. Therefore, we examined how T6SS-mediated competition may regulate *V. fischeri* strain distribution within the LO of *Euprymna scolopes* (ES). The T6SS is a molecular syringe that delivers toxic effectors to targeted cells and can be regulated in some *V. fischeri* strains by environmental viscosity and pH changes encountered within the host. To determine how T6SS-killing affects symbiont distribution in ES hosts, candidate T6SS-encoding strains were co-cultured with T6SS-deficient strains. Six strains exhibited a lethal phenotype against competitor strains under neutral pH. Additionally, we examined how adaptation to seawater (T6SS off) or host-like conditions (T6SS on) might affect symbiont fitness in our lethal strains by experimentally evolving lethal strains for 1000 generations. Lethal strains exhibited decreased fitness under host-like conditions, but increased fitness under seawater conditions when compared to their ancestor. These findings indicate that lethal strains can extirpate competitors via a T6SS, but continued expression of this weapon in the host ensues a fitness cost that may place a selective pressure on lethal strains to abolish such costly weapons.

The role of learning in speciation by sexual selection

Marco González-Santoro, Corinne Richards-Zawacki, Yusan Yang

After 150 years of research, the topic of speciation by sexual selection remains a frustrating endeavor, in which, empirical evidence fails to meet theoretical predictions. An outstanding progress was recently achieved by showing that sexual learning at early stages of development (imprinting) facilitates mating traits divergence and the preferences for them, the initial steps of reproductive isolation. Imprinting leads females to mate with males that look like their parents (and usually themselves), promoting assortative mating, which in turn drives divergence between traits. Importantly, imprinting also leads males to compete against males that look like themselves, releasing rare phenotypes in a population from competition pressures, promoting traits divergence. Much less understood is the role that social learning at other life stages and by different tutors – a common process in social animals – plays in the facilitation of reproductive barriers. To fill this gap in the current state of knowledge, we performed female mate choice and playback experiments in males in both field conditions and a captive bred colony of the poison frog *Oophaga pumilio*. I will show preliminary results of two experiments that suggest that individuals' social learning can modify imprinted behavioral biases based on the individuals' social environment (i.e., the phenotypes that they are allowed to see) both during development and after sexual maturity.

Identifying nematocyst sequestration genes in the nudibranch *Berghia stephanieae*

Jessica Goodheart, Robin Rio, Rose Fiorenza, Deirdre Lyons

The intracellular uptake and storage of structures from one organism inside another is fundamental to eukaryotic origins, and multiple lineages have evolved to sequester such structures secondarily from their prey. The processes of recognition and storage of sequestered cells is well characterized in cnidarian-dinoflagellate symbiosis, but little is known about the mechanism of sequestration in organisms that sequester defensive structures like cnidarian nematocysts. We investigated genes that may be involved in nematocyst sequestration in the nudibranch *Berghia stephanieae*, well known for its predation on the anemone *Euaiphtasia diaphana*. During digestion in *Berghia*, *Euaiphtasia* nematocysts move into a structure called the cnidosac in the *Berghia* ceras, where they are phagocytosed and stored by cells called cnidophages. We performed differential expression analyses between the distal ceras (where the cnidosac is located) and the proximal ceras and identified 188 upregulated distal ceras genes, all of which produced blast hits to other organisms. Using HCR, we find some upregulated genes expressed in

the cnidosac of *Berghia* juveniles, including known phagocytosis genes and receptors that may be used by cnidophages to identify nematocysts. Since *Exaiptasia* is a well-studied species, the development of molecular tools in *Berghia* provides a valuable system where both sides of sequestration can be investigated. This work also provides a framework for comparative analyses of convergent intracellular sequestration across eukaryotes.

Cold recruits: impacts of temperature on motor control during lizard locomotion

Colin Goodman, Stephen Deban

Limbed locomotion is a dynamic process, relying on the cyclical shortening and lengthening of muscles active during different portions of the stride. In nature, species must contend with changing environmental conditions, implying that either locomotor patterns must change or performance will suffer. Motor control in lizards is well studied in myriad ecological contexts, but little is known about the response of motor control to thermal variation. To examine this, we used electromyogram (EMG) recordings of the Peter's rock agama (*Agama picticauda*) and brown basilisk (*Basiliscus vittatus*), from three muscles active during different phases of the locomotor cycle: the m. caudofemoralis longus (stance; femur retraction), the m. puboischiofemoralis (swing; femur protraction), and the m. ambiens pars dorsalis (stance; knee extension). EMGs were synchronized with two high-speed cameras (500 Hz), which captured sprinting performance at three temperatures (20, 25, 33 °C). To test for thermal effects, we measured the timing and intensity of EMG bursts relative to the active phase of each respective muscle. We found no significant effects of temperature on maximum or mean burst amplitude for any muscles in *A. picticauda*. However, we found significant increases in both maximum and mean burst amplitude at lower temperatures for the two stance-phase muscles in *B. vittatus*. This suggests that some species employ compensatory motor recruitment at lower temperatures, thereby mitigating the effects of temperature on performance.

Opsin expression indicates brine shrimp embryos see before developing eyes.

Alexander Goodrich, Amalie Mattison, Joseph Covi, Meghan Ford

The brine shrimp, *Artemia franciscana* is one of many species that undergo a programmed state of reduced metabolic activity and arrested development called diapause. Diapause occurs during the gastrula stage of development in the brine shrimp. Environmental cues break the diapause state and trigger further development. Light is among these cues in many species including brine shrimp. It is unclear what sensors detect light in embryos of zooplankton while they are in diapause. One option is opsins, which are proteins that detect specific wavelengths of light. Published data indicates that the response of diapause *A. franciscana* to light is wavelength dependent. RNAseq data indicates that opsins are differentially expressed during early development. Data on the impact of light on opsin expression in early development of *Artemia franciscana* will also be presented.

Macrophagous planktotrophy in actinotroch larvae of the marine horseshoe worm, *Phoronopsis harmeri*

Chloe Goodsell, George von-Dassow

Phoronids are tube-building marine worms with a biphasic life cycle characterized by a planktonic larval stage – the actinotroch – that metamorphoses into a sessile benthic juvenile. The actinotroch larva is planktotrophic, and is known to graze on unicellular algae using both locally-reversible ciliary currents and muscular appendage movements. Observations of wild-caught actinotrochs with zooplankton in their stomachs suggest that they may subsist on more than just single-celled phytoplankton. Macrophagous planktotrophy may augment grazing to help actinotrochs achieve relatively large body size before metamorphosis. We raised actinotrochs of *Phoronopsis harmeri* from coelomic eggs on a diet of *Rhodomonas lens*, then offered them potential prey from wild plankton or cultures. Actinotrochs consistently and selectively captured a variety of prey items in the ~100 µm range under lab conditions, suggesting a natural diet of zooplankton and protists in addition

to phytoplankton. Acceptable prey items included large autotrophs (dinoflagellates *Ceratium* and *Gyrodinium*), ciliates (*Tiarina* and tintinnids), conspecific embryos and larvae, and other small ciliated zooplankton (rotifers, polychaete trochophores). Capture of large prey was qualitatively similar to unialgal particles, although we noted the role of the muscular hood in retaining large highly-motile objects. Opportunistic macrophagy may illuminate the complex anatomy of the actinotroch, while demonstrating unexpected interactions amongst marine plankton.

Hungry Hungry Tadpoles: Begging influences Behavioral and Neural Response in Poison Frog Parents

Billie Goolsby, Tony Chen, Ashlyn Callan, Lorianze Rogers, Julie Butler, Mark Cutkosky, Lauren O'Connell

Establishing a strong social bond with our caregivers lays the foundation for a healthy life. As offspring often cannot regulate their nutrition, signals of need require accurate and precise interpretation by their caregivers to provide care. However, little is known about the neural basis of communication between parents and offspring. Poison frog tadpoles are altricial and rely entirely on parental investment for healthy development. In the Mimic poison frog (*Ranitomeya imitator*), mothers provide more food to tadpoles that beg (vibrate) more intensely, suggesting somatosensation (touch) is an important component of offspring signaling need. To test this hypothesis, we created a robotic tadpole with modifiable characteristics to emulate offspring signals, along with a customized camera trap that is specifically designed for ectotherms. We found that parents, depending on sex, may use different offspring signals to make care decisions. In parallel, we explored how parental brains respond to the begging behavior of their offspring. We found that offspring touch coincides with activation of opioid pathways and suppression of nociceptive pathways. When opioid signaling is perturbed, we found that fathers modify their contact with offspring, suggesting an evolutionarily ancient role for these neural pathways in social adhesion on a somatosensory and behavioral scale. Using these two advances, we are beginning to decipher which infant communication components contribute most to a parent's choice to nurture or to neglect.

The Pace of Life in the Wild West: Lizards across an Elevational Gradient

Akhila Gopal, Guillermo Garcia-Costoya, Noa Ratia, Karla Alujevic, Madison Glenwinkel, Shea McKendree, Kaitlyn Napier, Cody Chapman, Allison Dorny, Michael Logan

An organism is an assemblage of many traits, each of which may be adapted to different aspects of the local environment. However, traits do not evolve independently and can (often) co-adapt into "complex phenotypes". The Pace-of-Life Syndrome (POLS) is a framework that links covariation across physiological, behavioral, and life history traits, and has recently been extended to incorporate the temperature dependence of biological processes (extended POLS). Under extended POLS, life-history traits vary along a slow-fast axis, behavior varies along a shy-bold axis, thermal physiology varies along a cold-hot axis, and these axes, in turn, covary with one another. While some of these trait associations have been found in laboratory experiments, to our knowledge, no studies have investigated if these trait axes exist and covary in nature. Here, we combine intensive mark-recapture data with both field and lab measures of life history, behavior, and thermal physiology, to examine trait covariation in western fence lizards (*Sceloporus occidentalis*) across an elevational gradient in the Great Basin Desert. We assess whether traits align and covary in a manner consistent with the predictions of extended POLS and compare trait values between sites, sexes, and life stages. We discuss the relevance of our results to understanding the evolution of integrated phenotypes and their potential to constrain adaptation to climate change.

Aquatic amniote limbs converge on a common morphology beyond terrestrial morphospace

Caleb Gordon, Christopher Griffin, Jacques Gauthier, Bhart-Anjan Bhullar

Aquatic mammals and reptiles provide striking examples of convergent evolution. They tell a story, repeated more than thirty times in the last 300 Myr, of how an originally terrestrial group adapts to life in the water, progressively optimizing its behavior, anatomy, and physiology for an aquatic lifestyle. Reconstructing the life habits of these groups can provide insight into the ecological drivers of recurring evolutionary transformations and the developmental processes that underlie them. However, reconstructing the life habits of extinct amniotes continues to thwart paleontologists, as different modalities of data often disagree and have destructive sampling consequences that limit sample size. We developed a new, non-destructive workflow based on limb proportions for determining the aquatic affinities of extinct amniotes. We generated the largest dataset of amniote limb measurements to date (>10,000 original measurements from >500 extant tetrapod samples), scored all extant samples for aquatic affinity, fit binomial logistic regression models to the data, and fed contentious fossil taxa into our models to predict how aquatic they are. We find that limb proportions accurately predict aquatic habits in all sampled extinct amniote clades. Our model predictions let us reconstruct the aquatic habits of extinct lizards, sauropterygians, and theropods with disputed ecomorphologies, revealing a remarkable morphological convergence among unrelated semi-aquatic and fully aquatic lineages.

Examining the role of diverse sexual behavior and conflict on eco-evolutionary dynamics

Swanne Gordon, Yusan Yang, Caleb Axelrod, Andrés López-Sepulcre

We have known for decades that ecological variation can lead to evolution in nature. Recently we have learned that the process can also operate in the opposite direction: evolution can change ecosystems by altering how organisms interact with each other and their environment. However, little is known about how diversity in sexual selected traits can affect such eco-evolutionary feedbacks. In this talk, I explore through a subset of experiments how sexual behaviors or traits lead to varying evolutionary and ecosystem outcomes using Trinidadian guppies. I use each experiment as case studies to highlight the importance of intertwining the study of diversity in nature with the value of diversity in academia. This reflects upon the study systems we use, the questions and perspectives we explore, and the people granted opportunities to be the knowledge drivers in our field.

Insights into the cellular and molecular mechanisms of a highly regenerative tunicate

Tal Gordon, Tal Zaquin, Noam Hendin, Omri Wurtzel, Lucia Manni, Ayelet Voskoboinik, Noa Shenkar

Regeneration is widespread in the animal kingdom, and a variety of model systems are employed to better understand the principles and genetic programs underlying this process. Ascidians are remarkable for their regenerative abilities, and while the majority of regenerative studies focused on well-known model species, our recent work suggested a new model: the solitary ascidian *Polycarpa mytiligera*. In vivo experimental observations revealed this species extraordinary ability to regenerate all body parts following their removal, including the central nervous system (CNS). Phylogenetic analysis demonstrated this species close affinity to colonial species, suggesting the ability for regeneration as an exaptation feature for colonial lifestyle. Our current study further describes *P. mytiligera*'s impressive regenerative potential and presents the morphological, cellular, and transcriptomic dynamics that lead to entire CNS regeneration. Our results revealed the expression of key neuro developmental markers that are not otherwise present in the adult CNS. Removal of the entire CNS resulted in high cell proliferation in the regenerated area. Transcriptome analysis revealed enhanced stem-cell related gene activity, with high expression of P53 and piRNA pathways preceding the activation of Notch, Wnt, and Nanos pathways. Our new findings provide an in-depth characterization of *P. mytiligera*'s regeneration process, presenting

insights into the cellular and molecular aspects of CNS regeneration, further emphasizing the importance of this new model system in the study of the evolution of chordate regeneration.

Drug-induced changes to spider behaviors in the changing landscape of the web during web assembly

Andrew Gordus, Darya Task, Abel Corver

Psychoactive drugs provide a window into neural circuits involved in sensory perception, learning and memory, and motor behavior. Experiments in various orb-weaving spiders have shown that drugs can alter distinct components of a spider's web. However, the geometry of the web is fundamentally the result of a behavioral algorithm that is influenced by the geometry of the web it builds. Due to the challenges of recording spider leg movements, prior drug studies with spiders had a limited ability to connect changes in web structure to changes in web-building behavior. Using machine vision tracking and computational modeling methods established by our lab, I am investigating the effects of psychoactive substances on behaviors underlying different stages of web building in *Uloborus diversus*. Prior work in insects has shown dopamine and the steroid ecdysone have opposing effects on behavior, mediated through the GPCR DopEcR. We find these modulators also appear to have opposing effects on orb-weaving behavior, and appear to gate the transition between distinct phases of web-building. We are currently investigating which specific behaviors are altered in different drug-contexts, and how these drugs alter how the spider interacts with the changing web landscape as it builds.

Carbonic Anhydrase Localization in Roach Ceca, as a Potential Source of Acidification.

Erika Gordy, Gregory Grabowski, Kennedy Dunlap, A'Tearea Boggan, Jolani Perez

Hansson's technique was used to histologically localize carbonic anhydrase (CAH) activity within ceca, Malpighian tubules, and tracheal airways in Madagascar roaches. Ceca are blind ended finger-like projections contributing to gastrointestinal acid secretions of pH 4.89, that was neutralized by the CAH inhibitor, acetazolamide. Ten-micron cecal sections floated on Hansson's media supported CAH's conversion of carbon dioxide and water into bicarbonate and protons. Larger tracheal airways penetrate cecal walls and branch into smaller airways within the mucosal cecal folds. CAH activity was found only in the smaller airways within the folds, and not in the larger airways, nor in cecal fold tissue. Because no CAH activity was found in cecal folds it is assumed that the small branching airways are the source of protons. Larger tracheal airways are closely associated with cecal surfaces. Malpighian tubules intertwining with the large surface tracheal airways demonstrated CAH activity. The PAS-Orange-G technique identifies acidophilic granules within endocrine cells. Orange-G staining cells were located adjacent to larger airways within the cecal wall, as well as within the apical region of Malpighian tubules. The proximity of these cells indicates possible paracrine control of acid secretions within the ceca and possibly between the larger surface airways and Malpighian tubules. Future research will involve using proton pump and bicarbonate/chloride exchange inhibitors to define the role of small airways in cecal acid secretion.

Optimal Migratory Speed of Large Oceanic Capital Breeders

William Gough, Max Czapanskiy, Matthew Savoca, Elliott Hazen, William Oestreich, James Fahlbush, Daniel Palacios, Lars Bejder, Jeremy Goldbogen

Long-distance migrations are one of the most energetically demanding behaviors observed within the animal kingdom and are undertaken to find seasonally variable food sources or move between spatially separated foraging and breeding grounds. Minimizing the cost of transport throughout these migrations can have a large impact on an individual's annual energetic economy. This is especially true for species that rely on the energy from a defined feeding season to last them throughout the year, a strategy known as capital breeding. Mysticete whales (Mysticeti) are a group of capital breeders that perform long-distance migrations across ocean basins, likely necessitating a low cost of transport throughout those journeys. Using estimates of

foraging and swimming energetic intake and costs for mysticetes of varying body sizes, we estimated the energy gained during a foraging season and the energy lost during migration. We used these values to parameterize both stereotypical and speed-dependent annual cycles that modeled the energetic cost of swimming at various speeds along a migratory route. From this model, we found optimal migratory speeds between 1-2 ms⁻¹ for a range of migratory distances. We also determined that longer migrations are more costly and occur at higher speeds than shorter migrations. In a rapidly changing ocean, our findings suggest even small differences in the distance or speed of a migration could have major impacts on individual fitness.

Evaluating climate disruption on avian species and communities in the southern Rocky Mountains

Lily Gowens, Caitlin Wells, Benjamin Freeman

Climate change is altering the phenology and distribution of organisms across taxonomic groups. Natural variation in abiotic factors along elevational gradients may be exacerbated by climate change, especially temperature. At high elevations, temperature can limit species distributions and upward range shifts occur as a response to climate warming. Contemporary and historical survey data can assess such range shifts and identify changes, declines, and extinction risks. This study assessed regional species abundance trends at one elevation in Gothic, Colorado using 23 years of annual survey data and resurveys points along an elevational gradient (2,469-3,421m) in the southern Rocky Mountains to evaluate changes in community composition and shifts in species elevation range compared to 2011 survey data and historical ranges. In addition to climate disruption, human disturbance in Gothic, Colorado has increased over the survey period and may contribute to impacts on avian species. We found a complex relationship between climate disruption and trends in species abundance. Our elevational gradient survey analysis indicates that avian species communities significantly differ in composition between survey sites and years at different elevations. Point-count survey observations suggest that species may be shifting their ranges upwards when compared to historical ranges, however, comparison to 2011 survey observations produced a variety of results and indicates that avian species are not uniformly responding to climate disruption with an upward range shift.

Health surveys as indicators for eelgrass meadow resiliency

Olivia Graham, Baylen Ratliff, Audrey Vinton, Tina Whitman, Jeff Gaeckle, Bart Christiaen, Catherine Harvell

Eelgrass (*Zostera marina*) forms foundational habitats in coastal regions worldwide, but is at-risk from environmental stressors, including climate warming and disease. *Labyrinthula zosterae* (Lz), the causative agent of seagrass wasting disease, threatens the many ecosystem services eelgrass meadows provide. Disease outbreaks are associated with recent, dramatic declines in eelgrass meadows throughout the San Juan Islands, Washington in the Salish Sea. Given that warmer temperatures favor many pathogens including Lz, we evaluated if levels of disease in meadows could serve as an indicator of eelgrass resiliency to climate and pathogenic stressors, since high disease levels had preceded declines in intertidal meadow density and extent (Aoki et al. 2023). We surveyed 11 subtidal meadows throughout the San Juan Islands for disease and changes in meadow extent. Surveys showed disease levels varied among sites with lowest levels in deeper meadows. Previous work has also suggested that deeper eelgrass meadows might be at least partial refugia from climate stress impacting intertidal meadows (Graham et al. 2023). We suggest sites with the lowest disease levels will be more resilient to future warming and one-time surveys of meadow health may offer an additional metric for wider scale assessment of priority in future conservation efforts. Our approach of using disease as a barometer for resiliency to multiple stressors can be applied to other systems to inform conservation and management decisions.

This Tongue is Made for Walking: The Parrot's Tongue Serves as a Mechanoreceptor During Climbing.

Michael Granatosky, James Virga, Stratos Kantounis, Reuben Jacobson, Melody Young, Edwin Dickinson, Callum Ross

Parrots exhibit a unique locomotor strategy, using their head as a limb while climbing. This locomotor strategy demands precise sensorimotor feedback, as the animal must coordinate movements of the hindlimbs and head while mitigating inter-"limb" interference and resisting falls. To date, the manner in which parrots gather the necessary sensory information to control head movements during tripedal locomotion remains unknown. Although both their mandible and maxilla have the potential to provide this feedback, the parrot's tongue stands out due to its remarkable concentration of sensory mechanoreceptors. In this study, we use a series of placebo protocols and temporary nerve blocks to assess the role of tongue mechanosensation during vertical climbing the rosy-faced lovebird (*Agapornis roseicollis*). Across trials, manipulation of the tongue either through saline injections, application of topical lidocaine, or a combination of topical lidocaine and a bupivacaine injection involved slower gaits. Further, the incidence of slips and falls increased significantly during trials that involved temporary nerve blocks of the tongue. These findings provide compelling evidence suggesting that the parrot's tongue serves as a mechanoreceptor during tripedal locomotion and represents the first instance of the tongue contributing to a locomotor behavior. This remarkable exaptation illustrates the importance of neuromuscular flexibility when species are faced with anatomical constraints and highlights the suite of adaptations required by parrots to co-opt their head into a functional limb.

Are You Stronger Than a Lemur? Assessing the Efficacy of an International Outreach Program.

Michael Granatosky, Melody Young, Noah Chernik, Stratos Kantounis, Reuben Jacobson, Matthew Cannata, James Virga, Jon Gustafson, Edwin Dickinson

Our understanding of Science, Technology, Engineering, and Math (STEM) education underscores the importance of active learning to enhance knowledge retention. However, most STEM outreach initiatives lack plans to evaluate effectiveness and ensure long-term impact. Bearing this in mind, we have developed the "Are you Stronger than a Lemur?" outreach program. Each session, lasting 45-60 minutes, commences with a pre-test containing six questions. These questions use language suitable for different grade levels and cover topics such as statistics, physics, muscle physiology, human evolution, primatology, and interest in STEM. Subsequently, we deliver a concise 10-15-minute presentation tailored to the grade level, followed by a standardized 10-20-minute activity measuring grip strength. The gathered data is then directly compared to results from live lemur experiments and previously collected student data. A post-test is administered to assess knowledge retention. To date, we have introduced this program to over 1,000 students in the United States and Mongolia. Notably, there has been a substantial increase in knowledge across all question categories, with the most pronounced improvement seen in primatology-related questions. While a slight rise in interest in pursuing STEM careers was observed, it did not reach statistical significance. "Are you Stronger than a Lemur?" serves as an interactive, adaptable activity that effectively enhances knowledge in biomechanics, anatomy, and evolution. It accommodates various ages, reading levels, and cultural backgrounds.

Nearest Neighbors as a foraging cue: Modeling the impact of collective movement on foraging

Jesse Granger, Gabrielle Nevitt, Sonke Johnsen

Recent research on collective movement (i.e. flocking, schooling, herding, etc.) suggests that it improves navigation; however, this research has not been applied to foraging accuracy. It is currently unknown if and how animals use the location and headings of their nearest neighbors as a navigational tool during foraging, though field work suggests that certain species of sea birds may use conspecifics to locate food, based on their proximity to conspecifics at food sources. Here we develop an agent-based model to explore how collective movement impacts foraging success in a patchy resource environment, where agents can use both visual and olfactory search strategies. We

simulated an environment with one stable resource patch that released a filamentous odor plume. Agents search for the resource using both olfactory and visual search strategies. In the olfactory search strategy, agents employed a typical upwind, zig-zag search pattern when they encountered the plume. The visual search was broken into three cues that the agents could attune to: 1) the resource patch, if it was in range, 2) conspecifics, and 3) circling birds that already located the resource patch. We then explored whether collective movement increased foraging success under different environmental conditions and examined the impacts of climate change and population loss on agents which rely on collective movement.

How does whisker shape affect mechanics?

Robyn Grant, Eugene Starostin, Victor Goss, Tom Allen

The whisker system is a specialised sense in mammals. When a whisker is deflected, precise vibrotactile information is encoded in its sensitive follicle. Whiskers vary in size and shape, which affects signals at the follicle. However, we do not yet know which shape features meaningfully affect whisker mechanics. Modelling would allow us to systematically explore the effect of whisker shape parameters on mechanical signals for the first time.

Using finite element analysis, we will present here a series of models, based on the whisker shapes of three species of Carnivora – red fox (*Vulpes vulpes*), Eurasian otter (*Lutra lutra*) and grey seal (*Halichoerus grypus*). These represent terrestrial, semi-aquatic and aquatic species, respectively, with grey seals also representing the Phocid seals, which have undulating whiskers with oval cross-sections. From 2D and 3D scans of whiskers, we found significant differences in whisker shape, with the grey seal having shorter, thicker whiskers than the red fox, and the otter being intermediary. We systematically explored the effect of shape (taper, curvature, cross-sectional shape and other 3D features) on whisker bending stiffness in representative whiskers from each of the three species. We compared our model results to experimental deflection data and show that certain shape features impact mechanics. Models of this nature will improve our understanding of whisker shape and inform the basis of future mechanical modelling studies.

Histological Analysis of the Uterus in Viviparous Shiner Perch (*Cymatogaster aggregata*)

Jordan Graves, Stacy Farina, Paola Correa-Alfonzo, Janine Ziermann-Canabarro, Jaquan Horton

In general, the vertebrate reproductive tract consists of gonads and their associated ducts and glands. It has three to four layers that surround it termed the mucosa, submucosa, muscular, and serosa. This is a common surpetch found in lagoons, estuaries, and coastal streams along the Pacific coast from Baja California to Alaska. Surfperches (Embiotocidae) are a live-bearing family that also exhibits superfetation, which is the presence of multiple stages of developing offspring at once due to multiple paternity. Here we aim to determine which biological materials can be found within the uterus of the shiner perch (*Cymatogaster aggregata*). We analyze the organization of the uterine layers in this fish. Uteri were isolated from pregnant perch, embedded in paraffin, and serial sectioned. Sections were stained using the Abcam Elastic Stain Kit and analyzed under a light microscope with a camera. The uterus of the shiner perch has an outer membrane made of collagen, smooth muscle, and other materials including elastin fibers. The uterus contains numerous inner folds that consist of epithelial tissue anchored to a collagen base surrounding the developing offspring. This work will serve to learn the structural relationships of live-bearing teleost uteri. A detailed understanding of its histology will be used to comprehend how the tissue properties of the uterus allow it to expand and accommodate developing

Combining behavior and mechanics to understand how mantis shrimp withstand impacts

Patrick Green

Many organisms produce and withstand impact forces, from archerfish stunning prey with jets of water to bighorn sheep colliding their horns during contests. Quantifying these impacts and how organisms withstand them

helps us understand the evolution of organismal structure and function. Mantis shrimp (*Neogonodactylus bredini*) exchange high-force impacts called strikes onto each other's coiled telsons (tailplates) during contests over territory. Previous work tested how the telson exoskeleton withstands strikes by dropping a steel ball onto the telson of dead shrimp, finding that the exoskeleton dissipates 68% of impact energy. However, behaviors like the "telson coil" used by mantis shrimp might allow for more energy to be dissipated than the telson exoskeleton alone. I analyzed strikes from live, competing animals filmed with 40,000 frames/second high speed video. By tracking appendage velocity before and after contact with the telson, I found that the telson dissipated 85% of strike energy—significantly more than the ball-drop test. This increase in energy dissipation is likely because postures like the telson coil let the entire body flex, dissipating more energy than the telson alone can. I explore whether variation in coil posture, strike velocity, or other factors predict variation in energy dissipation. Overall, this work reveals how combining approaches from animal behavior and mechanics can lend further insights to our understanding of organismal evolution than each field can do alone.

Introduction to the Symposium: Modeling Organismal Responses to Changing Environments

Kendra Greenlee, Dianna Padilla

Throughout their lives, organisms must integrate and maintain stability across complex developmental, morphological, and physiological systems, all while responding to changing internal and external environments. Determining the mechanisms underlying organismal responses to environmental change and development is a major challenge for biology. This is particularly important in the face of the rapidly changing global climate, increasing human populations, and habitat destruction. This symposium will highlight current efforts to use modeling to understand organismal responses to short- and long-term changes in their internal and external environments. The goal of this symposium is to facilitate collaboration and communication between modelers and organismal biologists, one of the major aims of the Organismal Systems-type Modeling Research Coordination Network. The contributed talks and papers will enhance education and contribute to building a new community of scientists to tackle these important questions.

Caenorhabditis elegans life history in a microbial world and the consequences for mating system evolution

Jennifer Gresham, Levi Morran

Why biparental sexual reproduction (outcrossing) persists as a common mating strategy remains a mystery. The costs are high and the benefits can be fleeting compared to self-fertilization or the myriad of asexual strategies. Mixed mating species (can outcross and self-fertilize) offer unique opportunities to examine what factors select for outcrossing rather than self-fertilization. The nematode *Caenorhabditis elegans* is an established mixed mating model host species for studying how pathogens may select for outcrossing. While natural populations of *C. elegans*, and most other host species, likely encounter a diverse group of microbes, most studies investigate host adaptation against a single pathogen species. Very few studies investigate multiple parasites concurrently or pathogenic microbes alongside non-pathogenic microbes. We reveal the results of an ongoing experiment that documents the consequences of multiple bacterial species on the life span and reproductive success of *C. elegans*, a preliminary step to experimental evolution. Fecundity and life span were documented for individual *C. elegans* hermaphrodites reared on lawns of either one single or combinations of multiple species of bacteria for their entire lives. Preliminary data suggests that extremely virulent bacteria strains remain equally virulent even when interacting with other bacteria. However, less virulent parasitic bacteria act synergistically when paired together. This project advances our knowledge of the potential role of microbial interactions in mating system evolution.

Modulation of the crustacean cardiac system by the SLY neuropeptide family

Grant Griesman, Daniel Powell

Central pattern generators (CPGs) are networks of neurons that produce rhythmic output in the absence of sensory stimuli. In decapod crustaceans such as the American lobster (*Homarus americanus*) and Jonah crab (*Cancer borealis*), heartbeat is controlled directly by a CPG known as the cardiac ganglion (CG). The CG consists of four premotor neurons that stimulate five motor neurons, which synapse onto cardiac muscles and trigger a heart-beat with each action potential burst. The CG is modulated by neuronal and hormonal inputs that enable adaptation to physiological needs, such as altered activity level or temperature. One such modulator is the SLY neuropeptide family, which is released both locally from the CG and globally throughout the organism. SLY neuropeptides have an unusually high peptide copy number in the preprohormone. Given the intriguing release patterns and processing of SLY neuropeptides, we sought to characterize the family's effect on the crustacean cardiac system. We found that SLY neuropeptides have an excitatory effect on crab and lobster whole heart contraction frequency and amplitude. We are currently characterizing their effect on the isolated CG. This work furthers our understanding of how neural circuits that produce stereotyped outputs can generate flexibility in response to modulatory input.

Skin Color Development and CRISPR Genome Editing in the Brown Anole Lizard (*Anolis sagrei*)

Zoe Griffin, Bonnie Kircher, Richard Behringer

Animal communication varies across species. Anole lizards comprise a large genus that rely almost exclusively on visual communication cues. Anoles have a colorful skin flap on their neck called a dewlap that lizards unfurl repetitively to communicate in mating and territorial interactions. The color of the dewlap is a complex signal, though it has been hypothesized to be important for species-specific communication in complex environments. The development of this coloration is not well understood, but recent development of CRISPR/Cas genome editing techniques in this system have provided the opportunity to study the genetic underpinnings of reptilian skin pigmentation. Here, we investigate the role of Scavenger Receptor Class B Member 1 (*scarb1*) in skin color development of the brown anole. *Scarb1* expression has been correlated with yellow coloration in anole skin and has been found to regulate the development of yellow pigment in other vertebrate species. The end goal of this project is to utilize these CRISPR guide RNAs to create mutant individuals. We hypothesize that disruption of *scarb1* signaling will lead to a lack of yellow pigment in the skin. These data will further our understanding of whether color patterning genes play a conserved role in development across tetrapod species, strengthening our understanding of color-based signaling and the development of these signals in an understudied phylum.

Investigating the convergent evolution of adhesive pads in lizards through comparative embryology

Aaron Griffing, Tony Gamble, Tim Higham, Greta Keller, Thomas Sanger

One goal of evolutionary developmental biology is to understand the role of development in the origin of convergent morphologies, such as the adhesive toe pads of lizards. Often considered a key innovation, toe pads have evolved at least 16 times in lizards. Despite their morphological diversity and functional importance, few studies have investigated the origins of these structures. Herein we detail three investigations of toe pad development. First, we describe the digital development of seven lizard taxa, including ancestrally padless species and independent origins of toe pads. We compared the development of these digits and found striking similarities in the early development of toepad lamellae between geckos and anoles, suggesting developmental constraint in the evolution of digital adhesion. Second, we describe adhesive pad development in a species with two types of adhesive structures: toe pads and tail pads. We found similar onset and morphological patterns of early toe pads and tail pads, which are distinct from padless toes and tails, suggesting serial homology of toe and tail pads. Finally, we investigate toe pad development within a single evolutionary origin to describe how variation is generated in the adhesive apparatus. We describe patterns of toe pad reduction and

how they mirror experimentally disrupted pad development in a separate species. Taken together, we demonstrate how morphologies derive and converge from ancestral patterns through embryonic development.

Quantifying the evolutionary potential for Delta Smelt persistence in a warming habitat

Joanna Griffiths, Andrew Whitehead, Md Moshir Rahman, Amanda Finger, Brittany Davis, Tien-Chieh Hung, Nann Fanguue

Long-term persistence of the endangered Delta Smelt (*Hypomesus transpacificus*) will depend, in part, on whether the species harbors the genetic variation necessary to adapt to changing environmental conditions such as warm temperatures. Temperatures in the San Francisco Bay-Delta are increasing and extreme temperature spikes are becoming more common. The availability of additive genetic variation for traits that affect fitness directly determines the ability to evolve; however, nothing is currently known about the presence of genetic variation for resistance to elevated temperature in Delta Smelt. We performed a quantitative genetics experiment to test whether Delta Smelt harbor the necessary genetic variation for adaptation to elevated temperatures. We established crosses between wild and hatchery-reared (domesticated) fish and raised siblings from each family in both an optimal temperature (15°C) and an elevated temperature (18°C). We estimated thermal tolerance (Critical Thermal Maximum; CTM) of 3,000 fish raised in these two thermal environments. Our experiments show that fish reared at warmer temperatures had higher CTMs and fish that had longer ancestry in the hatchery also had higher CTMs. We genotyped 3000 fish and identified SNPs important for thermal tolerance using GWAS. Together, this information can be used to manage the refuge population and inform supplementation by maintaining ecological genetic variation that may be crucial for resilience in a warming Delta.

Rewrite the star's... Genome: Population structure and adaptations to life in the Southern Ocean

Candace Grimes, Kyle David, Damien Waits, Andrew Mahon, Kenneth Halaných

Open-ocean barriers, such as thermal fronts, present obstacles to gene flow, but many marine invertebrates have adapted to overcome barriers and survive throughout their environmental ranges. For example, representatives of the brittlestar *Astrota agassizii* are morphologically indistinguishable throughout its geographic distribution, and previously reported admixture between populations on the South American shelf and Antarctica supports gene flow across the Antarctic Polar Front (APF). Here, we present a chromosome-level genome assembly for *A. agassizii* in 24 scaffolds with an N50 of 90.7 Mbp and a 95.5% metazoan BUSCO completeness score. Further, 23 individuals of *A. agassizii* from either side of the APF were sequenced at low coverage and mapped to the complete genome to investigate population connectivity. Individuals from both sides of the APF were sequenced to identify regions of selection within the genome. Because *A. agassizii* displays varying reproductive strategies (brooding in South America and lecithotrophic larvae in the Southern Ocean), we focused on single nucleotide polymorphisms (SNPs) in genes linked to reproductive strategies. We also concentrated on SNPs in genes related to thermal stress due to the drastic difference in temperature when crossing the APF between the southern Atlantic and Southern Oceans. These genomic analyses will allow us to better illustrate the potential impacts of the APF on population connectivity of benthic invertebrates across open-ocean barriers.

Long-term effects of paternal deprivation in a biparental bird

Jennifer Grindstaff, Kiley Penwell, Angela Riley

Although biparental care is common among birds, within and among species there is substantial variation in the amount and types of care provided by fathers to offspring, including the absence of care when fathers are lost through predation or opt to pursue additional mating opportunities. To explore if paternal care provides long-term benefits to offspring, we paternally deprived lab-reared zebra finches (*Taeniopygia guttata*). We hypothesized that paternal deprivation would act as a form of early life adversity and that this would be

reflected in adult offspring behavior and physiology. To test this, we set up groups of zebra finches that experienced paternal deprivation at hatching, paternal deprivation at fledging, and a control group where both parents were present throughout the nestling and fledgling stages. Once these sons and daughters were adults, we recorded their social behavior in response to novel, same-sex conspecifics and their responses to novel objects. We also quantified several physiological metrics. Our results show that offspring paternally deprived at hatching displayed more aggressive behavior. This suggests that paternal care might be needed for the development of normal social behavior.

Quantifying Goatfish Feeding Patterns over Rocky and Sandy Substrates

Olivia Grobmyer, Chloe Nash, Mark Westneat

The Goatfishes (Mullidae) are a family of 104 species found in association with coral reefs. They have evolved a unique sensory morphology that consists of two highly sensitive barbels, which are used in searching for and excavating food within the seafloor. Despite their common occurrence, many questions remain regarding the biomechanical flexibility of the barbel along its length, the kinematics of barbel motion during searching and excavating, and the variability of barbel function on different substrates. This study quantifies the feeding behavior and function of the barbels of the manybar goatfish, *Parupeneus multifasciatus*. We used 3D high speed video to quantify fish movement and barbel behavior during feeding. Using custom StereoMorph software in R, we digitized 30 landmarks over 260 timepoints to compare the feeding kinematics between rocky and sandy substrates. Results show that goatfish barbels are highly mobile, moving in complex motion through angles of at least 180 degrees. Barbel stiffness is variable, with the flexible tips of the barbel used for searching and the stiffer bases used for digging. Additionally, we observed that goatfish barbels function in three phases, scanning, feeding, and ascent. The central conclusion of this study is that barbel use during foraging differs between substrate types. Although all phases were present during foraging on both substrates, we identified quantifiable variation in phase duration and barbel movement frequency between substrates. The robust quantification of these unique feeding behaviors increases our understanding of the evolution of feeding morphology and habitat across the goatfishes.

Sensory tuning in subterranean environments

Joshua Gross, Tyler Boggs, Alyssa Hamm, Kaitlyn Reimer

Organisms must collect sensory information from their habitat. As habitats change, sensory systems assimilate to new environmental pressures. The cave presents challenging features including the absence of light and minimal nutrition. Resident animals commonly demonstrate regression (eye loss), but non-visual sensation is commonly subjected to constructive changes. Two sensory systems – touch and taste – change dramatically in convergent cavefish populations. The lateral line system is mediated by mechano-sensory neuromast organs, which indirectly alter the facial bones of cave morphs via a role in osteo-induction (which in turn influences bone positions). Interestingly, different eyeless populations harbor different patterns of morphology, indicating bone fusions are not a simple consequence of visual regression. Similarly, different cavefish populations show different patterns of taste bud distributions beyond the oral cavity. Although the modalities differ, here we suggest the local environment likely involves key pressures influencing organismal response. In the case of touch, different bone patterns (particularly across the left-right axis) may be important for lateralized behavioral preferences. Taste likely changes as a consequence of diverse nutritional environments at each locality, particularly for the umami (savory), bitter, and sweet qualities. Collectively, these changes illustrate the complex, and sometimes hidden, constructive changes necessary for animals to adapt to diverse natural environments.

A novel method for measuring phenotypic convergence

David Grossnickle, William Brightly, Lucas Weaver, Kathryn Stanchak, Rachel Roston, Spencer Pevsner, C. Tristan Stayton, P. Polly, Chris Law

Tests of phenotypic convergence can provide evidence of adaptive evolution, and the popularity of such studies has grown in recent years due to the development of novel, quantitative methods for identifying and measuring convergence. These methods include the commonly applied C1–C4 measures from Stayton (2015), which measure morphological distances between lineages. We test the performance of C-measures and other convergence measures under various evolutionary scenarios. We reveal a critical issue with C-measures: they often misidentify divergent lineages as convergent. Further, we find that all tested convergence measures are influenced by the position of focal taxa in morphospace, with morphological outliers often statistically more likely to be measured as strongly convergent by chance. We help to address the C-measure issues by developing novel convergence measures (Ct1–Ct4-measures) that quantify distances between lineages at specific points in time, minimizing the possibility of misidentifying divergent taxa as convergent. Ct-measures are most appropriate when putatively convergent lineages' evolutionary histories include considerable overlap in time (e.g., extant taxa). Ct-measures were incorporated into an updated version of the *convevol* R package, along with new plotting functions and other functionality. Although our new Ct-measures provide researchers with improved comparative tools, we emphasize that all available convergence measures are imperfect, and researchers should recognize the limitations of these methods and use multiple lines of evidence when inferring and measuring convergence.

Effects of Scented Sucrose on Subsequent Choice in Honey Bees (*Apis mellifera*)

Stella Gruenes, Siyea Redd, Riley Wincheski, Charles Abramson

The purpose of this study was to expand the literature on olfactory communication in honey bees at the free flying individual level. Previous literature looked at the colony level using pre-exposed hive foraging and the harnessed individual level utilizing the Proboscis Extension Response (PER) with pre-exposed odors. In Greece, we used two hives to determine if pre-exposure to one of two odors would affect foraging patterns. We used 5,3, and 1 pre-exposure trials and then the honey bees were tested using the ABBA paradigm. Our results showed that the bees who had 3 pre-exposure trials did better than those who had 5 pre-exposure trials. In contrast, bees who had 1 pre-exposure trial did not exhibit an effect on their foraging patterns. Overall, this shows that 3 pre-exposure trials is the ideal number for influencing foraging patterns at the individual level. This adds to the literature by focusing on the individual level in a natural setting as opposed to the collective hive or within a laboratory setting.

Cryopreservation of *Anopheles* mosquitoes

Courtney Grula, Arun Rajamohan, Joseph Rinehart

Cryopreservation of *Anopheles gambiae*, the primary mosquito vector responsible for transmitting malaria, holds significant potential for advancing research and control efforts. Cryopreservation allows for long-term storage and has been utilized in many organisms including insects. Cryopreservation is a technique that cools organisms to ultra-low temperatures and this process typically requires carefully controlled freezing techniques to prevent damage from ice formation to the cells. Our goal is to cryopreserve the first instar larval stage of *A. gambiae* utilizing vitrification, which is a process that allows cells to be rapidly cooled without the formation of ice. Utilizing the cryopreservation technique of vitrification, we hope to improve survival by avoiding damaging ice crystal formation during freezing. Through the use of cryoprotectants and dehydration, we aim to vitrify the larvae for preparation for cryo-storage. The larval stage was chosen due to the difficulties of cryoprotectant permeation during the embryo stage. Preserving these mosquitoes could be a valuable resource for future genetic research, reducing the cost of colony maintenance and developing strategies to combat malaria transmission.

The energetics of diurnal and nocturnal tropical small mammals in a changing world

Abigail Grunwald, Eric Brown, Danielle Levesque

Decades of study into the energetics of temperate endotherms has led to the belief that most endotherms operate at temperatures below their thermoneutral zone (a range of ambient temperatures where an endotherm does not need to produce energy to maintain their body temperature). However, mammal biodiversity is highest in the tropics where temperatures are consistently warm and the climate is relatively aseasonal. Therefore, unlike temperate species that must consistently generate heat to maintain elevated body temperatures, low latitude species spend more time at thermoneutrality or at temperatures where the costs of thermogenesis are low. To challenge the temperate paradigm, we tested the costs of living for both a diurnal (tree-shrew) and nocturnal (tarsier) small mammal living in the equatorial tropical forests of Sarawak Malaysia using mechanistic niche-based modeling. We discuss how relatively higher average ambient temperatures and the lack of consistently low temperatures allow for a wider range of normothermic body temperatures. We argue that knowledge of these phenotypes are essential for predicting vulnerability to future climate change.

Functional variation across cryptic coral lineages in Palau

Carsten Grupstra, Kirstin Meyer-Kaiser, Matthew-James Bennett, Maikani Andres, Hannah Aichelman, James Fifer, Shantelle Bartley, Paola Gonzalez, Carlos Tramonte, Alexa Huzar, Annabel Hughes, Aden Nagree, Hanny Rivera, Sarah Davies

Reef-building corals are threatened by rising seawater temperatures. Recent genetic sequencing efforts have uncovered a widespread pattern of “cryptic speciation”—the presence of genetically distinct but morphologically similar coral lineages. Here, we investigate adaptive variation in three cryptic lineages (L1-L3) of the coral *Porites* living across environments that vary from non-marginal reefs to marginal lagoon habitats in Palau. Thermal challenge experiments demonstrate that lineages dominating marginal environments (L2,L3) exhibited higher thermal resistance than those dominating non-marginal reefs (L1). Lineages also differed in their algal and bacterial communities. Differences in energy stores, pigment concentrations, and reactive oxygen species production between lineages demonstrate distinct symbiotic strategies and functional variation between lineages. Lastly, to disentangle whether this variation was driven by genetic or environmental mechanisms, we conducted a 12-month reciprocal transplantation experiment between non-marginal and marginal habitats using colonies from each lineage. Transplantation from non-marginal to marginal environments resulted in high L1 mortality, demonstrating that environmental filtering shapes lineage distributions. Thermal tolerance following transplantation was primarily explained by lineage, followed by colony origin, suggesting that thermal tolerance differences between lineages are genetic. Together, these findings suggest that genetic differences between cryptic *Porites* lineages may explain variation in coral bleaching observed across environments in Palau and may inform restoration efforts for genetic rescue as reefs are further threatened by rising temperatures.

Neither wing morphology nor body mass predicts degree of gene flow in bats

Edu Guerra, Sharlene Santana

Genetic differences among populations often increase in tandem with spatial distance (i.e., isolation by distance, IBD), and species’ dispersal abilities can further contribute to these differences by affecting gene flow. Yet, little is known about species-specific dispersal abilities, prompting reliance on proxies like body mass. Bat wings are multifunctional structures that are primarily dedicated to mobility, therefore wing morphology could be a reliable predictor of IBD. We explore how dispersal, mediated by wing morphology, relates to gene flow in Neotropical leaf-nosed bats (Phyllostomidae), and tested the prediction that species with lower dispersal ability exhibit steeper IBD gradients, indicative of reduced gene flow relative to better dispersers. We obtained 4903 COI gene sequences on GenBank and data on wing morphology and body mass from the literature for 32 phyllostomid species cooccurring in the

Guiana Shield, and estimated the slopes between geographic and genetic distances. We identified evident IBD patterns in 20 species ($p < 0.01$); however, the overall strength of this association remained weak across all species ($R^2 < 0.16$; slope < 0.04). We employed model selection, comparative analyses between linear, quadratic, and null models integrating body mass and wing morphology to explain IBD trends. None of these outperformed the null model, underscoring the complexity of IBD dynamics in bats, and the potential that bat wing morphology might not be a strong predictor of dispersal ability.

Structure and function of the vertical septum and neural-haemal spines of burrowing wrasses

Olivia Guerra, Mark Westneat, Linnea Lungstrom

Sand diving is a burrowing behavior exhibited by some wrasses (family Labridae) in which an individual completely conceals itself under the substrate as a startle response, to avoid predation, or to sleep. Previous studies showed that sand diving wrasses differ from non-diving species in morphological traits such as body shape and depth, head shape, vertebral prezygapophyses, and neural spine overlap. Here we analyze the diversity of backbone geometry and morphometrics in the neural and haemal caudal spines of sand diving and non-sand diving wrasses, and explore the collagenous membrane of the vertical septum, to test ideas of vertebral force transmission and develop models of the vertical septum. We performed 2D geometric morphometrics of vertebral images of multiple species of burrowing and non-burrowing labrids using StereoMorph and geomorph in R. The system of collagen fibers in the vertical septum was visualized using polarized light. Results show that burrowers and non-burrowers occupy different regions of vertebral morphospace, and that spine angles and overlap with pterygiophores are different between functional groups. Crossed fiber arrays of collagen fibers in the septum suggest variable modes of force transmission in a proposed tensegrity model of backbone function.

High temperature impairment of reproduction in fish: impacts on the female HPG axis and oogenesis

Teresa Guerre, Madeline Housh, Sean Lema

Reproductive function in fishes can be impaired at elevated temperatures. With aquatic habitats experiencing higher average temperatures and more frequent extreme heat wave events with climate change, it is expected that reproductive output of some fish populations will be harmed. To better understand how elevated temperatures influence fish reproduction, we are investigating thermal effects on reproductive processes in a eurythermal fish, the Amargosa River pupfish (*Cyprinodon nevadensis amargosae*). A closely related subspecies of *Cyprinodon nevadensis* has among the highest thermal limits for reproduction of teleost fishes; even so, reproduction in this taxon is impaired above ~32–34°C. Here, we examined variation in gonadal status and reproductive endocrinology in Amargosa River pupfish from two populations: 1) the Amargosa River – a variable temperature stream, and 2) Tecopa Bore – a groundwater-fed marsh with consistently elevated temperatures. Wild-caught females from Tecopa Bore had lower ovarian mass and ovarian gonadosomatic index (GSI) values than females from the Amargosa River. Males from these habitats, however, did not differ in GSI. Tecopa Bore females maintained at 35°C under controlled laboratory conditions for 75 d showed lower GSI and reduced plasma 17 β -estradiol (E2) compared to females at 25°C. Taken together, these observations suggest that gametogenesis in female pupfish is more sensitive to elevated temperature than in males, with effects in females occurring in part via temperature modulation of HPG axis endocrine signaling.

Thermal plasticity does not change with latitude

Alex Gunderson

In his classic “Mountain Passes” paper, Dan Janzen proposed that organisms living at high latitude should have greater plasticity in thermal physiology than those at low latitude (the Latitude Hypothesis). The reasoning is straightforward: high latitudes have more seasonal thermal variability, and therefore selection should favor greater thermal plasticity there. The Latitude Hypothesis enjoys general support among evolutionary and ecological physiologists, and is frequently invoked in macrophysiological and global warming

predictions. But how well is the Latitude Hypothesis supported empirically? I argue that it is not broadly supported by available evidence and synthesize data to that effect. I describe several non-mutually exclusive hypotheses that can explain why, covering themes such as behavioral thermoregulation, evolutionary constraint, and relationships between the evolution of constitutive and inducible responses to temperature. I also present theoretical results demonstrating how differences in constitutive versus inducible thermal tolerance can influence latitudinal patterns of plasticity. By widening the scope of our expectations, we can build a greater understanding of the forces that shape physiological evolution at global scales.

Increased fetal blood space in the placenta protects fetal growth from hypoxia in adapted mice

Kai Gurnoe-Brantley, Kathryn Wilsterman

Environmental hypoxia, found at high elevations (>2500m ASL), decreases birth weight in eutherian mammals. However, populations of humans and deer mice that have adapted to high elevation, over an evolutionary time scale, can protect fetal birth weight. One mechanism by which the placenta may contribute to fetal growth protection is an increased size in the functional zones responsible for nutrient and gas exchange between maternal and fetal blood. In this study, we evaluate fine-scale structural differences in the labyrinth zone of the placenta between adapted and non-adapted populations of deer mice gestating in hypoxia or normoxia. Utilizing immunohistochemical analysis of the placental structures, we examined the differences in volume and surface area of the maternal and fetal blood spaces within the labyrinth zone, where nutrient and gas exchange occurs. We found an increased area and surface area of fetal blood vasculature in the adapted population gestating in hypoxia while maintaining a relatively constant number of fetal blood spaces. These findings are important in furthering our understanding of how reproductive physiology adapts to novel environmental challenges and has translational potential to human health. By increasing fetal blood vasculature surface area in the placenta, we speculate that highland deer mice may better optimize oxygen delivery to the fetus.

Developmental mismatch of pCO₂ levels in a second generation of northern bay scallops

Samuel Gurr, Shannon Meseck, Meghana Parikh, Lisa Guy, Genevieve Bernatchez, Gabriella Panayotakis, Chen Yin Walker, Chris Pearce, Gary Wikfors, Dianna Padilla, Katherine McFarland

Cellular-molecular underpinnings of resilience to environmental changes, especially coastal acidification, remain poorly understood. It has a basis in cell physiology that is influenced by both historical conditions experienced by the population and conditions under which an individual develops. A developmental “mismatch”, sensu Gluckman (2019), is a contrast between the phenotype of an individual and that which otherwise confers optimal responses (fitness) under a different environment post-development. We leveraged a rare multigenerational experiment that produced adult northern bay scallops, *Argopecten irradians*, grown under three pCO₂ histories: one grown for two generations in low pCO₂, one with two generations in moderately-elevated pCO₂, and a third where scallops were initially raised under low pCO₂ followed by a second generation under severe acidification (high-pCO₂). Each lineage was then exposed to matched and mismatched pCO₂ and hemocytes were extracted at 24 hours and 14 days for flow-cytometric analyses. Independent of exposure, scallops that historically experienced severely-elevated pCO₂ had greater hemocyte mitochondrial superoxide (mtROS), whereas scallops with a moderately-elevated-pCO₂ history had greater hemocyte mitochondrial membrane potential ($\Delta\Psi_m$) and cell viability. In contrast, two weeks of pCO₂ mismatch increased $\Delta\Psi_m$ and decreased cell viability in scallops with low-pCO₂ history, suggesting a linkage between mitochondrial function and resilience to elevated pCO₂. Analyses of gill tissues for ATP, oxidative damage, and gene frontloading will further describe cellular mechanisms of rapid acclimation to coastal acidification.

Characterizing the SLC17 gene family expansion in Cephalopoda: Insights into octopus intelligence

Taryn Gustafson, Robert Fitak

Cephalopods are ideal for understanding the evolution of complex cognition, yet the genomic basis of cephalopod cognition is poorly understood. While the capabilities of the octopus nervous system are similar to those of vertebrates, cephalopod brains evolved independently of the vertebrate brain. The evolution of nervous system complexity in the octopus likely included several gene family expansions, such as the SLC17 family. The SLC17 family is composed of four clades of organic anion transporter proteins. While SLC17 proteins are involved in neurotransmission and are expanded in the octopus genome, their role in cephalopod evolution is unknown. In this study we characterized the evolutionary history of the SLC17 family across cephalopods and other major bilaterian groups. We found a significant expansion of SLC17s within the octopus lineage despite a loss of SLC17s in other cephalopods. Interestingly, the SLC17 sequences from octopus species also showed the highest levels of phylogenetic instability compared to other species analyzed. The rapid SLC17 family expansion as well as their instability suggest that SLC17s are under adaptive selection in the octopuses, perhaps for their suspected role in neural functioning. Future work aims to explore the relationship between SLC17 protein function and cephalopod cognition.

Two central pattern generators in the spinal cord of birds?

Cristian Andres Gutierrez-Ibanez, Douglas Wylie

To generate lift and thrust, birds move their wings up and down in coordination. The coordinated movements of the limbs during flight differs greatly from that of other types of locomotion, like swimming and walking, which entails alternating activity of the left and right sides. Neural circuits in the spinal cord, referred to as central pattern generators (CPGs), are the source of basic locomotor rhythm and pattern. Given the differences between wing flapping and other types of locomotion, it is likely that the neuronal components and connectivity of the CPG that coordinates wing movements differs from those that coordinate other types of locomotion. Here we use an in-vitro preparations of embryonic chicken spinal cords (E10-E14) to compare the neural response of the spinal CPGs that control and coordinate wing flapping with those that controls alternating legs movements. We found that in response to NMDA and serotonin (5-HT), the intact chicken spinal cord produces rhythmic outputs that are coordinated bilaterally and between the wing and leg segments. Despite this, we found that this rhythmic output is disrupted by strychnine, a glycine receptor antagonist, in the lumbosacral (legs) but not the brachial (wing) segments. Our results provide the first evidence that differences exist in the organization of the two CPG in the spinal cord of birds.

The ancestral function of β -catenin in ctenophores.

Lucas Gutierrez, Mark Martindale, Joseph Ryan

β -catenin is a key protein for axis determination and mesendodermal germ layer specification in many marine invertebrates through its nuclear translocation and initiation of downstream gene transcription. β -catenin is also a key player in cadherin-mediated cell-cell adhesion, thus providing an interesting target for understanding the evolution of protein function. The cytoplasmic concentration of β -catenin in bilaterians is controlled by both a constitutively active destruction complex, regulated by the Wnt signaling pathway, and the ability of the cadherin to bind free cytoplasmic β -catenin. Ctenophores are a group of carnivorous marine animals among the most ancient extant metazoans. Genome sequencing in several ctenophore species has cast doubt on the existence of the destruction complex due to the absence of key (axin) binding domains in the β -catenin protein, focusing attention on the role of cell adhesion in the regulation of cytoplasmic β -catenin availability in ancient metazoans. A recent publication studied the conservation of this adhesion complex in ctenophores. They showed that the cytoplasmic domain of one cadherin doesn't have the β -catenin binding site, suggesting an absence of the interaction between the cadherin and the β -catenin. We made a genome wide search using 3 ctenophore species in order to know if the β -catenin binding site was truly missing. Our bioinformatic analysis revealed the presence of additional cadherins that possess a conserved p120 binding site in one cadherin in both *Mnemiopsis leidyi* and *Hormiphora californiensis*, but,

strangely, not in *Beroe ovata*. However, the β -catenin binding site, essential for linking the cadherin and the actin cytoskeleton, looks absent in all ctenophore cadherins. These results raise important questions about the cellular role of β -catenin in metazoan development and evolution that we are using functional approaches to understand.

Employing novel microsurgical techniques in combination with XROMM to assess mastication in mice

Josiah Guynes, Kelsey Stilson, Sacha Sides, Susan Williams, Elizabeth Brainerd, Anna Wolff, Erika Tavares

X-ray Reconstruction of Moving Morphology (XROMM) uses marker-based X-ray motion tracking combined with CT reconstruction to visualize in-vivo skeletal motion in 3-Dimensional space. XROMM's utility has been limited primarily by size, with smaller animals requiring increasingly specific accommodations to achieve high-quality imaging. These size constraints also present challenges on the methodological side, requiring minimally-invasive microsurgical implantations of radio-opaque markers.

We describe the development of the first mouse XROMM methods for mice, including bone marker implantation, anatomical constraints during surgery, and its application in analyzing hemimandibular kinematics during mastication in *Mus musculus*. We explored this system by implanting 0.25 mm tantalum markers in the zygomatic arches and hemimandibles of standard lab mice (C57BL/6J). We found that the hemimandibles are extremely mobile when the animal is sedated, making the carotid artery, jugular vein, vagus nerve, and trachea highly susceptible to compression. Successful surgeries require the mouse to be placed in a specialized "bed" to distribute drill force and a counterforce behind the mandible when drilling occurs. Drilling involves a combination of high-speed electric drill and hand-held, and magnification is essential.

This project reports defined steps that can be applied to a range of mouse-size taxa, overcoming a limit that has prevented the progression of in-vivo kinematic assessments in smaller organisms.

How spotted lanternflies feeding on phloem sap flick sticky honeydew droplets

Nami Ha, Jacob Harrison, Elio Challita, Miriam Cooperband, Saad Bhamla

Spotted lanternflies (*Lycorma delicatula*) belong to the Fulgoridae family and are insects that feed on phloem sap rich in nutrients and sugars. Unlike xylem feeders that expel water droplets spotted lanternflies (SLF) are phloem feeders, excreting sticky honeydew droplets, which remain as residuals on leaf surfaces after evaporation. Understanding the excretion behaviors of SLF can inform how insects at small scales can overcome surface tension and viscous forces. SLF fling droplets using a stylus to expel their viscous liquid waste, seemingly similar to the catapult system of sharpshooters which feed on xylem sap. The scaling analysis, however, suggests that droplets expelled from SLF are governed by inertia rather than surface tension ($We \sim O(10^1)$) and viscous force ($Ca \sim O(10^{-1})$), implying the ejection concept differs from sharpshooters that exploit the surface tension-driven droplet propulsion. Here we explore how adults of SLF can flick sticky honeydew microdroplets without viscous residuals left near the stylus and surrounding appendages. By measuring rheological properties of honeydew such as viscosity and surface tension coefficients, we try to elucidate dynamic characteristics during super-fast droplet excretion. We show how their nymphs can expel droplets without styli and discuss the difference in excretion mechanics between adults and nymphs of SLF.

Effect of Formalin Preservation on Lizard Toe Pad Shape

Travis Hagey

Formalin (i.e. formaldehyde) preservation is a common herpetological approach to preserving specimens. Previous research has found that this preservation approach shrinks specimens over time, up to 3.5%, with different shrinkage rates for bony versus soft tissues (Maayan et al 2022). Understanding how preservation modifies specimens' morphology is important for studies incorporating morphological data, especially if both live and preserved specimens

are considered. Our project will investigate how size and shape of lizards' adhesive toe pads are affected by formalin preservation. We will ask if the adhesive toepads of select geckos and anoles shrink isometrically or if some dimensions of toepad shape are affected more strongly. Lizard toepads are supported internally by phalanx and sometimes paraphalangeal bones. As a result, we suspect length, which is supported internally by bone, may shrink less than width. We will use images of lizard toepads, focusing on three species (one gecko and two anoles). Specimens were imaged while alive and reimaged after formalin preservation and four years in ethanol. Using geometric morphometrics, principal component analyses, and trajectory analyses, we will find major axes of shape change and statistically compare changes across species.

The Mechanical Properties of Ray Skin

Madeleine Hagood, Joseph Alexander, Marianne Porter

In rays, dorsoventrally flattened elasmobranch fishes, dermal denticles vary among phylogenetic groups, and some rays lack denticles altogether. Collagen fiber angles in ray skin lie within a conserved range from 70-90°, relative to the longitudinal body axis. Previously, the mechanical properties have been described for one species of devil ray (*Mobula mobular*), which lacks denticles. Their skin is mechanically anisotropic, performing differently along alternate stress axes, and is stronger longitudinally and more extensible along the hoop axis (perpendicular). Collagen orientation is known to impact skin stiffness and extension among sharks. Here, we quantified the mechanical properties of ray skin. We dissected skin from 34 rays across 6 species (4 families, 2 orders), cutting longitudinally from the tip of the snout to the caudal edge of the pectoral disc. We sectioned 5x5cm skin squares and quantified the denticle density (if applicable) and collagen fiber angle for each square using stereoscopic microscopy and ImageJ. From each square, we dissected four pieces (2/orientation) for mechanical testing in uniaxial tension to failure at a 3 mm/s strain rate on an Instron E1000. We quantified mechanical properties between stress axes and among species. We found significant differences in the mechanical behavior among species. These data provide a comparison of mechanical behavior among rays with varying swimming styles and skin morphology, and for understanding the differential impacts on skin mechanical properties.

Post-spinal Transection Muscle Activation In The American Eel (*Anguilla rostrata*)

Jeffrey Hainer, Emily Standen, Keegan Lutek

Outside of any signalling from the brain, sensory feedback can activate central pattern generators (CPGs) in the spinal cord to produce rhythmic motor patterns that drive locomotion. While normal swimming body motion and muscle activation is seen after the removal of top-down signaling via spinal transection in both lamprey and dogfish. The lack of post-transection muscle activation in even basal ray-finned fish (ropefish) suggests that this capacity was lost early in ray-finned fish evolution. Little evidence exists in more derived fishes, except for a brief mention of spontaneous muscle activation occurring in a transected American eel, *Anguilla rostrata*. Revisiting the muscle activation and swimming kinematics of transected *A. rostrata* is therefore needed to confirm this long-assumed trait. We recorded muscle activity and swimming kinematics of 21 *A. rostrata* before and after transections of the spinal cord. Four transection treatments at different locations along the body were performed: high transections (~18% BL; body length), middle transections (~50% BL), low transections (~63% BL) and double transections (~18% BL and ~63% BL simultaneously). Muscle activity and body motion was observed anterior and posterior to transection site in middle and low transection treatments, suggesting a mechanical coupling between the anterior and posterior sections of the fish. Spontaneous movement and muscle activation posterior to transection site was observed in all transection treatments, characterized by decreases in tail amplitude and beat frequency resulting in slower swimming speeds. These kinematic changes are driven by changes in muscle activation, with muscles activating for greater durations at less intensity. While *A. rostrata* is able rely on local sensory feedback and CPG networks to swim, decreased swimming performance suggest that top-down signaling must play an important role in augmenting CPG output.

Kinematics of turning flight in bats of the Chiricahua Mountain Range

Jonas Bengt Carina Håkansson, Hamid Vejdani, Abigail Shultz, Sharon Swartz, Aaron Corcoran

Over two summer field seasons in Arizona, we studied turning behavior in seven species of native Arizona bats. We used a multi-camera setup in a large flight enclosure to film the bats during free flight. Analysis is ongoing and consists of using a deep learning-powered 3D-videography workflow to capture the large- and small-scale kinematics of the bats as they perform turns, followed by reduced- and extended-order modeling based on the videography results. Our goal is to quantify the different turning mechanisms the study species employ, and construct performance envelopes of their flight. Preliminary analysis has been performed on three species occupying different areas in the morphological parameter space, namely *Myotis auriculus* (low wing loading, low aspect ratio), *Corynorhinus townsendii* (intermediate wing loading, low aspect ratio) and *Tadarida brasiliensis* (high wing loading, high aspect ratio). Our preliminary analyses show *T. brasiliensis* to be less capable of tight turns, and that they rely on higher roll angles during turning, furthermore, early analysis suggests that they employ a higher degree of wing movement asymmetry to achieve turns that the two others achieve with less pronounced differences in wing movement. Of *M. auriculus* and *C. townsendii*, the latter appears to be closer to *T. brasiliensis* in terms of reliance on roll and wing movement asymmetry during turning, but these differences are less pronounced.

Antarctic marine benthic communities: Image transects reveal lower abundances in Eastern, than Weste

Kenneth Halanych, Candace Grimes, Kyle Donnelly, William Farris, Sarah Gerken, Madison Gott, Coral Halanych, Conor Judge, Harrison Mancke, Nusrat Noor, Samantha Schrieter, Lindsay Uzarski, Damien Waits, Jessica Zehnfpennig, Andrew Mahon

Antarctica, one of the most rapidly warming places on the planet, hosts stark contrast between barren frozen lands above the water and a rich diversity below. Not surprisingly, much of the understanding of Antarctic marine systems has been driven by accessibility issues. Thus, regions like the Antarctic Peninsula, Ross Sea, and northern Weddell Sea dominate our perceptions of Antarctic biodiversity and abundance. In March and April 2023, the RV/IB Nathaniel B. Palmer hosted a cruise, focused on benthic organisms, to remote regions of the Eastern Antarctic. Using video and photo transects, facilitated by a frame housing a 35-mm SRL and GoPros, we obtained images along the continental shelf. Organismal abundances were lower in Eastern Antarctica than comparative regions of the Western Antarctic. Eastern regions lacked the numbers of sea stars, brittle stars, urchins, and crinoids seen in similar depths in Western regions. Sea cucumbers, on the other hand, appeared more abundant in Eastern regions. Pycnogonids, or sea spiders were another dominant fauna in Eastern communities. Benthic environments in Eastern Antarctica also seem to have more limited sedimentary cover of hard substrates, presumably due to ice scour. Understanding the geospatial variation along the Antarctic coastal shelf will be important for predicting how this environment will continue to change in the near future. (This talk will be filled with gratuitous images of the Antarctic seafloor.)

Control Theory Analysis of Rice Root Response to Frequency-dependent Environmental Stimuli

Madison Hales, Yu Yang, Aradhya Rajanala, Christopher Pierce, Mingyuan Zhu, Philip Benfey, Noah Cowan, Daniel Goldman

Control theory has been important for understanding the algorithms underlying biological stimulus responses and providing hypotheses for mechanistic implementations of these feedback algorithms in diverse animals. Plants respond to environmental stimuli via physical sensing (statoliths), chemical signaling (auxin release) and differential cell elongation. From these processes emerge tropisms activated by stimuli including light (phototropism), touch (thigmotropism), and gravity (gravitropism). Tropisms play key roles in roots' ability to navigate soil heterogeneities during elongation and penetration, but quantitative mechanistic links between sensing and behavior remain unclear. To

elucidate mechanisms and algorithms underlying tropisms during root growth, we employ the "system identification" framework (using periodic forcing to probe how systems follow a particular control target) to study how rice roots (*O. sativa*) generate corrective gravitropic feedback during growth. We developed our plant System-ID apparatus to image roots growing in a transparent gel while subjected to periodic gravitational stimuli via sinusoidal container oscillation. At all stimulus frequencies (corresponding to periods from 2 to 20 hrs) root tip growth angles (relative to the vertical) oscillated at the stimulus frequency. However, as frequency increased, the magnitude of root oscillation decreased by half and the lag in time between forcing and response also increased to nearly 90 degrees out of phase. Such behavior is reminiscent of animal control systems and indicates an approximately linear control model captures the dynamics involved in rice root gravitropism.

Genetic underpinnings of developmental plasticity and acute thermal responses in a coastal copepod

Alison Hall, Manali Rege-Colt, Melissa Pespeni

Marine invertebrate populations exhibit varying capacities to withstand rising environmental temperature, but the genetic bases of differential tolerance remain an important area of investigation. Physiological plasticity, such as changes in gene expression, can be critical for the maintenance of physiological function under rapidly changing conditions. In particular, developmental plasticity is the phenomenon when environmental conditions experienced during development induce a differential ability to tolerate higher temperature. In this work, I quantify the effect of developmental temperature on adult upper lethal temperature in the coastal copepod *Acartia tonsa*. To understand potential genomic mechanisms underlying these changes, I test for baseline differences in gene expression between adults that developed under ambient (18 C) and elevated (22 C) temperatures. I also quantify differences in gene expression after two sublethal temperature challenges in both developmental treatment groups to understand how developmental temperature affects the response to an acute challenge at the genomic level. These results improve our understanding of phenotypic variation in upper thermal tolerance limits and reveal gene expression changes that may underlie the functional basis of acclimation in copepods.

Uncovering Principles and Consequences of Recombination Hotspot Evolution by Mapping PRDM9 Binding

Robert Hall, Rachel Cosby, Todd Macfarlan

Meiotic recombination (MR) ensures proper chromosome synapsis and segregation during gametogenesis. MR occurs at sites of programmed DNA double-stranded breaks called "hotspots", which are located at promoters in some species and at sites bound by the protein PRDM9 in others. PRDM9 binds specific DNA motifs via its tandem zinc finger array and deposits a unique combination of histone marks (H3K4me3/K36me3) at hotspots. Although this role is critical, the zinc finger array of PRDM9 is rapidly evolving within and between species, leading to differential hotspot usage between individuals. Despite this, the binding sites have only been mapped for a few mouse and human alleles, leaving the full impact of PRDM9's rapid evolution unknown. We hypothesized that PRDM9 alleles with divergent zinc finger arrays would specify unique genomic hotspots. We mapped the binding of 52 murine PRDM9 variants in PRDM9-transfected cells and found that previously characterized variants bound their known hotspots and motifs, suggesting our assay recapitulated *in vivo* binding. Uncharacterized variants with similar arrays bound similar motifs and sites, whereas those with different arrays bound different sites. We also observed that several mouse PRDM9 variants bound promoter regions, suggesting that the evolutionary forces driving PRDM9-dependent hotspot specification may differ between mice and humans. Going forward, we will generate transgenic mouse models to assess the consequences of putative nonfunctional PRDM9 alleles for fertility *in vivo*.

Effect of elevated temperature and air exposure on chiton behavior

Adriana Halvonik-Sanchez, Daniel Speiser

Rising global temperatures associated with climate change can have a severe negative impact on the development, fitness, and survival of many marine organisms. Ectotherms are especially vulnerable to these environmental changes, with some species more susceptible than others, depending on their thermal optima. One way to assess the effect of elevated temperature on ectotherms is to study their behavioral performance under different environmental conditions. Intertidal invertebrates are anticipated to exhibit wider thermal tolerances than subtidal ones because they are adapted to a habitat with greater fluctuations in temperature and humidity. We hypothesized that intertidal chitons would be less negatively affected by high temperature and air exposure than subtidal chitons. Behavioral performance was evaluated by the time it took chitons to right themselves onto a hard substrate under three conditions: submerged control (14°C), submerged high temperature (20°C), and dry heat (21.5°C). When exposed to the experimental treatments, subtidal chitons species were slower and less successful at righting themselves than intertidal chiton species. Out of all species tested, *Katharina tunicata* was the least affected by elevated temperature and air exposure, and the only species able to right itself on a flat surface, an ability not previously reported for any chitons. This study demonstrates that even though chitons can exhibit broad thermal tolerances, their behavioral performance can be hindered when temperatures exceed their thermal activity threshold.

Don't Spill the Krill: Flow Patterns of Filter-Feeding in *Mobula Rays*

Leandra Hamann, James Strother

Mobula rays (manta and devil rays) feed by engulfing large volumes of seawater and capturing plankton with specialized gill rakers. The gill rakers employ a unique filtration mechanism, in which small captive vortices cause particles to impact and ricochet away from the surface of the filter lobes. This filtration mechanism appears to be resistant to clogging and capable of operating at high flow rates. However, little is known about the effects of behavioral and morphological differences on the flow patterns and filtration performance. To address these questions, we constructed a 3D model of a mobulid ray body and buccal cavity in a natural feeding posture, using a combination of video recordings and computed tomography (CT) data. The flow fields that develop around the filter-feeding structures were then simulated using computational fluid dynamics (CFD). We next examined how these flow fields were affected by model parameters, including swimming speed and filter pore size. Our results suggest that complex flow patterns form within the buccal cavity, which subsequently influence the vortices and particle trajectories at the scale of individual filter pores. A deeper understanding of these processes is crucial for the development of bio-inspired filters and would provide valuable insights into the ecology of these important and threatened animals.

Long-Term Melatonin Induces a Seasonal Switch in Aggression in Siberian Hamsters (*Phodopus sungorus*)

Yuqi Han, Gregory Demas, Matt Lansing

Many animals display marked changes in physiology and behavior on a seasonal timescale. Previous studies suggest that the pineal hormone melatonin acts via steroid hormones to regulate seasonal aggression in Siberian hamsters (*Phodopus sungorus*), a species in which males and females display increased winter aggression. Our lab previously identified the importance of the adrenal hormone dehydroepiandrosterone (DHEA) in regulating territorial aggression in males and females. Here, we examine whether long-term melatonin administration influences offensive aggression and explore the underlying neuroendocrine mechanisms. We hypothesized that hamsters would experience physiological and behavioral alterations following long-term melatonin and short-day photoperiodic manipulations. To test this, we individually housed adult hamsters in long or short days, and administered daily timed injections of melatonin or saline two hours before lights off for either two or ten weeks. We used a resident intruder paradigm to examine defensive agnostic behavior in a home cage and a novel neutral cage to

assess offensive aggression. Blood samples were collected prior to and after behavioral assays to assess changes in hormones. We also examined the neural mechanisms underlying behavioral changes by quantifying the expression of the immediate early gene *c-fos* via immunohistochemistry. Collectively, findings from this study confirm the importance of melatonin in the seasonal switch in aggression and contribute to our understanding of the neural mechanisms underlying seasonal aggression in both sexes.

Bendy branches: impacts of substrate diameter and compliance on gecko locomotion

Maxwell Handen, Andrew Moura, Brooklynn Campbell, Teresa Liu, Austin Garner

Many species of gecko are well-known arboreal specialists that use adhesive toe pads to attach and move in the complex and heterogeneous environment of treetops. Arboreal substrates (e.g., branches) can vary widely in their properties, such as their diameter and flexibility, or compliance. The effects of substrate diameter on locomotion in squamates is well documented, yet there is little known on the individual and interactive effects of substrate compliance on locomotor performance. This work aims to examine the effects of substrate diameter, compliance, and their interaction on the locomotor performance of the Oceanic gecko (*Gehyra oceanica*). We recorded locomotion of *G. oceanica* on ropes of different diameters and tension to alter diameter and/or compliance. We found that maximum velocity decreases with substrate diameter but is unaffected by compliance. However, substrate compliance has a significant impact on acceleration capacity and stopping behavior but is dependent upon body size. Our findings suggest that substrate compliance is an important feature of the locomotor substrates exploited by arboreal lizards but its complex effects on locomotion require further interdisciplinary study.

Impacts of Competitive Interactions and Thermal Stress on the Reproductive and Stress Physiology of

Rich Hang, Brian Tsukimura

Community composition is highly dependent on temperature as it is known to affect the distribution of species. As global temperatures increase, it is critical to understand how organisms will respond behaviorally and physiologically to better predict future conditions. In direct response to increased temperatures, *Petrolisthes cinctipes*, a species of porcelain crab that occupies the upper to mid intertidal zones is predicted to shift into the cooler lower intertidal zones, where its congener *Petrolisthes manimaculus* resides. As a result, both species will experience higher densities and novel interactions. In this study, I address how temperature, density, and species interactions affect the reproductive activity and stress response in *P. cinctipes* and *P. manimaculus*. Reproductive activity was measured through mRNA expression levels of the yolk protein vitellogenin (*Vg*) while mRNA expression levels of heat shock proteins (40 and 70) were used to measure stress response. To simulate natural and experimental conditions, female crabs were exposed to low-density (333 crabs/m²), medium density (1000 crabs/m²), high-density (1334 crabs/m²) at ambient (12°C) and stress temperatures (20°C) in either inter- or intraspecific conditions. Current data suggests that *Vg* in both *P. cinctipes* and *P. manimaculus* is downregulated in response to increased temperature and density. Additionally, HSP 70 has been seen to be upregulated in these conditions highlighting the importance in understanding how thermally transduced stress can impact community composition.

Northcutt collection online: a novel resource for comparative neuroscience and developmental biology

James Hanken, Doug Boyer, Matt Gage, Brendan Haley, Jocelyn Triplett, Stephen Turney, Julie Winchester, Jon Woodward

We have launched a project to digitize and make widely accessible the entire R. Glenn Northcutt Collection of Comparative Vertebrate Neuroanatomy and Embryology. The collection, which comprises approximately 33,000 histological slides representing more than 240 genera and 270 species, is a unique, irreplaceable resource for comparative studies of the evolution and development of the vertebrate brain, especially for the current generation

of neuroscientists using molecular and genetic approaches to elucidate mechanisms underlying evolutionary innovations. The project utilizes a high-throughput, semi-automated, whole-slide imaging (WSI) workflow developed jointly by Harvard's Museum of Comparative Zoology (MCZ) and Center for Brain Science. Digital media are available principally via two Internet portals configured with software tools for image viewing, downloading and analysis: MCZbase, MCZ's permanent specimen database; and MorphoSource, an NSF-supported online repository for specimen digital imagery. Open access to the estimated 500,000 serial sections and wholemount preparations enables their routine use in fields ranging from comparative neurobiology to evolutionary developmental biology, from vertebrate morphology and systematics to computer science. Moreover, by employing both a WSI workflow that is applicable to other slide collections and protocols for access and visualization that utilize a shared online resource, we provide an exemplar method for rapid and cost-effective digitization that can be used by other natural history and biomedical institutions, whose slide holdings number in the millions, most of which remain dark data.

Range-wide landscape genomics of the federally threatened spotfin chub (*Erimonax monachus*)

Austin Hannah, Jason Mays, Crystal Ruble, Nathan Whelan

Rivers and streams in southeastern United States harbor the highest levels of biodiversity and endemism for fishes in North America. Yet, widespread environmental degradation has caused catastrophic losses in regional fish biodiversity, with more than one-third of freshwater fishes risk extinction. The spotfin chub (*Erimonax monachus*) is a federally threatened minnow that was once distributed throughout the Tennessee River Basin. Landscape-scale habitat alteration, specifically the construction of hydroelectric dams, has restricted the species to a handful of disjunct stream reaches across four river systems in North Carolina, Tennessee, and Virginia; yet, to date, a range-wide population genetic assessment has not been conducted for the species. This limits our understanding of landscape genetic structure, historical demography, biogeography, and extinction risk. We generated a dataset of thousands of single nucleotide polymorphisms (SNPs) and assessed population structure and genomic diversity of *E. monachus* in the Emory River (Tennessee), the North Fork Holston River (Virginia), the Buffalo River (Tennessee), and the Little Tennessee River (North Carolina). We hypothesized that gene flow is limited within and across river drainages. Thus, populations exhibit high levels of genetic structure, significant genetic differentiation, and low genetic diversity. Population genetic patterns revealed in this study will greatly enhance our understanding of the overall biology and conservation status of *E. monachus*. Our data will guide recovery efforts for the species.

Spiders got the blues: Rh2 prevalence in salticid secondary eyes with species specific localization

Sophia Hanscom, Mireille Steck, Tom Iwanicki, Megan Porter

Jumping spiders (Salticidae) exhibit complex visual capabilities through the use of four pairs of eyes. While the primary Anterior Median Eyes (AME) have been studied both anatomically and molecularly, the three pairs of secondary eyes remain largely understudied. Previous studies in *Hasarius adansoni* identified four visual opsins: Rh1 (green-sensitive), Rh2 (blue-sensitive), and Rh3 and Rh4 (UV-sensitive). We confirm expression in *H. adansoni* and characterize the visual opsins and their localization in all four pairs of eyes for two additional species, *Habrocestum africanum* and *Menemerus bivittatus*. Transcriptomes confirmed that *H. africanum* and *M. bivittatus* both express Rh1-3, but lack Rh4. Immunohistochemistry indicates the AMEs of *H. africanum* and *H. adansoni* only express Rh1 and Rh3, while *M. bivittatus* AMEs express Rh1-3. The secondary, lateral eyes of *H. adansoni* only contain Rh1 while both posterior eyes, and all secondary eyes of *H. africanum* and *M. bivittatus* express all three visual opsins. Blue-sensitivity in salticid photoreceptors is more common than previously thought, with more specialized localization patterns than Rh1 and Rh3. The variation in localization patterns among species in the secondary eyes suggests diverse color detection capabilities within multiple Salticidae species. These findings further our understanding of color vision in salticids and also provide a baseline for comparisons of opsin localization patterns and the evolution of color vision across the family.

Scientists in the Making: Undergraduate Service-Learning to Inspire the Next Generation

Alexandria Hansen, Myung Shin

The diversity of the Science, Technology, Engineering, and Mathematics (STEM) workforce is still unrepresentative of the larger United States population. Research shows that an early interest in STEM is crucial for persisting and pursuing a STEM career. This study explores the impact of an undergraduate Service-Learning course that partnered Biology majors with elementary school students in afterschool programs to engage in interactive STEM activities. Using a mixed-methods research design, surveys and course artifacts were used to analyze the impact of this experience on both the undergraduate students and the participating children. Results indicated that the Service-Learning course significantly increased the undergraduate students' interest in STEM and teaching STEM. Additionally, participating children showed slight increases in their interest in STEM and a strong desire to continue in the program. Recommendations and implications for this work are shared to advance equitable and inclusive STEM education for all.

Ontogeny of anatomical and ecological adaptations in Hawaiian large-bodied flightless waterfowl

Michael Hanson, Helen James

Large flightless island endemic birds have been characterized as paedomorphic with respect to their flighted relatives; "frozen" in earlier growth stages resembling juveniles. Prior to human arrival, Hawaii was home to several such birds: The moa-nalo species of Kauai, Oahu, and islands of Maui-Nui, descended from dabbling ducks, and a flightless goose of the genus *Branta* on the Island of Hawaii. These waterfowl convergently adapted to a flightless, fully terrestrial, herbivorous lifestyle. They exhibited an enlarged abdominal region, and graviportal body structure, with modifications to the hindlimb, pelvis, and vertebrae to support their mass, their wings and thoracic girdles highly reduced, and bills large, robust, and in some cases serrated to process tough plant matter. Fecal analyses indicate they processed this diet via hindgut fermentation, at least in the case of the moa-nalo. Juvenile and adult skeletons of moa-nalo (*Ptaiochen pau*) found in Maui lava tubes provide a growth sequence revealing differences in timing of development in different skeletal regions, with the juvenile skull similar to that of the adult moa-nalo, while limb proportions resemble hatchling mallards. Based on egg size, we show that moa-nalo hatched at a much larger body size than expected for typical ducks, potentially facilitating acquisition of efficient hindgut fermentation. Finally, we address a growth series of the flightless Hawaiian goose (*Branta rhuax*), which was converging upon similar ecological niche.

Fast and forceful: comparative scaling analysis of mantis shrimp strikes

Sophie Hanson, Erica Staatterman, Mireille Steck, Thomas Claverie, Megan Porter, Sheila Patek

Mantis shrimp (Stomatopoda) use their small raptorial appendages to produce intense impacts at high accelerations. While strike force has been studied across a few mantis shrimp species, it has not been analyzed across the phylogeny of this group, which encompasses a range of body sizes and appendage morphologies ("smashers" and "spearers"). To understand the relationship between size and strike force, we ask: how do these intense impacts scale within and across species of this diverse group? We prompted individuals from 21 mantis shrimp species (18 smashers, 3 spearers) to strike a force sensor. We analyzed the impacts in terms of three metrics: peak force, impulse, and impact duration. Using a phylogenetic comparative approach, we then examined scaling relationships within and across species. We found that peak force and impulse increase with body size yet impact duration shows no correlation with body size. Comparisons of within-species scaling reveal variable, species-specific scaling rates for peak force and impulse. These results establish the variation of strike force within and across species and provide insight into the upper limits of high force impacts in the mantis shrimp striking mechanism.

The Subtidal Environment at Auke Bay between Spring and Fall 2021

Masaki Hara, Michael Navarro

Many studies show subtidal hydrography is influenced by environmental and biological processes. Phytoplankton blooms in spring and growth continue until late summer and early fall when light becomes limiting and phytoplankton growth fades. The study site is Auke Bay located at a confluence of environmental processes including tidal exchange and freshwater runoff, in Juneau, Alaska. The research addresses two main hypotheses: (1) Semidiurnal, diurnal, and seasonal physical processes affect the subtidal hydrography, specifically temperature, and salinity, and (2) Phytoplankton growth events (e.g. blooms, and growth declines) will be detected by sensor pH and O₂ measurements. In the research, three sensors: SeapHOx, Pendant, and TidbiT were used to measure environmental factors, such as water temperature, salinity, dissolved oxygen, pH, pressure, and light, and show physical dynamics (based on temperature and salinity) and biological dynamics (based on pH and dissolved oxygen) in spring and fall. Throughout data analysis, tidal processes have dominant nearshore hydrographic properties. Moreover, biotic events were observed on April 20th and again on April 26th when pH and O₂ remained elevated. Furthermore, seasonal effects are driven by both physical and biotic mechanisms as both were observed. Thus, the subtidal ecosystem hydrography is heavily impacted by semidiurnal cycles (tides) and by seasonal biotic processes (blooms). Future research steps should include interannual measurements and measure the impacts of nearshore currents.

A Tide Simulator Helps to Study the Role of Sirtuins in Intertidal Mussels

Alexandra Hardcastle, Lars Tomanek, Robert Brewster, Sam Conti, Sarah Martin

Climate change models predict increasing variability in environmental stressors which will require many organisms to live close to their physiological limits. Lab experiments often lack the ability to simulate natural habitats due to the difficulty in regulating multiple stressors simultaneously. For organisms that live in dynamic environments, such as the intertidal zone, it is necessary that studies include a wide range of controlled parameters in order to accurately simulate ecological contexts. The Cal Poly Center for Marine Science's tide simulator recreates tidal conditions by controlling water levels, water and air temperatures, light levels, food availability, dissolved oxygen and pH. In addition, the body temperatures of organisms may be manipulated through use of a feedback loop with infrared lights. This level of control has proven useful in studies aimed at understanding the molecular responses of California mussels (*Mytilus californianus*) exposed to multiple environmental stressors over variable time points. Sirtuins, key regulators of the cellular stress response and metabolism, have been the focus of previous studies involving proteomic responses to environmental stress. Here we present the latest version of the tide simulator and its computational control and report on the effect of temperature acclimation on the levels of sirtuins in *M. californianus*, a keystone species of the intertidal zone along the Pacific coast.

Geometric morphometrics of the Eastern newt's vertebrae across polyphenic life stages

Aaron Hardgrave, Richard Carter

Eastern newts (*Notophthalmus viridescens*) are a ubiquitous member of eastern North America's caudate fauna. Unlike the common amphibian with two major life stages, their life cycle is typically split into three phases, commonly called a triphasic life cycle. The larvae of eastern newts are fully aquatic, eventually metamorphosing into terrestrial juveniles called eft. Upon sexual maturity, the eft will metamorphose into a semi-aquatic adult whose external morphology resembles other aquatic salamander species. The eastern newt is considered polyphenic and possesses alternative life cycle strategies that are not as common, including an aquatic juvenile stage and a facultatively paedomorphic adult stage. Since the different life stages of these salamanders occupy different ecological niches (terrestrial vs. semi-aquatic vs. fully aquatic) throughout their lives and, therefore likely experience various physical forces on their skeletons, they provide a unique model to study

musculoskeletal changes across ontogeny and ecology. We hypothesize that ontogenetic niche shifts and the associated shifts in locomotion biomechanics will coincide with shifts in the morphology of the axial skeleton. Using micro-computed tomography (μ CT) and geometric morphometrics (GMM), we quantified shape changes of vertebrae across different life stages (terrestrial juvenile, aquatic juvenile, paedomorph, adult). Our findings indicate a correlation between the form and function of specific vertebrae, with ecological differences and the associated biomechanics being the main contributors to these variances.

Quantifying intraspecific variation in host tolerance and resistance to a lethal pathogen

Bennett Hardy, Erin Muths, W. Chris Funk, Larissa Bailey

Due to the ubiquity of disease, hosts have evolved strategies of disease resistance and tolerance to defend themselves from further harm once infected. Resistance mechanisms directly limit pathogen growth while tolerance mechanisms limit the damage caused by the pathogen. Testing for intraspecific variation in host populations is important for informing decisions about captive breeding, translocation, and disease treatment. Here, we test for intraspecific variation in boreal toad (*Anaxyrus boreas boreas*) tolerance and resistance against the fungal pathogen *Batrachochytrium dendrobatidis* (Bd). Boreal toads have severely declined in Colorado (CO) due to Bd, but populations in western Wyoming (WY) appear to be less affected. We used a common garden experiment to expose individuals from four populations (2 in CO; 2 in WY) to Bd and monitored for two months. We used a multi-state model to estimate survival and transition probabilities between infected and cleared states to reveal the dynamic process that traditional approaches fail to capture. We found that WY toads are tolerant to Bd infection with higher survival probabilities than those in CO when infected with identical pathogen burdens. WY toads also had lower probabilities of reinfection, suggesting resistance. Our results provide new insights into the study of host defenses, how scientists measure host tolerance and resistance, and demonstrate that describing an entire species as "tolerant" or "resistant" is unwise without testing for intraspecific variation. Due to the ubiquity of disease, hosts have evolved strategies of disease resistance and tolerance to defend themselves from further harm once infected. Resistance mechanisms directly limit pathogen growth while tolerance mechanisms limit the damage caused by the pathogen. Testing for intraspecific variation in host populations is important for informing decisions about captive breeding, translocation, and disease treatment.

Tooth Origins and the Convergent Evolution of Sensory Structures

Yara Haridy, Karma Nanglu, Neelima Sharma, Sam Norris, Javier Ortega-Hernandez, Neil Shubin

There is limited information about the origin of the fundamental tissues of the vertebrate skeleton. Dentine, enameloid, and bone appear simultaneously in the odontodes of jawless vertebrates in the middle Ordovician. Yet little is known about the impetus for the initial evolution of odontodes, historical hypotheses propose scenarios ranging from defense, to hydrodynamics, to sensation, but consensus has yet to emerge. Early vertebrate fossils are scarce and understudied, with fragmentary plates of the putative earliest vertebrates with odontodes such as the controversial Cambrian *Anatolepis* being key. *Anatolepis* reportedly has vertebrate tissues akin to those of heterostracans, and a pore-system like in ostracoderms. Despite these observations, *Anatolepis* has also been argued to be an arthropod, rendering its significance for early vertebrate evolution controversial. We examined *Anatolepis* and related material, high contrast and high-resolution scan data aligned with descriptions of *Anatolepis* but also allowed visualization of novel complex structures which were revealed to be a complex pore system of Cambrian Aglaspid arthropod sensillae. The histological and morphological similarities between vertebrate odontodes and arthropod cuticle sensillae are remarkable and reveal that odontodes are convergently akin to mechanoreceptors. Additionally, using immunofluorescence labeling we show innervation of

external odontodes in several modern fishes, this preliminary study lends additional support to a parallel mechanosensory function of dentine, thereby lending credence to the sensory origins of odontodes.

Fractals and Filters: Using fractal analysis to describe function in filter feeders

Anna Harner, Shirel Kahane-Rapport, Jonathan Huie, Cassandra Donatelli

Fractal analysis is a mathematical method used to describe complex patterns that may be difficult to parse with other methods. The Fractal Dimension (FD) is a unitless value that describes complexity; the higher the FD, the more complex the system. This number describes how a physical system can take up space from a macro to a micro level. FD has been explored for image and signal processing, and even to describe morphology, but mainly in 2D. We have modified the 2D fractal dimension framework used in the past for 3D analysis of micro computed tomography (μ CT) scans. Using Python, we created a module in 3D Slicer that allows a user to calculate the Fractal Dimension of 3D μ CT scans. The module allows for a quick and consistent calculation of the fractal dimension of 3D morphology using the huge diversity of μ CT datasets available online through repositories. In this work, we have primarily focused on filter-feeding organisms, as we predict that the fractal dimension will correlate with other filter feeding metrics like filter mechanism, prey size, and Reynolds number (Re). In whales that use an oral filtration structure known as baleen to forage, we found a positive correlation between FD and Re and a negative correlation between FD and buccal volume. These results show that FD can be used to describe function as well as morphology.

Meadow Katydid as Habitat Indicators

Nathan Harness, Kenneth Hellmig, Haley Flowers, Erica Pearson

We attempt to use meadow katydid species diversity and population density as indicators of grassland habitat quality. We identified grasslands of various quality and performed exhaustive surveys of katydids in the genera *Orchelimum* and *Conocephalus*. We assessed species diversity and population density using various techniques.

These sort of assessments could have significant impact on conservation and restoration efforts by entities all over the world looking to quickly assess habitat quality of grasslands. Meadow katydids of various species are native to every grassland on earth. They are easy to identify, and make conspicuous advertisement calls that are species specific. We discuss the feasibility of using these calls to rapidly assess population size and thereby habitat condition.

Pandemic Impact Factors: COVID-19 as a chronic stressor for the endocrine community

Breanna Harris

The COVID-19 pandemic was a stressor for all academics. Certain groups, however, were disproportionately affected, particularly in the currency of academia: publications. Early in the pandemic, journal editors posted on social media about a precipitous decline in submissions, particularly by women authors; this trend was then backed by published data from many fields. For the current study, I obtained data (date of submission, author names, corresponding author country, final decision, and publication date for accepted manuscripts) from Elsevier for General and Comparative Endocrinology (GCE) from 1 May 2019 through 30 April 2023 (1 yr pre pandemic, 3 yrs during pandemic). First and corresponding authors were coded for binary gender (using internet searches and/or Gender API software) for the 1,688 manuscripts. I examined how 1) COVID-19 impacted submission and acceptance, and 2) if author gender or geographical location impacted outcomes. The number of manuscripts submitted to GCE dropped during the pandemic and submissions have not yet recovered; there was no effect of gender. Men and women authors saw a decrease in acceptance during the pandemic. Overall, GCE had gender parity in first-author position, but men outnumbered women as corresponding author. Region of manuscript origin impacted submission and acceptance rates; authors from Asia submitted the most manuscripts and North American authors had the highest acceptance rate. Women authors from Asia and the Middle East were the most negatively impacted by the

pandemic. Given the importance of publications for career advancement, the pandemic will have long-term impacts on the population dynamics, composition, and landscape of our academic ecosystem.

Behavioral evidence for di- and trichromatic color vision in jumping spiders using gaze tracking

Olivia Harris, Imogen Watts, Alex Winsor, Jenny Yi-Ting Sung, Nathan Morehouse

Researchers have long relied on associative learning paradigms to test color vision, wherein animals are trained to relate a color stimulus to a consequence or behavior. However, learning-based methods may exclude or underestimate the color vision abilities of animals less capable of or motivated to engage with color-based learning. In animals with mobile eyes/retinae, gaze tracking could be a better behavioral approach to studying color vision.

Molecular and microspectrophotometry data suggest that the front-facing, mobile retinae of jumping spiders are capable of color vision, with all species exhibiting at least ultraviolet (UV) and green sensitive photoreceptors. Additionally, the subtribe *Harmochirina* have evolved an intraretinal filter, resulting in a population of cells responsive only to long wavelengths, creating the possibility of trichromatic vision.

We characterized color vision in two species, putative dichromat *Phidippus audax* and putative trichromat *Habronattus pyrrithrix*, using ophthalmoscopic imaging of retinal responses to isoluminant stimuli in a UV-vis video playback paradigm. We find these spiders do respond to UV/green contrasts, at color distances as low as 3 dS, and *H. pyrrithrix* respond to red/green contrasts as low as 15 dS. These results provide the first systematic behavioral evidence for color vision in these species of jumping spiders, and suggest that gaze tracking as a behavioral paradigm may hold promise for testing color discrimination in the absence of associative learning.

Controlling ultrafast jumps: how furca morphology affects jump dynamics in springtails and robots

Jacob Harrison, Adrian Smith, Hungtang Ko, Baekgyeom Kim, Je-Sung Koh, Saad Bhamla

Springtails (Collembola) are a group of small, non-insect hexapods that use an ultrafast jumping appendage called a furca to launch themselves off substrates at millisecond timescales. Springtails use their jumps to effectively evade predators, disperse in their environment, and even migrate. However, in spring-latch systems like the springtail, the ultrafast release of elastic energy can make controlling the jump incredibly challenging. Therefore, in this study, we investigate how furca morphology and kinematics affect jump dynamics in springtails and whether certain aspects aid in controlling the jump. Using high-speed video, we analyzed the takeoff kinematics for four terrestrial springtail species leaping off rigid substrates: *Folsomia candida*, *Dicrytomina minuta*, *Homidia sauteri*, and *Pogonognathellus nigrinus*. Across species, we found that *F. candida* has a relatively small furca (~20% body length), while the other species have relatively long furcas (~50% body length). Interestingly, both *H. sauteri* and *P. nigrinus* possess a joint in their longer furca that bends significantly during a jump (< 40 degrees). This joint slows down their jump but also reduces take-off angle and angular velocity. We use mathematical and robotic modeling to explore whether including a joint is sufficient to reduce take-off angles and angular velocity. Our findings offer novel insights into how ultrafast biological systems control their jumps and inform potential constraints on the flow of elastic energy through materials at millisecond timescales.

The Jungle Biomechanics Lab: interdisciplinary field research experience for early career scientists

Jacob Harrison, David Hu, Geoffrey Gallice, Johana Reyes, Saad Bhamla

The Jungle Biomechanics Lab is a two-week field research experience for early-career scientists, from undergraduates to postdocs. Selected participants will embark on a journey to conduct field research and community outreach. This NSF-funded program takes place sometime during September and October at Finca Las Piedras in the Peruvian Amazon Rainforest. The goal of this program is to help give early career researchers the tools for

conducting quantitative biomechanics and behavioral experiments on diverse organisms in their challenging natural environments. With the help of a team of experienced researchers, each participant will develop and execute an independent interdisciplinary research project into one of the diverse biological systems found in the Amazon, the planet's largest and most richly diverse rainforest. Individuals will collaborate with their cohort of early career researchers, pooling experiences to achieve novel insights at the crossroads of biology, animal behavior, materials science, mathematics, evolution, robotics, physics, and engineering. As part of this program, participants will also engage in several science outreach initiatives in Peru. They will help lead outreach events at several local Peruvian schools and collaborate with Peruvian undergraduate students to increase intercultural connections where scientists conduct field research.

Affect of body size and colonial complexity on body and brain metabolic rates in *Pogonomyrmex* ants

Jon Harrison, Craig Perl, Robert Johnson, James Haas, Meredith Johnson, Leland Graber

How body size and social complexity affect the evolution of body and brain metabolic rates are central questions in comparative biology. *Pogonomyrmex* ants are mostly seed-harvesters with a significant role in seed consumption and dispersal across the southwestern U.S. *Pogonomyrmex* species are frequently sympatric, while differing significantly in body size, colony size, and foraging strategy. We found that body size, colony size and foraging strategy covary, with smaller-bodied species having smaller nests and less complex modes of foraging. Brain mass, whole body resting metabolic rate and brain metabolic rate of workers scales hypometrically with body mass, but brain metabolic rate scales much less steeply than body metabolic rate. Measured with an Agilent Seahorse, brain metabolic rates scale with a slope on a log-log plot of 0.08 compared with a slope of 0.56 for whole body metabolic rates. In the smallest species, the brain accounts for 43% of resting metabolic rate, and this declines to 14% in the largest species. One interpretation of this pattern is that smaller ants with their smaller brains must utilize their brains more intensively, allowing them to maintain commensurate brain performance with larger species with which they compete for seeds. Supported by NSF IOS-1953419.

Conserved and divergent aspects of segment polarity in the tardigrade *Hypsibius exemplaris*

Taylor Harrison, Frank Smith

The Segment Polarity Network is highly conserved between Arthropoda and Onychophora. We tested for conservation of this network in *Hypsibius exemplaris*, a representative of Tardigrada, the outgroup of the Arthropoda + Onychophora lineage within Panarthropoda. Engrailed was expressed in a segmental pattern in the ectoderm before morphological segment boundaries developed, in a posterior ectodermal region of each trunk segment during the segmentation stage, and in limb buds later during embryogenesis. *H. exemplaris* is missing a wingless ortholog. However, two other Wnt ligand-coding genes were expressed in segmental patterns. *Wnt4* was expressed in the endomesodermal pouches during segmentation, and *Wnt16B* in the posterior region of each limb bud. We also investigated the Hedgehog pathway genes *hedgehog*, *cubitus interruptus*, *patched*, and *smoothened*. During segmentation, *hedgehog* was expressed in the endomesodermal cell layer. Later in development, *hedgehog* was expressed in the limb buds. *Cubitus interruptus* was expressed broadly before segmentation, in the anterior ectodermal cells of each trunk segment during segmentation, and in the limb buds later in development. *Patched* was expressed in posterior ectodermal cells during segmentation and was broadly expressed during the limb bud stage. Comparing to previous studies, our results may reveal a developmental pattern of early divergence and later conservation in terms of the segment polarity network in *H. exemplaris*.

Breathing Under Pressure: Gill Pumping Kinematics and Temperature in Teleost Fishes

Jasmine Harry, Jasmine Renteria, Jennie Jannssen, Stacy Farina

Aquatic pump gill ventilation involves pumping water over the gills using cyclical pumping of both the buccal and branchial chambers. While this basic mechanism is present across all teleosts, the kinematics of pumping can vary considerably among and within species. Teleost species with more capability of modulating their ventilation kinematics may be better able to acclimate to changing temperatures as their habitats shift during climate change. We visited the closed-system aquarium habitats at National Aquarium and obtained 90 videos of 44 species using cell phone cameras and collected metadata for each video, including species, behavior, temperature, and estimated fish size. Using DeepLabCut, we tracked the movements of the jaws for individuals of species under different conditions. After training, our DeepLabCut model was able to accurately track jaw movement for most videos, and we developed a custom R script to calculate the duration of inhalation and exhalation. We will correlate kinematic variation with ecological variables such as temperature, geographic range, and habitat.

Vertebrate evolution in the face of extreme conditions: Cavefishes (Percopsiformes)

Pamela Hart, Melissa Rincon-Sandoval, Ricardo Betancur-R, Jonathan Armbruster, Matthew Niemiller, Dahiana Arcila

Fishes have successfully colonized numerous physiologically challenging environments, including the deep sea, sulfidic environments, and caves. Caves and cavefishes offer an ideal system to study adaptations to harsh environments since multiple lineages have independently colonized these habitats, turning them into "natural laboratories" with replication. Species within our study group—the North American amblyopsid (Percopsiformes) fishes—contain three levels of cave-obligate evolution (cave, surface-cave, and surface), with at least three independent colonization events into cave environments. While there has been significant progress in understanding the evolutionary relationships among amblyopsid species, the timing of their evolution, their colonization to caves, and the genetic mechanisms underlying their extreme morphological phenotypes (e.g., lack of pigmentation, blindness) remain unknown. To tackle this gap of knowledge, we estimated a new phylogeny using 1105 single-copy exon markers, which aligns with previous phylogenomic reconstructions. Our findings reveal the nesting of an eyed surface-cavefish genus within a clade of blind cavefishes. Employing a total-evidence dating Bayesian approach and integrating both extant and fossil percopsiform species as terminal taxa, we proposed a new time of evolution for the group. Furthermore, we explored signatures of evolution in 328 candidate loci by generating whole genome sequencing data for 12 percopsiform species. This study helps our understanding of organismal evolution in extreme environments.

Effects of mTBI on depressive-like behavior and blood brain barrier permeability in a jumping fish

Jenna Hartzler, Grace Bollinger, Ryan Earley, Kate Graziano, Cristel Ruiz, Helen Hoye, Julia Mooncotch, Mackenzie Woodward, Alexia Washington

Mild Traumatic Brain Injury (mTBI) is a prevalent neurological disorder in humans that is caused by blows to the head and can produce debilitating behavioral and cognitive effects. Other organisms experience infrequent mTBIs and lack significant protections (e.g., horns) outside the skull and associated musculature, which raises the question of whether and how such organisms protect the brain or recover from intermittent, low-level brain damage. We used mangrove rivulus fish to investigate the neural and behavioral impacts of mTBI. This fish can survive on land and performs tail-flip jumps to propel itself, often landing squarely on its head. We examined acute and chronic behavioral effects of mTBI and determined their association with changes in the permeability of the blood-brain barrier. First, standardized mTBIs of two weights (0.3g, 0.6g) were administered using a custom weight drop apparatus. Fish then underwent behavior trials to quantify locomotion, thigmotaxis, anhedonia, and lethargy, all of which are associated with depressive-like behavior in many vertebrates. Then, biocytin was injected intraperitoneally thirty minutes prior

to euthanization to stain brain vasculature and analyze blood-brain barrier permeability. Finally, brains were harvested and blood-brain barrier leakage was quantified as the amount of biocytin fluorescence in brain tissue outside of the vasculature. We present initial findings that highlight the relationship between morphological damage to the brain and the magnitude and persistence of TBI-induced behavioral deficits.

Species-specific variation in avian airfoil morphology

Christina Harvey, Rowan Glenn, Andrew Engilis-Jr.

Avian flight characteristics are dictated by morphology, behavior, and environment. Each of these elements can vary substantially across the phylogeny. Therefore, there is a need to establish a method that can extract each element's relative importance on flight performance. In this work, we build upon previous studies to explore the interaction of avian morphology and flight behavior. In this work, we focused on the airfoil, which is a 2D cross-sectional geometry of a wing and, unlike aircraft, is known to vary substantially along the avian wingspan. We used a 3D scanner to scan prepared wing specimens at the UC Davis Museum of Wildlife and Fish Biology to obtain a complete 3D model of the wing. We selected species that primarily glide or primarily flap only. Next, using an adapted slicing technique, the 2D cross-section of the wings (i.e., airfoil) are extracted along their span allowing us to visual how the avian airfoil changes from the proximal to the more distal morphology. This variance was quantified using a geometric morphometrics approach to define the major axes of variation and investigate differences between gliding and flapping species. The outputs of this work will define the airfoil shapes used by gliding birds and investigate how these shapes may be associated with flight behavior.

Wild and domesticated *Betta splendens* exhibit different behavioral responses in novel situations

Christina Harvey, Miriam Ashley-Ross

Captive populations are often used to make inferences about wild populations. However, captive organisms often have unique traits unlike those found in their wild counterparts. The betta fish has been a popular model system for exploring animal sexual selection and aggression. Behavioral responses in domestic bettas are potentially different from those in wild fish, as domestic strains have been selectively bred for their personalities since their original domestication roughly 700 years ago. In this study, we compared the behavioral responses of wild-caught and domestic bettas while exposed to a novel environment. Additionally, we attempted to classify betta personalities (behavioral syndromes) by identifying correlations between response variables commonly used in animal behavior studies. We compared wild bettas to two strains of domestic bettas (plakat and veiltail). We found no significant differences in behavioral responses between the domestic strains. In contrast, wild bettas exhibited a decreased willingness to explore novel environments and exhibited a greater variation in every response variable measured. These findings add to the growing body of literature highlighting the differences between captive and wild animals. Researchers should carefully consider the source population of their sample populations prior to making inferences about natural systems.

Opsins are differentially expressed during larval development in pteriomorphian bivalves

Md Shazid Hasan, Jorge Audino, Kyle McElroy, Jeanne Serb

Marine organisms exhibit biphasic life cycles involving mobile larvae and sedentary adults. At the end of the initial phase, larvae must locate suitable settlement sites, but the sensory receptors involved are largely unknown. We focused on opsins, a type of G-protein coupled receptor known for light detection and, more recently, light-independent functions. Previous investigating opsin genomic content in both eyed and eyeless pteriomorphian bivalves revealed significant variability across species, with the unexpectedly high genomic abundance of opsin in eyeless adults. Therefore, we investigated the diversity of opsin expression from the perspective of larval development. We analyzed opsin expression from publicly available transcriptomic data across larval stages (trochophore, veliger, pediveliger) in seven

species of four families, including scallops, mussels, oysters, and pearl oysters. Our results showed that some opsin genes, such as retinochrome, xenopsins, and Go-opsins displayed heightened expression during later larval stages, coinciding with substrate testing during settlement at the pediveliger stage. Exploring opsin gene expression throughout larval development provides pivotal insights into understanding how opsin receptors in these organisms respond to diverse environmental cues, crucially impacting the settlement process. Consequently, this research contributes to our understanding of the multifaceted settlement mechanisms in marine organisms with complex life cycles, offering a holistic perspective on the role of opsins in their survival and adaptation.

Evolution of animals from protozoan ancestors: The ins and outs of colony design

Tom Hata, Mimi Koehl

Choanoflagellates and animals share a common ancestor, so we use choanoflagellates that can form multicellular colonies as a model system to study selective factors in the evolution of multicellular animals from unicellular protozoan ancestors. Choanoflagellates have a single flagellum surrounded by a row of microvilli on which bacterial prey are caught, like choanocytes in sponges. Hydrodynamic studies of swimming, feeding, and susceptibility to predation of choanoflagellates have focused on single cells and colonies with flagella pointing outward, whereas flagella line cavities in sponges. We studied aspects of hydrodynamic performance that may have affected the evolution of sponges from choanoflagellate-like ancestors using *Choanoea flexa* cup-shaped colonies that can reversibly invert to form colonies with flagella covering the outer surface or lining the cup's cavity. We used microvideography and PIV to quantify flow fields and behaviors of these colonies. Flagella-in colonies captured 10X more bacteria-sized particles per cell per time than did flagella-out colonies. In contrast, flagella-out colonies were much faster swimmers (38um/s) than flagella-in colonies (5um/s). Although flagella-out colonies produced larger flow fields with velocities detectable by raptorial protozoan predators than did flagella-in colonies, they could swim away from benthic raptors. Our results suggest that having flagella on a colony's outer surface enhances swimming, and thus exploring the environment and avoiding predators, whereas having flagella lining a cavity enhances feeding at the expense of swimming performance.

Killer whale chemical tracers and trophic enrichment factors

Madison Hattaway, Paul Chittaro, Li-Jung Kuo, Kim Parsons, Brad Hanson, Dawn Noren, Candice Emmons, Jonelle Gates, Lydia Staggs, Steve Osborn, Todd Schmitt, Karen Steinman, Todd Robeck

With the lack of recovery of the endangered Southern Resident killer whale population being related, at least partially, to decreased quantity & quality of prey, an understanding of their diet is critical for their conservation. Stable isotope analysis can add insight on a longer timescale to the existing body of research on Southern Resident killer whale diets that has been conducting using genetic identification of fecal prey and prey remains. While stable isotope analysis is a powerful tool to reconstruct diets, its utility to study those of Southern Resident killer whales is limited because isotope fractionation between prey and killer whale tissues is poorly understood. Isotope fractionation is the enrichment of one isotope relative to another, which occurs with increasing trophic levels. This study focused on estimating isotope fractionation for killer whales fed a controlled diet at multiple oceanaria. Specifically, trophic enrichment factors were measured between killer whale feces, breath, and serum as well as the fish fed to killer whales in terms of both bulk ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) and compound specific stable isotopes of amino acids ($\delta^{15}\text{N}$). Fecal matter, breath, and serum integrate isotopes over different intervals of time and therefore we also explored the distinct temporal scales of consumed prey.

Salt, Fat, Acid, and Heat: the Impact of Acid Weed on the Green Sea Urchin

Rachel Haughton, Stephanie Crofts, Katie Dobkowski

Acid weed (*Desmarestia herbacea*), is a brown alga found in the San Juan Islands with an internal pH of 0.6 to 0.8, releasing this acid when stressed. The

green sea urchin (*Strongylocentrotus droebachiensis*) is a known consumer of *D. herbacea*, but prefers bull kelp (*Nereocystis luetkeana*). Our aim is to determine how these diets affect the green sea urchin. We collected urchins near Friday Harbor, WA, and divided them between a control group and three treatment groups: unstressed *D. herbacea*, stressed *D. herbacea*, and *N. luetkeana*. To assess how spines, Aristotle's lantern and teeth were affected, we microCT scanned the structures to measure their density after three weeks on the assigned diet. To assess plasticity or reallocation of resources, we measured gonad weight and Aristotle's lantern size. For all calcified structures, the control group, measured directly after they were collected from the wild, were significantly denser than those of urchins maintained in the lab. Calcified structures of urchins fed on stressed *D. herbacea* were denser than those of urchins eating the unstressed alga, and equivalent to those consuming *N. luetkeana*. Our results also suggest that the amount of food eaten has a greater impact on gonad weight than diet. Finally, our results suggest that diet acidity may not impact green sea urchins, however they may be more affected by caloric intake and habitat.

Thermal adaptations in embryonic metabolic rates and developmental times in a *Sceloporus* lizard

Benjamin Haussmann, Tiffany Hegdahl, Travis Robbins

Many ectotherms inhabit large latitudinal ranges with environmental factors, such as temperature, creating gradients to which species must adapt. Adaptations may result in phenotypic gradients with phenotypes, such as developmental time and metabolic rate, interacting to compensate for the shifting environmental conditions. Adaptive responses are thus complex coadaptations with both plastic and evolutionary mechanisms that must be examined simultaneously to elucidate how trait interactions occur on both proximate and ultimate scales. This study investigates adaptations associated with embryonic development in Prairie Lizards (*Sceloporus consobrinus*) across a latitudinal thermal gradient. Gravid adult females were collected from three latitudinally distinct populations. Eggs from each clutch were randomly assigned to two temperature treatments (25°C and 31°C) in a split-clutch design and checked daily for hatchlings to determine developmental time. Embryonic metabolism was measured as both respiration and heart rate at respective incubation temperatures. Results show the expected proximate response of warmer temperatures decreasing development time with increases in respiration and heart rates. Populations differed, however, in how developmental time, heart rate, and respiration rate collectively responded to temperature treatments, suggesting evolution has occurred in these traits. As we continue to witness climate change, understanding the effects of temperature on ectotherms and their physiology becomes crucial to conserving these species and their communities.

An integrative approach to studying magnetoreception

Hazel Havens, Kenneth Lohmann

After decades of successful research, in many ways the magnetic sense remains an outstanding mystery in the field of sensory biology. We know that the sense is phylogenetically widespread and provides many species with both directional and positional information. However, no receptor has been conclusively identified in any animal and as such, the physiological function, limits, and processing of this sense are largely unknown. Here, we present an approach developed to systematically study the magnetic sense in this context. We combine neural circuit modeling with iterative behavioral experiments and species-specific neural architecture information to develop and test narrow hypotheses of how aspects of this enigmatic sense may function. We demonstrate this approach in the Caribbean spiny lobster *Panulirus argus*, which shows remarkable abilities to navigate using Earth's magnetic field. In doing so, we eliminate some possibilities of how the sense may function in *P. argus* and bolster others. After this efficacy in studying magnetoreception in lobsters, we believe our approach may prove a promising tool for understanding magnetoreception more broadly.

Singing through time and space: spatial patterns of advertisement relative to neighborhood fertility

Carly Hawkins, Autumn Iverson, Jessica Malisch, Thomas Hahn, Gail Patricelli

Despite low overall reproductive variance in socially monogamous species, the categorization of "monogamy" conceals an enormous amount of variability in how individuals achieve overall reproductive success. 97% of "monogamous" birds are sexually promiscuous and engage in tactics that can lead to extra-pair paternity and/or loss of within-pair paternity. Investment in within-pair reproduction (e.g. parental care) comes at the expense of investment in extra-pair reproductive tactics (e.g. courtship) and vice versa. These trade-offs are unique to social monogamy, with striking differences in how males balance extra-pair vs within-pair choices leading to wide variation in reproductive composition (proportion of extra-pair to within-pair offspring).

I investigate behavioral differences among male white-crowned sparrows (*Zonotrichia leucophrys oriantha*), a socially monogamous songbird, that might favor or result from alternative mating tactics. Specifically, I examine how male singing locations (determined by acoustic localization) change in response to neighborhood fertility by comparing song locations over time to their overall home ranges (determined by GPS backpacks). Understanding how variation in reproductive composition impacts the overall reproductive success of animals can offer insights into overlooked subtleties of avian biology that allow us to further understand the evolution and biological implications of social monogamy.

The impact of caudal fin shape on the hydrodynamic performance of elongate foils

Olivia Hawkins, Megan Vandenberg, Eric Tytell, Cassandra Donatelli

Given the complex interactions between morphology and behavior in living organisms, simplified models are often necessary to answer questions about form and function. Foils in particular enhance our knowledge of fish swimming through manipulation of parameters such as swimming speed, stiffness, and fin shape. Historically, the effect of caudal fin shape on hydrodynamic performance has been investigated using short, deep bodied foils to reflect well-studied non-elongate fishes like the bluegill sunfish. For elongate fishes, we do not know how caudal fin shape differences affect hydrodynamic performance. Although elongate fishes typically reduce or lose their caudal fins, some species retain them and exhibit different shapes as observed in Zoarcoidei (pricklebacks, gunnels, and relatives). We tested three foils of varying stiffnesses cut into a rectangle and five representative zoarcoid caudal fin shapes. We attached foils to an integrated motor and 6-axis force transducer system with input frequencies of 0-3 Hz and filmed foils from below at 120 fps as they flapped in flow speeds ranging from 0.5-2.5 BL s⁻¹. We compared thrust and midline kinematics across tail shapes, stiffnesses, input frequency, and flow speeds. At low flow speeds and medium frequencies, small differences in caudal fin shape account for differences in midline kinematics within and across stiffnesses. Fins could provide subtle hydrodynamic advantages in elongate fishes, but functional differences during swimming should also be considered.

Post-settlement growth and staging of the sea slug *Hypselodoris festiva*

Makiko Hayashi, Hiroaki Nakano

The family Chromodorididae (Mollusca: Gastropoda: Nudibranchia) have attracted a great deal of attention for their bright, complex, and diverse body colorations. However, there are no reports of rearing chromodorids from eggs to adults in the laboratory. Therefore, the process of body coloration patterning during development remains to be elucidated. Furthermore, it is not clear how ecologically and taxonomically important adult chromodorid organs are formed, such as rhinophores, spicules, mantle dermal formations, and gills. In this study, we report on the successful laboratory rearing of the chromodorid species *Hypselodoris festiva* from eggs to adults and describe its coloration patterning and vital organ formation. Additionally, easily identifiable external characteristics were used to classify post-settlement growth into nine stages. Larvae fed with microalgae began to settle from 21 days after hatching,

followed by a rapid metamorphosis including casting off of their shells. Settled juveniles were fed marine sponges. Body coloration appeared around 36 days after hatching. Organogenesis of all vital organs began within 42 days after hatching. Laboratory reared individuals reached sexual maturity approximately 6 months after hatching, with mating and spawning being observed. This study is the first successful laboratory rearing of chromodorids from eggs to adults, and also the initial such report for sponge-eating nudibranchs. Through these methods and our comprehensive post-settlement staging, we propose *H. festiva* as a model organism for future chromodorid research.

Examining Associations Between Skin Infection and Skin Microbiome in Arkansas Snakes

Natalie Haydt, Lorin Neuman-Lee

Since the late 2000s, researchers have connected a handful of population declines in North American snakes to a skin fungal infection, ophidiomycosis, attributed to the fungus *Ophidiomyces ophidiicola*. However, emerging evidence demonstrates that multiple fungi from the order Onygenales may be responsible for ophidiomycosis-like skin infections, especially in reptiles. Thus, there is a demand to examine the impact of other infectious microbes on snake populations. To further understand how diverse microbial communities are impacting snake health, immunological research is key, yet few studies have examined both snake skin infections and immune function. For our study, we sampled snakes at six sites in Northeast Arkansas and obtained both skin swabs and blood. We characterized skin infection severity by recording the number of lesions, size of lesions or percent of body length infected, and lesion type. We detected the presence and relative frequency of microbes on snake skin using Illumina sequencing methodology. To examine immune function, we quantified microbial killing ability of plasma using microbial killing assays and leukocyte populations using flow cytometry. We found seven genera in addition to *Ophidiomyces* present in swab samples, and *O. ophidiicola* was detected in only one out of 100 samples. In preliminary analyses we found lesion numbers to be significantly associated with Onygenales microbiota in the genera *Arthroderma*, *Chrysosporium*, and *Zeloasperisporium*. We additionally found lesion numbers to significantly vary by species, site, and bacteria killing ability. By examining both immune function and microbiota of infected snakes, we can detail a more holistic view of the well-being of snakes or snake populations. Our research will allow us to identify the best microbiological or immunological variables to use to predict skin infection risk in snakes throughout Arkansas.

Tactile feedback enhances multi-legged locomotion on rugose terrain

Juntao He, Baxi Zhong, Esteban Flores, Zhaochen Xu, Daniel Soto, Daniel Goldman

When locomoting through complex environments, organisms leverage various sensory modes, from long-range vision to short-range tactile feedback. In cluttered and crowded environments, visual sensing becomes challenging and local responses (short-range and rapid feedback) could be effective. In legged robotic applications [Rogelio, Humanoids 2018], tactile sensing on feet has improved locomotion in complex terrain via decentralized foot trajectory planning. However, it remains unclear if foot-level tactile information could contribute to overall locomotion via centralized whole-body motion planning. To address this, we developed a centipede-like multi-legged robotic physical model ($L = 60$ to 160 cm, 3 to 8 segments each with four degrees of freedom for limb and body movement) with point-like feet and monitored locomotion performance on rugose terrains with heterogeneities of limb length scale. We developed a simple tactile sensor (one per limb) to detect binary ground contact. The sensing allowed estimation of local terrain complexity (via average duty factor). Building on observations that vertical body wave undulation amplitude affects open-loop locomotion speed, we used duty factor to modify the amplitude of the vertical wave. The scheme improved speed by 50% (to 0.17 BL/cycle) and reduced variance across trials by 60%, indicating that such control reduced sensitivity to environmental foot contact fluctuations. This approach to tactile information for multi-legged locomotion can improve understanding of how biological systems could use tactile sensing when long range sensing is unavailable.

"Come on Baby, Do the Locomotion": Male and Female Running Strategies in *Podarcis muralis*

Alyssa Head, Princeton Vaughn, Logan Fraire, Emma Foster, Maya Moore, Emily Virgin, Eric Gangloff

In many squamate species, males and females exhibit sexual dimorphism causing them to move through their environment differently. However, most conclusions about lizard morphology-performance relationships have been solely based on males, leaving unanswered questions about the female sex. Female ecology and performance are essential to understanding population dynamics, as females are often the limiting factor in reproductive output and therefore population persistence. This is especially important for introduced species. We addressed this knowledge gap by comparing morphology and performance in male and female Common Wall Lizards (*Podarcis muralis*) from established populations in Cincinnati, Ohio, USA. Specifically, we measured the dimensions of the head, limb segments, toes, tail, shoulder girdle, pelvic girdle, and trunk in male and female lizards. To quantify performance, we sprinted male, gravid female, and non-gravid female lizards on different substrates and on straight and curved paths. After examining sex-specific morphology-performance relationships using multivariate statistical tools, we found males were not faster sprinters than females despite having substantially larger limbs. To understand this result, we implemented deep neural networks to analyze lateral and longitudinal limb movements, joint angles, and stride lengths. The neural network analysis allowed us to test the hypothesis that stride kinematics vary depending on sex and reproductive status. This study provides insights into how sexually dimorphic male and female vertebrates may utilize limbs differently to better survive in novel environments.

Functional analysis of protein kinase G in the decapod crustacean molting gland

Talia Head, Donald Mykles

Crustacean growth is regulated by several molecular pathways which mediate the synthesis of molting hormones, ecdysteroids, which stimulate molting processes in peripheral tissues. Molt-inhibiting hormone (MIH) is synthesized and secreted by the X-organ/sinus gland complex (XO) and acts on the molting gland (Y-organ, YO) to inhibit mTOR-dependent the production of ecdysteroids. MIH signaling in the YO involves NO-dependent production of cGMP. The YO expresses two cGMP-dependent protein kinase (PKG) isoforms (PKG1 & 2). We hypothesize that PKGs phosphorylate Tuberous Sclerosis Complex1/2 (TSC1/2), a GTPase activating protein that controls mTOR-dependent ecdysteroid synthesis. The response of YOs to PKG inhibitors was quantified by measuring ecdysteroid secretion in vitro in *Gecarcinus lateralis* and *Carcinus maenas*. In the presence of MIH, a universal PKG1 and PKG2 inhibitor increased ecdysteroid secretion in both species, whereas a PKG2-specific inhibitor enhanced the effect of MIH. These data indicate that the two PKG isoforms have opposing roles with PKG1 having the dominant inhibitory role, as PKG1 mRNA level was two orders of magnitude higher than the PKG2 mRNA level. We hypothesize that the PKGs phosphorylate different sites on TSC, resulting in mTOR activation or inhibition. Proteomic analysis using LC-MS/MS will determine the effects of PKG activation and inhibition on phosphorylation of TSC and other proteins in the mTOR signaling pathway. Supported by NSF IOS-1922701.

Single Cell RNA Sequencing Gives Clues for the Developmental Genetic Basis of Syngnathid Adaptations

Hope Healey, William Cresko

Seahorses, pipefishes, and seadragons are extraordinary fishes from the family Syngnathidae. These fish have numerous derived traits including male brooding, elongated snouts and body, loss of teeth and ribs, dermal bony armor, and presumed loss of lateral line and Mauthner neurons. However, the evolutionary developmental basis for these trait changes is largely unknown. Recent syngnathid research has focused on identifying gene family expansions or losses to understand trait changes using new whole genome sequences. To build upon these findings and examine the developmental genetic basis of trait alterations, we created a single cell RNA sequencing (scRNAseq) atlas in Gulf pipefish. We found that, despite their unusual morphologies, Gulf pipefish

have largely conserved gene regulatory networks across cell identities. By investigating the spatial expression of select genes from gene networks, we discovered *bmp4* expression in the ethmoid plate and hyoid of pipefish's elongating snouts. Additionally, we observed *dlx3b* and *pitx2* expressed respectively around epidermis and muscle of the developing dermal armor. Finally, we did not find tooth primordia cells in our scRNAseq dataset, however we observed expression of genes involved with tooth formation in bone cells. Our findings suggest that syngnathid's skeletal evolutionary novelties have re-purposed existing gene regulatory networks to create highly modified and novel structures. We propose that further incorporating developmental assays into syngnathid evolutionary research will speed understanding of how their novelties evolved.

Does cranial morphology variation among domestic dog breeds reveal a functional mirage?

Nicholas Hebdon, Alexa Ortega, Alexander Orlove, Lindsay Waldrop

Inferring potential form-function relationships in animals after watching them excel at certain tasks is a common and often fruitful venture. Occasionally, humans use these inferred relationships as the basis for driving selective breeding of certain animals to further improve upon their performance in functions we already see as valuable. One of the most striking examples of this process are modern domestic dogs. Narratives about functional performance shape that are grounded in qualitative correlation form the basis for the physical standards that dogs are bred to meet. Which begs the question: Are these inferred form-function relationships consistent for given tasks and do they have real, measurable ramifications?

As a first step to tease this apart we have collected 40 landmarks from over 100 3D model canid skulls for use in a geometric morphometrics study. First, we compare how the task-based clustering of human breeding standards compares to k-means clustering of the skulls within PCA space. We also compare this to the clustering of an internal landmarking scheme devised to capture the shape of the internal nasal passages to assess if internal anatomy may be underlying perceived form-function associations. We ultimately find task-based clustering to be inconsistent and that it does not align well with the groups produced by clustering analyses. This reinforces the need to first quantitatively inferred form-function relationships using models before extrapolating even further.

Choose your research adventure: hypothesis cascades for aquatic biomechanics

Nicholas Hebdon, David Peterman, YunJi Choi, Mikelia Heberer, Garrett Butler, Jay Merril, Kathleen Ritterbush

Discussions of the form-function relationships in aquatic swimming animals often involve the nebulous concept of whether a change in form is improving or hindering swimming. But close interrogation shows swimming actually encompasses a host of different performance parameters. To tease apart the actual mechanics at play in a system requires stepping through hypothesis cascades that build towards a specific performance concept. These cascades require conscious choice at each level of the models input and outputs and how subsequent hypotheses build that understanding more deeply.

As an example of such hypothesis cascades in practice and how they can provide powerful explanatory frameworks we present computational fluid dynamics simulations, coupled to open-water experiments on life-size specimen analogues (propelled and inert). We test overall geometry and nuanced shape additions. We test size, velocity, and jet strategy. We test performance in terms of speed, efficiency, and maneuverability. Overall, we learn that supposed first-order limits on swimming potential are subject to radical intervention from presumed-minor anatomical or behavioral features.

Polar Body Derived Chimeras in *Patiria miniata*

Elise Hebert, George von-Dassow

In animals, female meiosis involves highly an asymmetric division, yielding one haploid egg and two small polar bodies (PBs). The PBs are cells, with functional centrioles and maternal chromosomes, but lack enough cytoplasm

to divide. However, if artificially enlarged with cytoplasm from the egg, the second PB can divide multiple times. We wanted to know if such activated polar body nuclei could go on to participate in embryogenesis, and whether they were restricted in capability or fate. By temporarily suppressing cytokinesis in oocytes of the sea star *Patiria miniata*, we forced them to retain their second polar body nuclei until first mitosis, at which point the retained nuclei cleaved into a third, smaller cell. These cells, here called PB2 clones, joined the larva, creating chimeric individuals with maternal clone tissue localized at the anterior left side. Such chimeras represent a violation of the one-cell bottleneck that is among the most widespread features of multicellular life histories.

Cosmopolitan to Complex: Species Delineation of *Octopus vulgaris* in South Florida

Colleen Hecker, Chelsea Bennice, Michelle Cavallo, W. Brooks

In the preceding two decades, western Atlantic octopuses underwent considerable taxonomic revisions. Long considered a cosmopolitan species, *Octopus vulgaris* is now recognized as a consortium called the *Octopus vulgaris* Species Complex (OVSC). While the hard-to-discern, cryptic OVSC members are closely related, evolutionary divergence, morphological discrepancies, and implausible gene flow between populations lend veracity to the thesis that the recently reinstated cryptic species, *Octopus americanus*, should supplant two OVSC taxa in the West Atlantic. However, *O. americanus* remains a dubious classification for *O. vulgaris*-like specimens in Florida, USA, which have not undergone morphological and molecular analyses. This study proposes such analyses for 5 specimens sampled from South Florida's Lake Worth Lagoon and 19 voucher specimens from Florida and the Caribbean to investigate this suggested name change. A comparative analysis of standard, octopus-specific morphological counts, measurements, and indices will be conducted. Mitochondrial genes 16s and COI will be used for identification and phylogenetic tree construction. These mitochondrial barcodes can yield discordant results among OVSC taxa; therefore, additional phylogenetic analyses of understudied nuclear genes (EF-1 α and Rhodopsin) as well as full genome sequencing will be performed in investigating the proposed name change. Preliminary morphological results indicate that this Floridian *O. vulgaris*-like species may represent the description of *O. americanus*. Ongoing genetic analyses will present definitive evidence. Accurate species identification is necessary to address octopus biodiversity and fisheries conservation.

The Effects of Age on Exploratory Behavior in Endler's Guppies (*Poecilia wingei*)

Sarah Heckmann, Kelly Diamond

Exploratory behavior can increase the probability of survival in animals, especially in complex habitats, by increasing an individual's spatial awareness of the environment. This spatial awareness is especially important for small animals, like Endler's guppies (*Poecilia wingei*), because they can use spatial information to avoid predators, increasing their odds of survival in the wild. Many animals change their behavior and activity patterns throughout their ontogeny due to changes in their anatomical and physiological phenotypes. The goal of this study was to examine how exploratory behaviors vary by habitat complexity and by age in a species of freshwater fish. We used machine learning (DeepLabCut) to track the position of 12 adult and 12 juvenile fish in both complex and open artificial habitats and compared movement behaviors between treatments. Preliminary results suggest that juveniles move longer distances but occupy a smaller area, compared to adults, regardless of habitat complexity. This indicates that adults are more exploratory, but juveniles are more active. However, adults were more exploratory in the open control environment, indicating that they could be more timid in a complex environment. This heightened exploratory behavior could suggest that adults may be better suited to survive in the wild but only in open environments because they are more spatially aware and better prepared to avoid predators.

City Living: Does Urbanization Increase Stress in Eastern Musk Turtles?

Brandon Hedrick, Florence Wen, Oliver Ljustina

Urbanization commonly leads to declines in species diversity and abundance due to a variety of stressors including habitat loss, environmental pollutants, road mortality, and inadequate nutrition. As urban centers increase in size, the effects of urbanization on natural habitats commensurately increase. Although the impacts of urbanization on vertebrates have been widely studied, this research has largely centered on birds and mammals with herpetofauna being comparatively unstudied, especially turtles. While many turtle species occur in urbanized areas, negative impacts on individuals and populations may be obscured by their long lifespans. We assessed stress in urban and natural populations of Eastern Musk Turtle (*Sternotherus odoratus*) in Louisiana and hypothesized that urban individuals would exhibit greater signs of stress and decreased body condition compared to those in natural areas. To measure stress levels, we examined body condition, heterophil/lymphocyte ratios, and fluctuating asymmetry of the carapace and plastron. We found that individuals from the urban population were significantly larger than the natural population, but that urban turtles had significantly higher heterophil/lymphocyte ratios and increased shell asymmetry relative to the natural population, suggesting both greater chronic and developmental stress. These results suggest that environmental factors other than lack of foraging opportunity act as stressors in urban turtles and that even populations which appear to persist in urban areas may be at increased risk of further decline or extirpation.

Biomechanics of insect flight stability and maneuverability

Tyson Hedrick, Haithem Taha

The small body size and minimal inertia of insects make them highly sensitive to external environmental perturbations such as gusts and endogenous perturbations such as slight asymmetries in neuromechanical pathways or wing shape. These underlying sensitivities are clearly displayed in the extraordinary in-flight maneuverability of many insect species. However, despite these challenges to flight stability, insects readily navigate their aerial environment leading to long term interest in the biomechanical, sensory, and neurobiological systems that enable their performance. Here we present a review of past and present research on insect flight stability and maneuverability, including theoretical, simulation, and experimental results. We particularly focus on possible biomechanical and fluid dynamic sources of passive stability, ranging from aeroelastic deformation to vibrational stabilization and added mass while also considering prospects for further experimental work in both animals and physical models.

Epigenetic variation of periphery and core populations of Prairie lizard (*Sceloporus consobrinus*)

Tiffany Hegdahl, Travis Robbins, Aaron Schrey

The prairie lizard (*Sceloporus consobrinus*) is a widespread species that can be found throughout the southern Great Plains of North America. The northern periphery of the species range can differ greatly from the central areas in biotic and abiotic factors, with shifts in both environmental means and variance. Populations near their adaptive limits often exhibit greater phenotypic variance generally prescribed to plasticity. Recent studies have suggested that this greater phenotypic variance may be associated with greater evolutionary potential when facing environmental threats and that epigenetic mechanisms may underlie the phenotypic variance. Indeed, differential gene expression through epigenetic mechanisms, such as methylation, can offer species ways of adapting to their environment more quickly than evolution. The objective of this study was to characterize phenotypic and epigenetic variation among three populations of prairie lizards, two within the core range and one on the periphery. We examined phenotypic variation in body size and utilized epiRADSeq to screen for DNA methylation variation ($n=43$). The peripheral population was found to have greater phenotypic variation. We expect this population to exhibit greater epigenetic variation, as well. This expected increase in DNA methylation variation could be contributing to the observed

phenotypic diversity through a difference in gene expression. These epigenetic mechanisms could play an important role in ensuring the survival of the species as climate change continues to change ecosystems.

Machine learning approaches delimit cryptic taxa in a previously intractable species complex

Haley Heine, Shahan Derkarabetian, Rina Morisawa, Phoebe Fu, Nathaniel Moyes, Sarah Boyer

Cryptic species are not diagnosable via morphological criteria, but can be delimited through analysis of DNA sequence data. A number of methods have been developed for identifying species based on genetic data; however, these methods are prone to over-splitting taxa with extreme population structure, such as dispersal-limited organisms. Machine learning methodologies have the potential to overcome this challenge. Here, we apply both unsupervised and supervised machine learning approaches to species delimitation, using a large dataset generated through hybrid target enrichment of ultra-conserved elements (UCEs). Our study taxon is the Aoraki denticulata species complex, a lineage of extremely low-dispersal arachnids endemic to the South Island of Aotearoa New Zealand. Previous attempts to delimit cryptic species within this complex used smaller, Sanger-generated datasets and conventional multispecies coalescent and distance-based delimited methods which yielded an unrealistic and extreme putative cryptic species count. Results from machine learning approaches, on the other hand, identify cryptic species with geographic ranges that are similar to those seen in other morphologically diagnosable mite harvesters in Aotearoa New Zealand's South Island. We conclude that applying machine learning approaches to the analysis of UCE-derived genetic data is an effective method for delimiting species in complexes of dispersal-limited cryptic species, and that the incorporation of training data from biologically relevant analogues through supervised machine learning can be critically informative.

The PROTEUS Underwater Saturation Habitat: A case for an International Space Station of the Ocean

Brian Helmuth, Fabien Cousteau, Mark Patterson, Angela Jones

Our entry into the International Decade of Ocean Science for Sustainable Development highlights the need for novel ocean research approaches. Perhaps even more importantly, it also emphasizes a critical need for new ways of reigniting the public's passion for the ocean and for marine science. With a team of fellow aquanauts, scientists and other professionals, explorer and filmmaker Fabien Cousteau is building PROTEUS™, a modular underwater saturation diving facility that includes multiple cutting-edge scientific laboratories and a broadcast studio. PROTEUS will be situated adjacent to an accreting coral reef within a marine protected area in Curaçao. The new habitat will foster a broad array of research and public engagement approaches, with a focus on using cutting edge science and engineering techniques to address critical challenges facing society. These will include (but are not limited to) coral reef health and maintenance of biodiversity; sustainable food production; human health and well-being; and robotics and technological innovation. The facility will also provide unfettered access to deeper mesophotic and rariphotic zone habitats by robots, submersibles and technical divers. PROTEUS will host multiple saturation teams with the ability to lock in and out of the habitat at different times, and will promote collaborations among academic researchers and educators with industry, government and non-profit organizations across the planet.

Human Exceptionalism hinders Socio-Ecological Systems Perspective in Environmental Science Education

Brian Helmuth, John Coley, Alegra Germain, Daria Healey, Jessica Holstein, Benjamin Dittbrenner

Students enter educational settings with complex and well-established conceptual understandings of the world around them. These powerful intuitive frameworks— arising through an interaction of cognitive structures, personal experience, culture, and evolution— provide fast and efficient but often fallible guidelines for dealing with complexity, and influence how students

learn. Although the interplay of intuitive conceptual frameworks and science learning has been investigated in several STEM disciplines, very little work has been done in Environmental Science. A “social-ecological systems” (SES) approach is the current state-of-the-art in how environmental scientists understand humans’ relations with the environment, and emphasizes that humans live as part of nature. This SES framework contrasts sharply with human exceptionalism, a widespread intuitive conceptual framework in western culture that involves beliefs that human societies exist independently of the ecosystems in which they are embedded, thereby promoting a sharp ontological boundary between humans and the rest of the natural world. Using surveys distributed to students in three undergraduate classes, we found that when asked to define “nature” over half of the students explicitly excluded humans from their definition. This tendency was, surprisingly, highest among environmental science students. Moreover, responses containing explicit human exceptionalism were much less likely to reflect a systems perspective than those which did not exclude humans, demonstrating that this cognitive construal is antithetical to a social-ecological systems understanding of human-nature interactions.

Developmental origins of adaptation to high elevation in the deer mouse placenta

Megan Hemmerlein, Kathryn Wilsterman

Adaptation to facilitate successful reproduction is vital in a new ecological environment. At high elevation, pervasive hypoxia adversely impacts fetal birth weight, which is directly linked to post-natal survival and thus fitness. Our lab recently demonstrated that elevation-adapted highland deer mice (*Peromyscus maniculatus*) can mitigate these negative outcomes through adaptations in placental development and function. These adaptations include substantial changes in placental gene expression under hypoxia during late pregnancy. However, comparison to early pregnancy is necessary to understand the developmental origins of these late-gestation transcriptional landscapes. Therefore, we used RNA sequencing to capture placental transcriptomes at both early and late gestation from highland-adapted and lowland-derived deer mice gestating under either normobaric normoxia or hypobaric hypoxia. I will investigate patterns of differential gene expression and alternative splicing to gain insight into how different molecular processes underlie adaptive phenotypes. These analyses will help link genetic mechanisms to the evolution of reproductive traits and expand our understanding of how placental physiology adapts to high elevation across mammals.

A Method for Testing Odor Valence in Bumblebees (*Bombus impatiens*)

Paige Henderson, Jordanna Sprayberry

Bumblebees are essential pollinators who use odor cues to find resources. Odor valence, the degree to which an odor is appetitive or aversive, has been peripherally observed but not concretely tested in this taxon. Here we present a forced-choice proboscis extension reflex (PER) paradigm for testing odor valence. The free-moving proboscis extension reflex (FMPER) has been used in many studies to study associative learning and generalization behavior in bumblebees. In this protocol, we remove the learning trials to test for innate odor-preference. These data are used to select stimuli for companion foraging arena experiments, which test preference in freely foraging bumblebees. Elucidating the structural boundaries of appetitive and aversive odors is likely a critical step in understanding bumblebee responses to odor pollution. Given the proliferation of research indicating negative impacts of odor pollution on pollinator foraging behavior in the past decade, this research could have broad conservation applicability.

Examining Genotype and Developmental Phenotype from CRISPR/Cas9 Editing in Annual Killifish

Isabel Henkes, Keria Moritsugu-Vandehey, Yekaterina Chmykh, Amie Romney, Jason Podrabsky

Genome editing to knock out gene function using CRISPR/Cas9 has shown continued success in model organisms such as zebrafish and the turquoise killifish. However, this technology has not been confirmed using the annual

killifish, *Austrofundulus limnaeus*. Using Cas9 protein and guide RNAs tailored to the tyrosinase genetic sequence - a pathway encoding for melanin pigment production - we examined if CRISPR/Cas9 could effectively target and edit this sequence in this species. To verify successful editing, we used DNA extracted from Cas9-injected embryos and analyzed the tyrosinase gene through Sanger sequencing and with alignment to the *A. limnaeus* genome. This knockout process produced the expected phenotype with little to no black pigment visible in injected embryos. We hope to generate a stable homozygous line for this knockout to maintain a mutant fish that lack black pigment and thus be useful for visualization of internal structures during development and larval life.

Chemical analysis of extrafloral nectar in western Australian *Solanum tudununggae* (Solanaceae) to ex

Kaitlin Henry, Melody Sain, Chris Martine

Solanum tudununggae Symon (Solanaceae) is a narrowly-endemic dioecious bush tomato species of the remote Kimberley Region of Western Australia. One uncommon trait that sets it apart from most other species of *Solanum* is its secretion of extrafloral nectar. In fact, structurally complex extrafloral nectaries (EFNs) are currently known to occur on the back of the corollas in only three species of Australian dioecious *Solanum*. Consequently, little is known about the characteristics of its nectar - although botanists have noted abundant ant activity around these nectaries. However, the relative lack of research on *Solanum* EFNs leaves a gap in knowledge regarding the nature of the relationship between these plants and ants. Previous literature has shown that the composition of extrafloral nectar, specifically the presence of essential nutritional amino acids, can cause ant populations to prefer certain nectars to others in other species of plants. This ex situ project uses high-performance liquid chromatography (HPLC) to analyze the composition of extrafloral nectar, specifically the presence of amino acids and sugars. Through HPLC, we hope to infer a possible correspondence between ant behavior and nectar content. We hypothesize that the presence of essential amino acids could suggest a mutualistic relationship between *S. tudununggae* and local ants, with the plant providing essential nutrients to the ant population and, in return, the ants offering protection against herbivores.

Urchin Delicacies: A Dietary Preference Study of Green Sea Urchins, Bull Kelp, and Acid Weed

Lucy Hensley, Katie Dobkowski

Understanding the future of kelp ecosystems, energetic relationships between grazers and seaweed, and marine communities is vital as population dynamics change from climate change. We performed feeding preference experiments with green sea urchins (*Strongylocentrotus droebachiensis*) to analyze how grazing might affect current and future kelp forest species composition. We compared urchin preference between *Nereocystis luetkeana*, a common food species for local herbivores, *Desmarestia* (strap-like morphology), a weedy species observed in similar subtidal habitats as *N. luetkeana*, and temperature-stressed *Desmarestia*. No-choice experiments revealed that *S. droebachiensis* will eat *Desmarestia* when it is the only available food source, suggesting future *Desmarestia* population control if *N. luetkeana* populations decrease. However, choice experiments demonstrated that urchins will choose *N. luetkeana* over *Desmarestia* when both are present. This suggests that *S. droebachiensis* will continue to consume *N. luetkeana* instead of providing population control to the weedy *Desmarestia* competing with bull kelp, even as kelp counts decrease in the Salish Sea. As ocean temperatures continue to rise, *Desmarestia* may become more palatable to *S. droebachiensis* due to acid release and tissue breakdown under temperature stress, and therefore preference for *Desmarestia* may change, possibly exerting a greater level of top-down control on the populations. Changes in trophic interactions, species composition, and abundance in the Salish Sea and other temperate subtidal kelp forests could possibly be affected by urchin dietary preference.

Avian maternal hormone transfer is mediated by limited food in urban environments

Jennifer Heppner, Jenny Ouyang

Urbanization alters organismal endocrine responses and parental investment; however, the specific ecological pressures driving these modifications remain relatively unknown. For instance, insectivorous urban birds are challenged with limited food, leading to reduced fitness across life stages. Specifically, urban house wren (*Troglodytes aedon*) eggs and fledglings have reduced mass, potentially mediated by hormones, such as elevated corticosterone. Since the endocrine system is sensitive to environmental cues, the transfer of maternal hormones may be a key mechanism integrating environmental information and manipulating offspring phenotypes. Therefore, to test the effects of limited food on maternal investment in urban birds, we supplemented breeding house wrens with live mealworms across an urbanization gradient differing in prey abundance for 3 years. We measured egg and yolk mass (532 eggs, 223 yolks) and extracted yolk hormones (corticosterone, testosterone, and thyroxine) using methanol and solid phase extraction to measure yolk hormone levels using enzyme immunoassays. We found urban eggs and yolks were smaller, yet food supplementation increased yolk mass similar to non-urban eggs. Additionally, urban yolks had higher corticosterone and testosterone concentrations, with levels reduced under food treatment, matching natural eggs. Our results show that urban mothers have different investment strategies with higher levels of steroid hormones maternally transferred, highlighting a possible mechanism shaping urban offspring phenotypes. Ultimately, we found that food limitation could be the cause of hormonal phenotypic differences in urban birds.

Mediterranean pollinators display limited acclimation capacity for heat tolerance

Natalie Herbison, Victor Gonzalez, Gabriela Robles-Pérez, Thomas Tscheulin, Theodora Petanidou, John Hranitz, Trisha Panganiban, Laura Haefner

Understanding the ability of pollinators to cope with extreme temperature changes is crucial for predicting their resilience to climate change. Herein, we measured short-term acclimation ability of the Critical Thermal Maximum (CTMax) in foragers of six bee species from the Greek island of Lesvos, which differ in body size, nesting habit, and level of sociality. We acclimated bees for 48 h in the dark at one of two temperature treatments, “average” and “warming”. We used 27 °C as the average temperature and a 5 °C increase from the “average” temperature as the “warming” treatment, which is within the range of natural variation recorded at the location sites. We used a dynamic protocol (ramping temperature) to measure bees’ CTMax. We found that acclimation did not significantly influence bees’ average CTMax, which differed among species and was significantly associated with body size across all species. We found no evidence for a trade-off between either average CTMax or body size and bees’ acclimation ability. Our results indicate that bees might have limited capacity to enhance their CTMax via acclimation, rendering them physiologically sensitive to rapid temperature changes during extreme weather events.

DNA methylation dynamics after long-term temperature acclimation in *Nematostella vectensis*

Alexandra Hernandez, Laura Baldassarre, Sebastian Fraune, Adam Reitzel

To acclimate to shifting environments, individuals must modulate their phenotypic responses to match their surroundings. DNA methylation, a chemical modification where methyl groups are added to nucleotides, has been hypothesized to alter gene expression in response to the environment, assisting in acclimatization. However, reports on the correlation between DNA methylation and gene expression have varied depending on exposure time and types of environmental stressors individuals have been challenged with. For our study, we aimed to investigate the long-term impacts of temperature variation on DNA methylation and gene expression using the sea anemone *Nematostella vectensis*. We acclimated clonal lines of *Nematostella* to low, medium, and high temperatures for a period of 76 weeks and analyzed differences in methylation and gene expression. We identified over 50 differentially methylated regions between animals acclimated to different temperatures.

Genes that were differentially methylated included those involved in redox reactions and enzymes that catalyze methylation. Our results showed that differences in methylation and gene expression were not correlated. Further, half of the differentially methylated regions were repetitive elements, corroborating previous findings that DNA methylation plays a role in protecting the genome from instability. This study provides an important point of comparison for future studies assessing DNA methylation and gene expression responses to environmental stressors under short- and long-term acclimation.

Bioinspired spines on an insect-scale robot facilitate locomotion on diverse terrains

Alyssa Hernandez, Perrin Schiebel, Robert Wood

To navigate complex environments, insects use diverse tarsal structures (adhesive pads, claws, spines) to reliably attach to and locomote across surfaces. This includes environments with terrains of variable roughness and inclination, which often require reliable transitions from ambulatory to scansorial locomotion. To evaluate how diverse tarsal spines may facilitate locomotion by increasing shear force generation, we used bioinspired physical models as a means for comparative research. For spine design, we considered a primarily terrestrial insect family known as ground beetles (Family Carabidae). Taking inspiration from certain species (*Carabus* and *Calosoma*), which have rows of rigid spines along their entire tarsus, we tested the relationship between shear force and spine aspect ratio (length over base width) and angle. We systematically evaluated these parameters using spines cut from stainless steel shim attached to a small acrylic sled loaded with various weights. The sled was placed on a 3D-printed model of natural terrain, randomly generated using fractal Brownian motion, while a motorized pulley system applied force to the spines. A force sensor measured the reaction force on the terrain, recording peak shear force before failure occurred. Using this data, promising spine arrangements were placed on the legs of our insect-scale ambulatory robot (HAMR). This allowed us to evaluate how the design of passive, interlocking contacts may enhance or diminish robot walking performance on various rough and inclined terrains.

Investigating Mutual Interactions in Coral-Seaweed Cocultures

Caleb Hernandez, Loretta Roberson

Coral reefs face significant threats from bleaching and climate change, underscoring the urgent need for research focused on bolstering their resilience. Notably, red seaweeds hold promise in enhancing coral health and mitigating stressors like nutrient excess and acidification. This research project investigates the impact of co-cultivation on the growth and health of specific corals, *Astrangia poculata* and *Acropora yongei*, in conjunction with red seaweeds *Eucheuma isiforme*, and *Gracilaria mammillaris*. Growth rates of both red seaweeds and corals were quantified by weekly weight measurements, while quantitative assessments reveal pronounced growth of *Gracilaria mammillaris* in association with *Astrangia poculata*, reciprocally driving *Astrangia poculata*’s calcification. Ongoing monitoring of pH, dissolved oxygen, and nitrate levels ensure stability and photosynthetic efficiency is assessed using pulse amplitude modulation (PAM) measurements. This study aims to uncover synergies in coral-seaweed dynamics, potentially influencing sustainable coral farming practices. The insights gained are significant not only for co-culture systems but also for a broader understanding of coral biology and ecology. The research strives to strengthen coral reef ecosystems by introducing innovative approaches to enhance coral health and productivity. Ultimately, this project elucidates interactions between corals and red seaweeds in co-culture systems, thus informing strategies for coral reef conservation and management.

Functional development of sensory and motor circuits for cardiac control in zebrafish

Luis Hernandez-Nunez, Joana Avrami, Florian Engert

Autonomic control of cardiac function is essential for survival, yet the functional diversity of the autonomic sensory and motor circuits of the heart remains

poorly understood. Here we take a multidisciplinary approach, combining systems neuroscience techniques, genetics, and control theory to study the role of autonomic sensory and motor circuits in larval zebrafish. While larval zebrafish's optic and genetic accessibility has made it a popular choice for studying how the brain processes environmental cues to modulate behavior, it has not yet been used to study organ control or the autonomic nervous system (ANS) from a systems neuroscience perspective. Thus, we use calcium imaging, optogenetics, pharmacology, and electron microscopy to map the developmental time course of anatomical and functional innervation of the heart. We identify the emergence of parasympathetic and sympathetic control of the heart, as well as the anatomically defined neural populations needed for heart modulation. We also show the onset of cardiac sensing and cardiac state feedback to the brain. Our study provides a timeline of developmental landmarks of the autonomic circuits for heart control and sets the stage for future mechanistic studies of neurocardiac circuits.

Characterization of the nervous system in larvae of *Osteopilus dominicensis* (Anura: Hylidae)

Isabela Hernandez-Rodriguez, Carlos Rodriguez, Martha Munoz, Marcell Pacheco

During ontogeny in Anura, the caudal structure is not destined to perform any function in the adult organism; therefore, it is lost through the metamorphosis process. Due to this, understanding the caudal innervations can provide insight into novel ontogenetic factors. This study presents the results from the identification and analysis of the peripheral innervations that emanate from the spinal cord during the different stages of larval development in *Osteopilus dominicensis*. 428 specimens were collected in seasonal lentic waters located in the Parque Mirador Sur (Dominican Republic). 149 individuals were classified based on their larval stages (Gosner, 1960). Morphological measurements were taken through different larval stages, to determine the development of the structural features in the tadpoles. The majority of the morphometric variables studied presented a high level of association, which demonstrated that most of the growing and reshaping of the individual are connected and (sometimes) dependent on each other. 94 individuals were selected for the observation of the peripheral nervous system. The innervations in the tadpole tail were quantified, using clearing and staining methods, to register the living variation in the number of nerve connections (ranging from 0 pairs in stage 44, to 16 pairs in stage 28). Additionally, this work leads us to discuss the adequacy of staining methods for the study of innervations.

What determines feeding and pollination efficiency in avian pollination interactions?

Amanda Hewes, Todd McWhorter, Alejandro Rico-Guevara

Animal pollination is a classic mutualism. Pollinators are attracted by floral rewards and in the process they facilitate plant reproduction by transferring pollen. Increased matching between floral morphology (e.g., corolla length) and pollinator morphology (e.g., length of a bird bill or butterfly proboscis) is thought to be beneficial for both parties, enhancing pollen transfer and increasing feeding efficiency. There is evidence that some avian pollinators, namely hummingbirds, tend to visit flowers that best match their bills; this suggests that morphological matching could influence the ecology and evolution of bird-plant relationships through pollinator foraging preferences, leading to bird-plant coevolution. A key gap in this story, however, is that we lack empirical information on whether increased morphological matching actually confers pollination and feeding benefits during real bird-plant interactions. We investigated this in Australian honeyeaters, the primary avian pollinators of Australian flora. Using manipulative field experiments, we examine feeding/pollination interactions between an endemic Australian shrub, *Eremophila maculata*, and its three primary honeyeater pollinators (*Acanthagenys rufogularis*, *Ptilotula ornata*, *Purnella albifrons*) at Gluepot Reserve in temperate South Australia. We found that these honeyeaters vary in their pollination and feeding efficiency on *E. maculata* flowers. Additionally, we found that the number of pollen grains deposited on the stigmas of *E. maculata* flowers declined as the morphological matching between the bill and flower decreased.

Tradeoffs between aerodynamic force production and sensing in insect wing and haltere morphology

Michelle Hickner, Urban Fasel, Tom Daniel, Michael Dickinson, Steven Brunton, Bingni Brunton

Insect wings serve both a sensory and aerodynamic purpose. While most flying insects have four wings, insects in the order Diptera have two wings and two halteres, which are derived from hindwings. Both hindwings and halteres sense body rotation due to deformation from gyroscopic forces. Hindwings generate aerodynamic force, but halteres are not large enough to generate meaningful aerodynamic force. We hypothesize that the divergence of hindwings and halteres has been driven by a fundamental tradeoff between strain sensing and force production in flapping wings. We investigated this question using a computational finite element model of stereotyped wing and haltere shapes with realistic flapping kinematics and body rotations. This modeling approach allowed us to determine how varying morphology across many wing-like and haltere-like shapes results in changes in strain sensing of body rotations, and which directions of strain are most useful for gyroscopic sensing. We show that wing-like shapes and haltere-like shapes with a bulb on a stalk may be good shapes for sensing body motions using shear strains.

Of Humans and Horseshoe Crabs

Eron Higgins, Aimee Mendoza

In this talk I will share observations and discuss my attempts to integrate innovative scientific research with effective science education, while avoiding the constraints of the grant-funded process. After completing my master's degree, the outlook for continuing on the path of a PhD and traditional professional science looked unappealing. The jobs for PhD's seemed to be overwhelming and stressful. I wanted to spend time with my children, and explore creative research topics, but being a professor at a major university did not realistically allow for either. Instead, I embarked on a career that would disprove the idea that significant scientific research had to be done by tenure track PhD's with big budgets. Over the last twenty years I've had the amazing opportunity to work with science learners at the middle school, high school, and undergraduate levels. I've been able to conduct research projects with science majors and non-majors, without the support of traditional funding. On our own, my students and I have made significant discoveries in horseshoe crab breeding behavior in the Tampa Bay Estuary, and in their learning and retention abilities through operant maze testing. It is my intention to share some of the results and wisdom of these experiences, which have culminated in my recent transition from being a science professor to being a Remotely Operating Scientist for Project Malu.

Recreating Movement Patterns of Flatback Sea Turtles (*Natator depressus*) Using Stable Isotope Analysis

Ricarda Hill, Calandra Turner-Tomaszewicz, Jeffrey Seminoff, Tony Tucker, Scott Whiting

Flatback sea turtles (*Natator depressus*) are a marine turtle species endemic to coastal Australia and Papua New Guinea. The data deficient status of flatbacks on the IUCN Red List complicates species management and necessitates further demographic and life history research. This project is part of larger, on-going research, and coupled carbon and nitrogen stable isotope (SI) ratio analysis with skeletochronology, the study of bone growth layers, to recreate movement and diet patterns over multiple years of a turtle's life. As part of the on-going research, the humerus bones were previously analyzed for age analysis (Turner Tomaszewicz et al. 2022 PlosOne); here, we sampled a subset of the bones from stranded turtles in Eastern Australia (n=13), Northern Territories (n=8), and Western Australia (n=16) using a micromill to extract 150 samples for stable isotope (SI) analysis. Turtle body size ranged from 11.3 to 96.0 cm curved carapace length (CCL); the estimated age of the turtles was between 0 and 48 years. Samples were sent for SI mass spectrometry analysis that produced: percent carbon, percent nitrogen, and the isotopic ratios of $^{12}\text{C}:^{13}\text{C}$ ($\delta^{13}\text{C}$) and $^{14}\text{N}:^{15}\text{N}$ ($\delta^{15}\text{N}$) for each growth layer. Carbon isotope values ($\delta^{13}\text{C}$) can help to indicate potential shifts between coastal and pelagic habitats while nitrogen isotope values ($\delta^{15}\text{N}$) can show changes in trophic level. The initial results show that

individual adult turtles are consistent in their foraging and habitat use patterns; but also that turtles in the same region may have different foraging strategies. These SI techniques, with previously published skeletochronology age and size data, are beginning to reveal spatiotemporal patterns of flatback sea turtle habitat use, thus informing species management and protection.

Embryonic development in *Ixodes scapularis*

Isaac Hinne, Hailee Ciccotti, Ben Faustino, Carolina Alonso, Michael Pham, Monika Gulia-Nuss

Despite their relevance as vectors of the most important vector-borne disease in the United States, much remains to be known about the biology of the black-legged tick, *Ixodes scapularis*. For instance, there is no existing literature on the embryonic development of this species. The recent development of tick embryo injection protocol and CRISPR/Cas9-mediated gene editing has expanded the molecular toolbox to study *I. scapularis* and provided new avenues for studying tick biology. However, to create heritable mutations, it is essential to understand the early embryonic events of tick development, particularly the formation and specification of primordial germ cells (PGCs). We have developed an embryonic staging system that identifies morphogenetic processes and developmental events in *I. scapularis*. (Stage 0 to 14). Our data shows BMP activity and suggests cumulus cell migration, which is vital in establishing the embryonic body axes. We are tracking the formation and specification of PGCs using *vasa*, *nanos*, and *piwi* genes. This would inform us of the timeframe and injection location for successful germline transformation, enabling the development of transgenic ticks and thereby further expanding the molecular toolbox to study tick biology.

Duvernoy's gland and associated structures in rear-fanged snakes (Lamprophiidae and Colubridae)

Kayla Hinnen, Ollie Safford, Megan Vandenberg, Michelle Shafer, Kate Jackson

Duvernoy's gland, a post-orbital oral gland of rear-fanged (aglyph and opisthoglyph) colubroid snakes, is the homolog of the venom gland of front fanged (proteroglyph and solenoglyph) snakes. Venom-delivery systems of rear-fanged snakes remain understudied relative to those of more medically relevant front-fanged species. Moreover, much of the existing literature on the morphology of rear-fanged venom-delivery systems predates current understanding of the phylogenetic relationships among rear-fanged colubroid lineages. Here, we use DiceCT (Diffusible Iodine-based Contrast-Enhanced Computed Tomography) techniques to visualize the cranial anatomy of rear-fanged snakes of the families Colubridae (sensu stricto) and Lamprophiidae, two lineages separated by 55 million years of evolution, with emphasis on the cephalic glands and associated structures.

Body size and hormonal responses to temperature changes in the tobacco hornworm, *Manduca sexta*.

Hannah Hirsch, Laura Park, Yuichiro Suzuki

Warming climates have had a profound impact on many different species. A common response seen in ectotherms is an increased growth rate with a smaller final body size, which is often associated with reduced fitness. The tobacco hornworm, *Manduca sexta*, exhibits such a response to temperature, but the cause of this trend is largely unknown. Infection and immune response, oxygen availability, larval density, gut microbiota have all been suggested as possible explanations, but in this study, we tested the hypothesis that hormones may play an important role in mediating this effect. We focused on the insulin/target-of-rapamycin signaling pathway, juvenile hormone (JH) and its response genes, and ecdysteroid titers. We found that higher temperatures result in elevated expression of Insulin receptor (InR) and genes coding for juvenile hormone (JH) degradation enzymes, such as juvenile hormone esterase (JHE). These findings suggest that hormones respond to temperature to impact growth rates and final body size.

Some Stars Like it Hot: Early life stage climate resilience in the endangered sunflower sea star

Jason Hodin, Fleur Anteau, Michael Brito, Fiona Curliss, Augustin Kalytiak-Davis, Chloe Schwab, Vanessa Valdez, James Peng

Starting in 2013-14 all known populations of the sunflower star (*Pycnopodia helianthoides*) suffered from an unprecedented outbreak of seastar wasting (SSW). This top predator, once common from Alaska to México, is now nearly extinct in the southern half of its range and rare from the Salish Sea northwards. Consequently, sunflower stars have been recommended for a 'Threatened' listing under the US Endangered Species Act. In 2019, at the University of Washington's Friday Harbor Laboratories, we began the first ever captive breeding program for sunflower stars. We aim to understand their ontogeny, behavior and ecology through all life-history stages, in order to inform conservation efforts including possible future wild reintroductions. A particularly vital concern is determining the impacts of climate warming on the recovery likelihood of the species in the wild. Here, we report on temperature optimum experiments with sunflower star embryos, larvae and early juveniles, and on potential carry-over effects of larval rearing temperature on juvenile temperature tolerance. Our findings indicate that optimum larval and juvenile temperatures for Salish Sea sunflower stars are well above mean summertime temperatures in the area. These results imply both that sunflower stars are likely to show resilience to future climate warming, and also that individuals derived from Salish Sea broodstock may be suitable for introduction to more southern waters.

Including low-quality individuals reveals a physiological basis of cost of reproduction in starlings

Brett Hodinka, Tony Williams

Parental care is assumed to require sustained, high-intensity activity sufficient to generate 'costs of reproduction' and these are assumed to have a physiological basis. Most studies have sampled birds late in chick-rearing under the assumption that physiological state during intense workload is predictive of subsequent reproductive success and fitness—but these studies only sample 'successful' birds, and these may be the very individuals that can 'work hard' while avoiding physiological dysregulation. To test this idea, we blood sampled free-living female European starlings on hatch day to include individuals which were subsequently successful and unsuccessful. We assessed hatch day body mass of adult females, current breeding success and productivity (e.g., number and size of fledglings), and future fecundity (e.g., productivity of second broods) and measured three physiological traits, including measures of oxygen-carrying capacity (hematocrit and hemoglobin) and total antioxidant capacity (OXY). We found that in a 'good' year (2022), hematocrit and hemoglobin were significantly lower for second broods (46%), when overall productivity is lower, compared to first broods (52%). Additionally, in a 'poor' year (2023), mean hematocrit, but not hemoglobin, was significantly lower for successful first broods (46%) relative to 2022 while second brood females that failed had hematocrit values indicative of significant anemia (~43%). Preliminary results therefore suggest that hematocrit measured early in chick-rearing is predictive of subsequent reproductive success.

Linking Form and Function in the Weberian Apparatus of *Noturus* (Siluriformes: Ictaluridae) using Dyn

Jennifer Hoeflich, Juan Liu

Our previous study revealed considerable diversity among the Weberian ossicles (hearing apparatus) of *Noturus* catfishes (Siluriformes: Ictaluridae). This study investigates whether morphological variation corresponds to functional variation using dynamic finite element analysis. We built finite element models of the Weberian ossicles using ANSYS software with realistic geometry from X-ray-based computed tomography of eight *Noturus* species (data processed using Avizo and GeomagicWrap). Harmonic analyses predicted peak frequency at the scaphium (in Hz): *Noturus maydeni* 542, *N. leptacanthus* 715, *N. furiosus* 766, *N. gilberti* 850, *N. nocturnus* 1534, *N. stigmosus* 2409, *N. miurus* 3409, and *N. munitus* 6368. The four catfish with the peak frequencies >1000 Hz possess less ossified claustra, smaller body size, and negative PC1 values corresponding to decreased concavity and increased height of

the concha of scaphium (principal component 1, from geometric morphometric PCA of the scaphium of 20 *Noturus* species). Although these high frequency peaks await validation, the models also showed a local optima between 300 and 1600 Hz. This overlaps with peak frequencies of the other models and behaviorally-tested best hearing range of *Ameiurus nebulosus*, another ictalurid. The maximum power output corresponding to sound intensity broadly ranged from -3.6 and -40 dB, which may relate to different swimbladder amplification abilities. Our results show that morphological variations of Weberian ossicles result in functional variability with the specific mechanisms to be further investigated.

More than gene editing: Using CRISPR-Cas12a for rapid, field-based, molecular organism detection.

Brandon Hoenig, Jakub Zegar, Michel Ohmer, Macie Chess, Brady Porter, Myah Madril, Corinne Richards-Zawacki

The field of ecology has undergone a molecular revolution, allowing researchers to probe the wonders of the natural world with a level of precision not possible with more traditional approaches. Unfortunately, as these approaches often require expensive equipment and specialized training, the sequence-level sensitivity of molecular methods has been largely out of reach for a great number of individuals – particularly those from underfunded research programs, field research teams, and community science endeavors. It is for these reasons that we have developed a rapid, inexpensive, and field-deployable approach for molecular organism detection to be used by any ecologist, regardless of their prior training. This technique – termed FINDeM (Field-deployable Isothermal Nucleotide-based Detection Method) – uses a centrifuge-free, ambient temperature extraction protocol alongside a body-heat inducible DNA amplification method to extract and amplify target DNA in under 30 minutes. The product of this amplification reaction is then used as the template for another 30-minute, body-heat inducible CRISPR-based reaction, which relies on the ssDNA degradation properties of Cas12a and ssDNA molecules that fluoresce upon degradation to indicate the presence of target DNA molecules. Here, we validate FINDeM through the detection of *Batrachochytrium dendrobatidis* – the causative agent of amphibian chytridiomycosis – and demonstrate that FINDeM can be used by researchers with limited training to detect subclinical zoospore concentrations with little more than a pipette, a modified salad spinner, and the human arm pit.

Plasticity in response to temperature magnitude and fluctuation—from coloration to thermal tolerance

Austin Hoffman, Zach Stahlschmidt

Warming associated with climate change has many direct and downstream effects on organisms. For example, warmer developmental temperatures can improve heat tolerance and reduce insect melanization, which is involved in coloration, thermoregulation, immunity, desiccation resistance, and potentially life-history traits, such as investment into reproduction and dispersal. Increased temperature variability is also a feature of climate change, and it may have a larger impact on animals than gradual warming. However, we have limited insight into the independent and interactive effects of temperature magnitude and variability on important traits and trait-trait interactions, such as tradeoffs. Thus, we used a factorial manipulation of temperature magnitude (mean: 20, 25, and 30°C) and daily temperature fluctuation (+/- 0, 5, and 10°C) during development in the variable field cricket (*Gryllus lineaticeps*) because it exhibits a wing dimorphism mediating a tradeoff between investment into reproduction and dispersal (flight) during early adulthood. We used linear model and path analyses to determine the roles of temperature magnitude and fluctuation in: (1) the plasticity of important traits associated with life history, coloration, and temperature tolerance, and (2) shaping trait-trait relationships (e.g., whether warmer temperatures promote heat tolerance directly, or indirectly via changes in coloration). Our factorial approach will allow us to compare the relative impacts and interactive effects of two increasingly common features of climate change—warmer and more thermally variable environments.

Sublethal effects of perfluorooctanesulfonate (PFOS) exposure on the immune systems of watersnakes

Elizabeth Hoffman, David Lee Haskins, Matthew Hamilton, Devin Jones, Melissa Lech, Abigail Valachovic, Youn Choi, Linda Lee, Jason Hoverman, Kristina Meichner

Per- and polyfluoroalkyl substances (PFAS) are a group of chemicals used in numerous consumer and industrial products, such as non-stick cookware and firefighting foams, whose persistence and accumulation in humans and wildlife has led to increasing concerns worldwide. However, our understanding of adverse effects is largely unknown in most wildlife, especially herpetofauna. While most research with wildlife has focused on acute toxicity trials with model species, sublethal exposure studies with environmentally relevant concentrations in non-model species are needed to understand the potential ecological impacts of PFAS. In this study, we exposed 66 juvenile Northern watersnakes (*Nerodia sipedon*) to one of three PFOS treatments: control (n=22), low PFOS (200 ppb; n=22), and high PFOS (2000 ppb; n=22). Watersnakes were exposed to PFOS through diet (PFOS-spiked fathead minnows), and treatment concentrations were based on PFOS concentrations reported in comparable prey items sampled from PFAS-contaminated sites. To evaluate hematologic and immune responses of watersnakes to PFOS, we assessed differences in packed cell volume, total solids, bacterial killing and hemagglutination assays, and white blood cell differentials. Our findings will form a basis for understanding how PFAS may impact reptile health.

Comparing sea turtle size to nesting parameters, clutch size and hatch success on the Gulf of Mexico

Arionne Holden, Jacob Lasala

Sea turtle morphological size is largely determined by foraging quality. Sea turtle clutch size is often linked to maternal size. However, less is known about how female and clutch size affect nest chamber parameters and the eventual hatch and emergence success of the resulting hatchlings. This study aims to identify whether loggerhead (*Caretta caretta*) and green sea turtle (*Chelonia mydas*) size has changed over time and whether that has impacted nest shape parameters and resulting hatch and emergence success. The study was prefaced by an analysis of growth patterns over time which revealed a sharp decline in loggerhead size in the last 20 years and a gradual incline in green turtle size. Several models were run to analyze 40 years of data recorded in Sarasota County, Florida, a growing sea turtle rookery in the Gulf of Mexico. These models reveal that loggerheads' cavity shape and clutch size together have a significant effect on both hatch and emergence success. Over time, clutch size has also increased in relation to turtle size, which is consistent with previous studies. This information could contribute to further research on how specific nest parameters and egg quantity encourage hatch and emergence success rates.

Determinants of, and constraints to, muscle performance in vertebrates and invertebrates

Natalie Holt, David Labonte, Nihav Dhawale

Skeletal muscle exhibits huge functional diversity despite a relatively common structure and mechanism of contraction. Overlapping contractile protein filaments, actin and myosin, are organized into sarcomeres, and force is generated by cyclical crossbridge interactions between them. This gives rise to the isometric force-length relationship where force is maximal at an intermediate length corresponding to maximal overlap, and declines at shorter and longer lengths. In vertebrates some of the observed functional diversity is achieved by varying muscle gross structure. Increased cross-sectional area increases crossbridges in parallel and so force, while increased muscle length increases crossbridge in series and so displacement capacity and velocity. Hence, for a given volume of muscle, force and displacement trade-off resulting in a largely constant work density. In addition to gross structure, invertebrates also vary muscle ultrastructure. Increased contractile protein length increases parallel crossbridges and so force, while decreased contractile protein length increases serial crossbridges and so velocity. However, the effect of contractile protein length on muscle displacement capacity, or force-length curve width, is unclear. We constructed a simple, 1D, sliding-filament model

of muscle and used this to predict sarcomeric and muscle force-length relationships for varying contractile protein lengths. Increasing contractile protein length increased predicted peak force and sarcomeric force-length curve width, but did not affect predicted muscle force-length curve width. Hence, it increased predicted work density. This finding highlights important differences in determinants of, and constraints to, performance across vertebrate and invertebrate muscle.

Biomechanics of prey capture in fish: exceptions to the suction feeding paradigm

Roi Holzman

Fish feed on a vast diversity of prey types, including phytoplankton and zooplankton, fish and vertebrates, hard- and soft-shelled prey, and algae. Yet, the functional mechanisms that support this trophic diversity are unclear. Moreover, virtually all fish rely on generating a flow of water into their mouth to capture and transport their prey, and this mechanism is highly conserved across species. In this talk I will review the key mechanisms that permit trophic diversity under strong functional constraints, focusing on the role of functional innovation and biomechanics trade-offs.

Life history, condition dependency, and energetic performance

Wendy Hood

There has been much interest in identifying the mechanisms responsible for the negative tradeoff between reproductive output and lifespan. With this presentation, we will synthesize findings from several studies conducted in the Hood lab on the bioenergetic underpinnings of intraspecific variation in the life history patterns of the house mouse (*Mus musculus*). These studies consider the impact of reproduction on oxidative stress, mitochondrial performance, mitochondrial DNA (mtDNA) mutation load, and the impact of environmental conditions on similar bioenergetic variables. Data will emphasize the effects of reproduction and the environment on the liver, skeletal muscle, and brain. Our results suggest individual variation in bioenergetic capacity appears to be positively correlated with a female's reproductive fitness and negatively correlated with mutation load, suggesting that this relationship is condition-dependent. Still, reproduction appears to have little negative impact on the bioenergetic variables thought to determine lifespan. We show exogenous stressors can have positive and negative effects on bioenergetic capacity. Our findings suggest environment and individual body condition are more important than lifetime reproductive output in determining the longevity of house mice.

Bioenergetic adaptations to migration in White-crown Sparrows

Wendy Hood, Emma Rhodes, Paulo Mesquita, Kang Nian Yap, Andreas Kavazis, Hailey Parry, Geoffrey Hill

Long-distance migration is among the most fascinating and energetically demanding animal behaviors. Work by several lab groups has shown that migrating birds transport more oxygen and nutritional substrates to their cells than birds that are not migrating. Within the cell, oxygen and energy substrates are targeted to mitochondria, where the fuel for powered flight—ATP—is generated. We hypothesized that the mitochondria of migratory birds also display adaptations that enhance their ability to maintain a high level of ATP production for extended periods during migration. Comparing migratory and nonmigratory subspecies of White-crowned Sparrows (*Zonotrichia leucophrys gambelii* and *Z. l. nuttalli*, respectively), we found that maximum mitochondrial respiration (state 3) was high before the onset of migration, and then appeared to increase further during migration, approximately doubling baseline, regardless of respiratory substrate (complex I: pyruvate, malate, and glutamate, and palmityl carnitine; complex II: succinate). Enzymatic activities of the mitochondrial complexes did not follow a similar pattern, suggesting that change in complex performance may play a limited role in the up-regulation of mitochondrial respiration for migration. In contrast, levels of mitochondrial fusion markers mitofusin 2 and optic atrophy-1 and mitochondrial fission markers

mitochondrial fission 1 and dynamin-related protein 1 were up-regulated in patterns that mirrored changes in mitochondrial respiration, suggesting mitochondrial dynamics may play a key role in the change in mitochondrial respiratory performance that occurs with migration.

Reshaping the past: geological deformation in *Diictodon* using 3D geometric morphometrics

William Hooker, Andrew Orkney, Brandon Hedrick

A major goal in comparative morphology is to understand the relationship between structure and function in biological taxa. Fossils, however, are taphonomically deformed, obscuring original biological shape (OBS). Therefore, systematic quantitative studies are needed to explore the influence of taphonomy on paleontological analyses and possibility of perceiving OBS. Shape can be decomposed into symmetrical and asymmetrical components, which can be further split into biological and taphonomic components. A large intraspecific sample of crania of *Diictodon*, a basal therapsid from the Late Permian Karoo Basin of South Africa, are used for investigating the effects of taphonomic compression on fossils using 3D geometric morphometrics. We classify crania into five taphomorphotypes – bilateral, dorsoventral, rostrocaudal, shear (left and right), and saddle-backed – which form distinct clusters in morphospace. We then evaluate the magnitudes of asymmetry for each taphomorphotype to determine how different taphomorphotypes may obscure OBS. Further, we examine the individual contributions of taphomorphotype to intraspecific asymmetry and find that individual taphomorphotype magnitudes are highest in the sheared crania, but that the dorsoventrally and bilaterally compressed crania have higher magnitudes of symmetric compression. Given that taphonomic deformation is large within our intraspecific sample, we will next examine how different taphomorphotypes impact biological trends. These data grant a novel view on addressing the problem of the influence of taphonomy on conducting effective morphological analyses on fossils.

Modeling the mechanospace of larvacean pumping and swimming

Alexander Hoover, Kakani Katija, Joost Daniels, Janna Nawroth

Far from the surface, the ocean's midwater presents a rich frontier of biodiversity that is not well understood. Part of this gap in our knowledge is the great expense involved in collecting data with remotely operated vehicles. In this presentation, we will discuss the pipeline of developing in-silico computational experiments in concert with in-situ experimental data. Using a combination of particle image velocimetry data, optical scans, and confocal microscopy, we will discuss the creation of fluid-structure interaction models for organismal pumping and fluid transport, with the goal of developing an intuition on the physical mechanisms that drive their success. Using a combination of simplified geometries and scanned body meshes, we will employ the immersed boundary/finite element (IB/FE) method to simulate the swimming and chambered, valveless pumping mechanism of a giant larvacean. All motion described in this model will emerge from the interaction of active muscular tension, passive elastic recoil, and the local fluid environment.

Functional heterodonty of pricklebacks (*Stichaeidae*) across diet types

Richard Hoover, Olivia Hawkins, Joseph Heras, Cassandra Ford, Karly Cohen, Cassandra Donatelli

Pricklebacks (*Stichaeidae*) are elongate fishes from intertidal and nearshore North Pacific waters. In several species there is a major dietary shift over ontogeny from carnivory in juveniles to omnivory or herbivory as adults. These dietary differences have been established through gut content analyses, changes in digestive tract length, enzymatic functions, microbiome, and transcriptomes. This wealth of dietary information makes this group valuable for connecting nutritional physiology to functional feeding morphology. Most vertebrates capture and process food with teeth, with a huge diversity of tooth form and function reflecting the diversity of diet types across fishes. The teeth needed for eating plants vary considerably from the demands of shearing

prey and so we hypothesize that tooth shape and dental battery function may reflect dietary evolution in this group. In other words, the distribution of stresses and forces across the teeth should reflect the different challenges presented by different prey. We examined the oral jaws in four pricklebacks ($n=15$). Using micro-CT scans, we segmented the dental battery and calculated a functional homodonty metric that measures the stress and leverage of each tooth based on its position in the jaw. All pricklebacks have functionally heterodont dentitions, with interspecific differences in tooth number, morphology, function, and high intraspecific variation.

Differences in skeletal muscle between desert pupfish populations from unique thermal habitats

Jordan Hopkins, Teresa Guerre, Sean Lema, Kristin Hardy

Geographically discrete populations of desert pupfishes often differ in morphological and physiological traits as a consequence of being confined to isolated aquatic habitats with distinct - often extreme or highly variable - temperature regimes. For example, Amargosa pupfish (*Cyprinodon nevadensis amargosae*) from a geothermally-heated bore spring outflow near Tecopa, California, exhibit a complete or partial loss of paired pelvic fins in ~34% of fish, which contrasts with conspecifics from the cooler, faster flowing Amargosa River, where >99% of fish have both pelvic fins. Activity levels of the anaerobic enzyme lactate dehydrogenase (LDH) are also higher in the skeletal muscle of Tecopa Bore (TB) pupfish compared to the Amargosa River (AR) pupfish. Herein, we investigated whether there are other differences in skeletal muscle properties between these isolated pupfish populations which could ultimately influence swimming performance and tolerance for surviving extreme flash flood events. To this end, we used stereological techniques to quantify fiber cross-sectional area/diameter and oxidative capacity of red and white skeletal muscle from succinic dehydrogenase-stained tissue sections of wild TB and AR pupfish. In the TB pupfish, skeletal muscle fibers of both types were significantly smaller, and oxidative capacity in the white muscle (though not the red muscle) was significantly lower. These observed differences are consistent with TB pupfish adapting to a slower flowing, warmer, therefore less well-oxygenated, habitat than AR pupfish.

Scaling Upstream Barriers: Evaluating Eel Ladder Efficiency for Dam Crossing

Amanda Hornung, Andrea Ward

American eels (*Anguilla rostrata*) have a unique life cycle with migration from marine to freshwater environments occurring during the early life stages and the reverse migration in later life stages. Migratory American eels face barriers posed by dams and rivers, necessitating interventions for safe passage. This study focuses on understanding how eels use eel ladders, a type of fish passage specifically for eels, as well as monitoring eel movement across a recently installed fish passage in Rockville Center, New York. To monitor the fish passage, eel mops were deployed at entry and exit points, with daily collections and photographic documentation to test how size differed between the upstream and downstream collections. Further, we investigated ladder efficacy using three ladder types: traditional pegboard, straw pegboard, and nylon mesh. While existing research emphasizes the positive impact of ladders on larger eels, the study extends this understanding by exploring ladder utilization patterns among smaller eels. Our study indicates that there's a difference in performance between the different eel ladder types. These findings provide valuable insights into ladder preferences and performances for both eel size categories. The significance of this research lies in the potential to develop more effective eel ladders. By shedding light on ladder suitability across diverse eel sizes, this study contributes to refining dam passage designs and informs conservation strategies for this ecologically important migratory species.

Navigating the breeze: unraveling insect odor plume tracking strategies in dynamic windscape

Jaleesa Houle, Floris van-Breugel

Odor plume tracking is an important ecological process that many species rely on to localize food sources and potential mates. Though the breadth of knowledge surrounding these behaviors has expanded over the past several decades through various wind tunnel studies, our understanding of how these tasks are executed in the great outdoors remains limited. To address this question, we first tackled another: what is the wind experience for insects tracking odor plumes in natural environments? Our recent exploration of near-surface microscale wind variability across sage steppe, forest and urban environments indicates a strong correlation between turbulent intensity and wind direction variability. However, most prior odor plume experiments in wind tunnels have been unable to investigate insect behaviors in directionally variable wind conditions due to physical constraints. To better mimic the real-world conditions that flying insects might encounter, we added auxiliary fans to our wind tunnel to create unilateral crosswinds. Next, we leveraged optogenetic tools in freely flying fruit flies to remotely control their olfactory experience with high spatiotemporal precision while they navigated the crosswind. Through flow field visualization techniques, we can estimate the wind experience during the fly's odor experience, allowing us to examine the behavioral strategies flies use to navigate a virtual odor plume in a spatiotemporally variable wind field. Our findings suggest that being blown off course may in fact help flies efficiently estimate wind direction.

Disentangling relationships between diet and gut microbial diversity in kestrel nestlings

Jennifer Houtz, Mercy Melo, Jean-Francois Therrien, Allison Cornell

Gut microbiota are increasingly recognized as important drivers of host health and fitness across vertebrate taxa. Given that gut microbial composition is directly influenced by the environment, gut microbiota may also serve as an eco-physiological mechanism connecting host ecology, such as diet, and physiology. Although gut microbiota have been well-studied in mammalian systems, little is known about how gut microbial diversity and composition impact morphological and physiological development in wild birds. Here, we characterized both diet and gut microbial diversity of free-living American kestrel (*Falco sparverius*) nestlings throughout development to test whether gut microbial diversity predicts host morphological and physiological traits in either contemporary or time-lagged manners. Gut microbial alpha diversity on day 21 of nestling development was positively correlated with diet alpha diversity representative of the majority of nestling development (days 5–20). Gut microbial alpha diversity early in development was negatively correlated with body mass in both contemporary and time-lagged manners. Gut microbial alpha diversity early in development was positively correlated with blood glucose later in development. As nestlings experience rapid growth demands in preparation to fledge, these time-lagged associations may indicate that gut microbial diversity at early critical developmental windows may determine the future trajectory of morphological and physiological traits underlying metabolism that ultimately impact fitness.

Morphological evolution across bulbous monocots

Cody Howard

Underground storage organs are found across many plant lineages: the corms of crocus, the stem tubers of potatoes, and the bulbs of onions. In addition to being the place for belowground bud placement, these organs are often swollen to store nutrients that can be used during periods of resource scarcity, regrowth after disturbance, and emergence at the start of the growing season. Therefore, the size of an underground storage organ may play a significant role in determining the ecological conditions in which a plant can grow, which can then influence their evolution. To investigate the relationship between underground storage organ size and other important plant traits, we collected available morphological data using taxonomic monographs for three major bulbous geophytic lineages, each of which evolved independently of one another. Additionally, to examine how the environment has influenced evolution within these lineages, we downloaded available occurrence data

for each taxon in order to obtain the associated climate data. Collectively, these data, when examined within a phylogenetic context, will provide us with a better understanding of how the environment has selected for these unique plant morphologies. Furthermore, we can then investigate whether similar morphological and ecological patterns are found across the different bulbous lineages.

Investigating extraocular photoreception in the skin of summer flounder (*Paralichthys dentatus*)

Maureen Howard, Lorian Schweikert

Vision is an important sensory system for some animals, facilitating predation, mate selection, and predator avoidance processes. While vision is best understood to occur using eyes, recent studies have shown that photoreceptor proteins, such as opsins, are present in the skin of certain animals, including those capable of dynamic color change. One such animal is the summer flounder (*Paralichthys dentatus*), but the function of opsins in flounder skin and the role they might play in dynamic color change remain poorly understood. Examining summer flounder (N=6), we set out to determine 1) where opsins are expressed in the structure of skin and 2) how expression differs over the dorsal (i.e., color-changing) and ventral surfaces of the body. Using anti-opsin immunohistochemistry, we labeled three opsin classes previously identified in summer flounder skin (i.e., RH1, SWS1, and LWS) and imaged their expression patterns using confocal microscopy. We then used these images to determine the location of opsin across skin layers, the co-expression of opsins within and between cell types, and any differences that exist between the dorsal and ventral skin surfaces. This study provides new insights into the sensory physiology of dynamic color change and may improve our broader understanding of visual systems that exist outside the eye.

Recombination rate and gene density are correlated with clines of diversity and differentiation

Jack Hruska, Joseph Manthey, Garth Spellman, John Klicka

Spatial patterns of genetic diversity and population differentiation are the result of extrinsic and intrinsic factors to which organisms are subject. Extrinsic factors include population genetic factors like gene flow, natural selection, and genetic drift. Intrinsic factors are inherent to genomes and include properties like recombination rate, mutation rate, chromosome size, and the density of gene content. To evaluate how these intrinsic and extrinsic factors interact to produce empirical patterns of genetic diversity and differentiation we leverage a whole-genome population re-sequencing dataset of 53 individuals of the Grace's Warbler (*Setophaga graciae*). The Grace's Warbler has a latitudinally broad distribution, extending from southern Nevada to northern Nicaragua. Specifically, we explored how latitudinal clines of diversity manifest in this species – across genomic, chromosomal, and sub-chromosomal scales. In addition, we explored heterogeneity in patterns of differentiation across the genome. Lastly, we evaluated how the strength and direction of these clines and patterns of differentiation are influenced by gene content and recombination rate. Leveraging the fact that recombination rate and gene density can both serve as proxies for the diversity-reducing effects of natural selection, we show that clines of genetic diversity that match the genome-wide pattern are more likely to be found in regions of the genome that are subject to high recombination and/or have lower gene content. This suggests that clines matching the genome-wide pattern are more likely to reflect 'neutral' processes, such as differences in population demography across individuals and populations. We find a negative correlation between recombination rate and FST and a positive correlation between recombination rate and dXY, suggesting that differentiation between populations in this species is in part mediated by selective forces.

Changing foot shape and function during locomotion on sand

S. Tonia Hsieh

Foot morphology, posture, and material properties can dramatically affect substrate interaction dynamics and locomotor performance. Among the

sand specialist zebra-tailed lizards (*Callisaurus draconoides*), elongate toes and their associated long tendons store large amounts of elastic energy, decreasing the cost of running across hard surfaces. Running across a softer surface such as sand is particularly challenging because granular materials fluidize and solidify in response to force application. On these surfaces, a prior study showed that the feet appear to function more as paddles and stroke through the medium. Our experimental results from high-speed fluoroscopy of running lizards, robot testing, and discrete element method models reveal that foot use and substrate interaction dynamics shift throughout the course of a step. During the initial impact and intrusion, the foot fluidizes the sand and intrudes deeply into the volume. Simulations show grain-grain dynamics generating overlapping regions of force chains slowing material flow between toes that functionally creates "virtual webbing", increasing vertical support and forward propulsive forces. Lizards then plant their toes and pivot over a largely fixed point to propel themselves forward—much as do sea turtles when moving on sand. Implications for inclined running and sand running performance among other lizard species will also be discussed.

Calcium Flux in Early Shell Formation of *Biomphalaria glabrata* from Trochophore to Veliger Stages

Chi Huang, Wenjun Yi, Jian Sheng, Wei Xu

Calcium waves and oscillation during embryonic development are key elements in the intricate process of molluscan shell formation. However, understanding calcium dynamics in the early embryonic shell formation in gastropod development is still insufficient. The present study explores the role of calcium flux in early shell formation within the embryo of gastropod *Biomphalaria glabrata*. We hypothesized that the role of calcium is not only in providing a critical element for shell formation but also in serving as a signaling molecule for the genetic regulation of calcification. The calcium flux was visualized using the Fura-2 and Fluo-4 calcium indicators through the trochophore (72 hours) and veliger (120 hours) stages of *B. glabrata* development. The dynamics of calcium signals were correlated to the rapid transition from motile trochophore to veliger, marked by cilia-mediated movement and premature shell and foot development. According to our observation, the intracellular calcium signals were attenuated from 72 to 120 hours of embryo development. The expression profiles of genes encoding calmodulin and related protein kinase following the calcium flux in embryos suggested a critical role of the calcium-binding proteins in the early shell development of gastropods. Although the embryonic calcium dynamics and the related signaling pathway of shell formation are under further observation and analysis, the role of calcium in the signaling pathway of shell formation has been demonstrated by this preliminary study.

Computational models of risk-aware bipedalism

Christian Hubicki, Jacob Hackett, Tianze Wang, Jason White, Monica Daley

Bipedal animals must balance many sources of risk to survive in natural environments with threats ranging from injuries to starvation to predation. This work details a continuing effort to build a computational model of bipedal behavior in a manner which links the locomotor dynamics at the biomechanics scale to the range of demands animals face at the ecological scale. Using trajectory optimization as a core component, this computational approach models all bipedal decisions from speed, to footstep placement, to ground reaction force patterns, as phenomena that emerge from minimizing apparent probabilities of failure in a given terrain environment.

This presentation shows the strengths and weaknesses of current computational instantiations of the model. Current results show that restricting the foot contact pattern to walking or running results in realistic gait patterns in nominal scenarios, but limits the creativity of extremely agile maneuvers (e.g. avoidance or escape patterns). Alternatively, allowing the foot contact pattern to freely deviate produces biologically plausible hopping results and agile escape maneuvers (e.g. kangaroo rats), but does not yet produce walking behaviors. As preliminary evidence of the physical plausibility of the modeled behaviors, the authors demonstrate a robotic implementation of candidate models on a human-scale bipedal robot.

Multiple New Pilargidae (Annelida: Polychaeta) Species

Sonja Huč, Greg Rouse

Pilargidae Saint-Joseph, 1899 (Annelida: Phyllodocida) is a small family of polychaete worms with only 12 genera and 104 accepted species. Based on phylogenetic results, we have added *Antonbruunia* Hartman & Boss, 1966, and removed *Hermundura* Müller, 1858 (17 accepted species) from the clade. We have contributed to the known biodiversity of pilargids with seven new species descriptions: one new species each of *Antonbruunia*, *Otopsis* Ditlevsen, 1917, and *Ancistrosyllis* McIntosh, 1878 from California, USA, and four new *Sigambra* Müller, 1858 species from Costa Rica, Croatia, and China. Additionally, the first complete mitogenome sequences for members of *Antonbruunia* and *Otopsis* enabled a revised classification within Pilargidae. Therefore, Synelminae Salazar-Vallejo, 1987 has been emended to reflect both the newly classified and newly included or excluded genera.

Mud Crab Metabolism, Feeding, and Survival Under Increased Temperature and Acidification

David Hudson, Lars Vikstrom, Alyson Lowell, Leah Reidenbach, Bradley Peterson

Mud crabs, *Dyspanopeus sayi*, help maintain community structure through top-down control on plant ecosystems by controlling grazer abundance. The combination of OA and warming could affect the foraging rate and behavior of this mud crab commonly found in seagrass meadows, oyster beds, and salt marshes. To address this question, we exposed individual *D. sayi* to low pH and +3°C increase in temperature to stimulate a future climate change scenario. Each treatment was fully crossed with two levels of temperature (26°C and 29°C) and two levels of pH (7.3 and 7.8), with animals exposed for 28 days. Mortality was quantified over the 28 days. Following this exposure period, metabolic demand was measured by respirometry, and food consumption rates of the blue mussel, *Mytilus edulis*, were determined for each individual. The laboratory experiment was designed to determine if there were interacting effects of OA and warming on food consumption of a common mussel prey item, respiration, and survival. To that end, results indicated that the crabs with two lesser stressors (higher pH and lower temperature) survived the best, whereas those with the highest stress (lower pH and higher temperature) consumed the most mussels and had the highest mortality over the exposure period (56%). Given that this is a species present in areas of coastal acidification, the effect of temperature appeared to be the more impactful both to metabolic demand and to survival of this species. Mud crabs, *Dyspanopeus sayi*, help maintain community structure through top-down control on plant ecosystems by controlling grazer abundance. The combination of OA and warming could affect the foraging rate and behavior of this mud crab commonly found in seagrass meadows, oyster beds, and salt marshes. To address this question,

Thermal stress reduces photosynthate metabolism and disrupts nitrogen cycling in coral larvae

Ariana Huffmyer, Jill Ashey, Eric Chiles, Emma Strand, Xiaoyang Su, Hollie Putnam

Recruitment of reef-building corals is critical for reef persistence under climate change. Rising sea surface temperatures cause breakdown in the nutritional relationship between corals and algal endosymbionts (Symbiodiniaceae), but the effect of thermal stress on metabolic exchange (i.e., transfer of fixed carbon photosynthates from symbiont to host) during sensitive early life stages remains understudied. We exposed symbiotic *Montipora capitata* coral larvae in Hawai'i to elevated temperature (+2°C for 3 days) and used stable isotope metabolomic tracing (¹³C sodium bicarbonate) to track changes in metabolism under stress. We observed altered metabolic activity during stress including shifts in ammonia assimilation and reduced metabolism glucose - a key photosynthate. There was increased ammonia assimilation at elevated temperatures indicated by the production of glutamate through the glutamate dehydrogenase pathway. Incorporation of free ammonia into amino acids reduces nitrogen availability to symbionts. This may limit their population growth and maintain carbon translocation, and indeed, there was equal translocation of glucose in ambient and high-temperature exposed larvae. Despite this, downstream metabolism was compromised in exposed larvae,

indicated by a reduction in glycolysis. Survival, settlement, and tissue physiology, however, were not affected, suggesting that these shifts in metabolism are early indicators of thermal stress in larvae. Compromised photosynthate metabolism under elevated temperatures may place larvae in an energetic deficit during critical recruitment periods with negative consequences for reef maintenance and replenishment.

Characterization of Histone Modifications in Response to Anoxia in Killifish Cells

Chelsea Hughes, Jason Podrabsky, Dietmar Kueltz, Elizabeth Mojica

Embryos of the annual killifish *Austrofundulus limnaeus* have the greatest tolerance to anoxia of all vertebrates, making them ideal to study the cellular mechanisms necessary for anoxia tolerance. This tolerance is supported by the ability of embryos to arrest development in diapause as part of their normal development and to enter into a state of anoxia-induced quiescence even during active development. While key RNAs have been described, the regulatory mechanisms that control anoxic gene expression have not. Gene expression is regulated by a variety of mechanisms, including alteration of chromatin through histone modification. Preliminary data in WS 36 embryos (anoxia LT50 of 65 days) identified 626 peptides across fifteen distinct histone proteins, representative of H1, H2A, H2B, and H3 isoforms. Since whole embryo patterns of histone modifications are an aggregate of multiple cell types, we are repeating this work using WS40NE cells, a neuro-epithelial cell line isolated from embryonic *A. limnaeus* tissue explant that can survive anoxia for 49 days. Mass spectrometry based proteomics will be used to quantify histone modifications that occur in anoxic, anoxia-recovered, and normoxic WS40NE cells and compared to modifications in WS 36 embryos. Understanding patterns of histone modification in response to anoxia will provide insight into how cells can utilize histone posttranslational modifications to alter gene expression and support stress tolerance. This work is partly funded by NSF (2025832, 2209383).

Locomotor kinematics and morphology of climbing *Aneides* salamanders

Jonathan Huie, R. Pyron, Sandy Kawano

Many climbing salamanders (*Aneides* spp.) are arboreal and renowned for their ability to climb trees as high as 88 m – a dangerous feat for the ill-equipped – but others are strongly terrestrial. The ecological variation within *Aneides* provides an opportunity to identify how morphological and biomechanical traits vary with ecotype. We hypothesize that arboreal *Aneides* possess multiple adaptations for climbing. Arboreal species may use different limb kinematics to move on vertical structures. They may also have specialized limb morphologies that prevent slippage. We will compare the 3D kinematics of the arboreal *A. aeneus* with that of the terrestrial *A. hardii* walking on flat surfaces at 0, 45, and 90 degree inclines. We will also compare the body proportions of *Aneides* salamanders and closely related plethodontids. Our preliminary data indicate that *A. aeneus* have greater limb abduction associated with a crouched posture, reduced locomotor speed, increased duty factor, and only one foot moving at a time on vertical surfaces. We predict that if *A. hardii* can climb vertically, it will have similar kinematic patterns but with more exaggerated changes due to the morphological differences between terrestrial and arboreal salamanders. Arboreal *Aneides* have longer limbs, larger feet, and expanded toe tips that likely improve their cling performance. These data suggest that biomechanical and morphological traits have evolved together to confer impressive climbing abilities.

Ecological and Life History Drivers of Galloanserae Skull Evolution

Eloise Hunt, Ryan Felice, Joseph Tobias, Daniel Field, Stephan Lautenschlager, Anjali Goswami

Our research aims to elucidate the drivers of Galloanserae (ducks, chickens, and relatives) skull evolution. We created and analysed a comprehensive 3D dataset of skull morphology for >230 species, including key extinct lineages, to uncover the extent of morphological variation exhibited by Galloanserae

as well as quantifying the factors driving this skull shape variation. Extant Galloanserae only represent a fraction of the remarkable morphological and ecological diversity they attained over ~67 million years of evolution, spanning from pseudotoothed Pelagornis to the giant flightless Gastornis. We tested the hypothesis that extinct Galloanserae will exhibit distinct cranial forms unrepresented in extant taxa. Additionally, we isolated the relative influences of phylogeny, ecology, diet, habitat density, developmental mode, and migration on skull shape variation and evolutionary rates. Principal Component Analysis (PCA) revealed that extinct clades greatly expand the breadth of Galloanserae morphospace occupation beyond what could be inferred from extant taxa alone. Preliminary analyses show significant relationships between Galloanserae skull shape and ecology ($P = 0.039$), habitat density ($P = 0.001$), and migration ($P = 0.0016$). Preliminary rate analyses demonstrate heterogeneous rates of evolution for different character states within habitat density, migration, diet, and developmental mode. Our results indicate that ecology, habitat density, migration, diet, and developmental mode are key factors influencing the skull evolution of Galloanserae and highlight the importance of fossils to inform estimates of phenotypic disparity.

What normal used to be: Reproductive cycles of WWII-era blue and fin whales inferred from baleen

Kathleen Hunt, Allie Case, Piper Thacher, Nadia Gray, Trent Grasso, Janine Brown, Matthew Savoca, John Ososky, Michael McGowen, Malia Smith, Alyson Fleming

Research and management of modern populations of baleen whales is hampered by numerous data gaps. Normal reproduction baselines are particularly difficult to determine, given that modern populations may already be experiencing impacts from reduced population size, climate change, toxins, and other anthropogenic influences. In an effort to examine reproduction of past populations of Antarctic blue (*Balaenoptera musculus*) and fin (*Balaenoptera physalus*) whales, we assessed patterns of progesterone (females) and testosterone (males) in baleen from a historic Smithsonian archive collected in the 1940s. We subsampled baleen powder at 2 cm intervals along the full length of twenty specimens of baleen, each representing a continuous ~6-7 year (blues) or ~4-5 year (fins) endocrine history of an individual adult whale. Hormones were then extracted and assayed with enzyme immunoassays. Most baleen from females contained widely spaced regions of sustained high progesterone, interpreted as pregnancies. Apparent calving intervals ranged from 1-3 years, with some “interrupted” cycles that may represent failed pregnancies. In males, regularly spaced regions of high testosterone (putative annual breeding cycles) were present in some but not all individuals; the regularity of these cycles allowed further inferences regarding differing migratory strategy in the two species. Endocrine data derived from historic museum baleen specimens can offer critical context for interpreting patterns of reproduction observed in modern whale populations.

Treading Through Change: Robust bipedal gait in a dynamic landscape

Nathaniel Hunt, Seongwoo Mun

The Ontogeny of Visual Fields in Alligator mississippiensis

Tyler Hunt

Visual fields delimit the maximum area over which an organism can extract near-instantaneous optical information from their environment. Despite the myriad behaviors vision subserves in vertebrates, visual field morphologies exhibit remarkable inter-specific tuning in birds and mammals. Nevertheless, little is known regarding how this sensory modality varies throughout ontogeny; especially among long-lived reptiles who traverse large size ranges. One such organism, the American alligator (*Alligator mississippiensis*), undergoes an ~4000-fold increase in body mass, substantial shifts in diet, habit use, predation pressures, and cranio-dental morphology during development. Given these drastic ontogenetic shifts, we hypothesized that these animals might exhibit similar shifts in visual field morphology. To investigate this, we constructed a custom gimble device enabling measurement of the in vivo visual fields via the ophthalmoscopic reflex technique over the full-size range

exhibited by *A. mississippiensis*. The results show visual fields do indeed vary substantially over ontogeny. Adult alligator visual fields are comparatively restricted in the dorsal, posterior, and ventral regions due to infringement of the palpebrals, the expanded pterygoid muscles, and intrusion of the rostrum, respectively. Conversely, hatchling visual fields are completely unobstructed by the body. When the unobstructed regions are compared between hatchlings and adults, they are statistically indistinguishable, suggesting the cranial visual field configuration is maintained despite significant ontogenetic shifts in morphology and ecology.

The Energetic Cost of Stress in Developing Fishes: Quantifying allostatic load indices (ALIs).

Ione Hunt-von-Herbing

Stress can exert negative effects on fish health through stimulation of the hypothalamic-pituitary-interrenal (HPI) axis and autonomic nervous system (ANS) and adjustment and physiological adaptation are required to achieve or re-establish homeostatic stability (allostasis). The energetic cost of exposure to fluctuations or heightened neural or neuroendocrine responses resulting from stressful events is defined as allostatic load. While measuring the sources of stress and assessing their consequences have resulted in the derivation of allostatic load indices (ALIs), most of this work has been conducted in adults (mostly humans and mammals), and no ALIs have been estimated for developing stages in fishes considered more vulnerable to change than older stages. From a series of experiments on 2 species from different aquatic environments, 1) zebrafish (*Danio rerio*) (tropical, freshwater) and 2) Atlantic cod (*Gadus morhua*) (cold-temperate, marine), in which larvae were exposed to acute and chronic stressors (high-temperature and/or hypoxia) ALIs were quantified based on biomarkers such as ontogenetic processes, growth, and metabolic activities. Results showed that compared to acute stress, chronic stress imposes a significantly greater allostatic load because of prolonged energy demand in the face of limited resources (e.g. yolk and poor prey capture ability). Under severe chronic stress, energetic costs were sufficiently large that energy-limited developing fish may not be able to fully compensate, resulting in maladaptive responses from allostatic overload leading either to death or alternatively, as proposed in the stress entropic model (Hunt von Herbing, 2023), to novel evolutionary physiological states possibly more resilient to environmental change.

The role of phylogeny in the relationship between hamate morphology and dexterity of haplorhines

Laura Hunter, Zeresenay Alemseged

Primates exemplify a formidable range of hand dispositions associated with a diversity of locomotor behaviors, tool use, and manipulation. For this reason, a considerable body of literature has analyzed hand skeletal morphology with the aim of inferring the behaviors of extinct taxa. These studies have identified features associated with specific movements of the wrist, including ulnar deviation, grip strength, and force resistance. An element of particular interest is the hamate, which articulates to manual rays four and five and serves as an attachment point for the flexor carpi ulnaris and hypothenar muscles. Features of interest include hamulus size and orientation, triquetral facet orientation, and metacarpal articular surface complexity. The role of phylogeny in how these features vary remains unexamined, however, obfuscating whether morphological differences are due to selection or phylogenetic inertia. To explore these relationships, we quantified these features in 65 haplorhine species and applied a Bayesian method for inferring adaptive shifts across a phylogeny. When examined in a phylogenetic context, it appears that these features have undergone morphological changes at differing rates across clades, consistent with selection. This analysis indicates that the relationships between these features and certain hand dispositions are consistent with those hypothesized in previous studies but that, for many taxa, morphology is more likely a result of genetic drift than selection for that behavior.

Movement, Habitat Selection, and Survival of Largemouth Bass in the Restored Kissimmee River

Reid Hyle, Rachel Grey, Jason O'Connor, Brittany Bankovich, Charles Hanlon, Steve Bousquin, Sam McPherson, Arthur Bernhardt

Partial restoration of the Kissimmee River has improved water quality, but the fish community indicated dissolved oxygen impairment going into 2019. We used radio telemetry of Largemouth Bass (*Micropterus salmoides*) to track movement, habitat use, and survival within the restored river and test for emigration/immigration during 2020 and 2021. We implanted radio transmitters in 50 adult (≥ 980 g TW) Largemouth Bass in January 2020 and 2021 distributed across the restored river, Lake Kissimmee, and Pool A C-38 canal. These were monitored by passive stations and active tracking. Bass readily used off-channel areas during spawning season when stages provided access. However, floodplain use was limited to near-channel sites and was restricted by dense invasive plant growth. Stage duration appears to influence the quality of water and habitat for fish in the restoration area. The two years differed in the magnitude and timing of flows and subsequent water quality. The result was total annual mortality of bass in the river of 89% in 2020 and 32% in 2021. All fish either perished or vacated the restoration area in 2020 but many survived the shorter hypoxic event in 2021. We plan a follow-up study in 2024 that includes archival accelerometry dataloggers to track fine-scale behavioral changes in bass experiencing variable dissolved oxygen availability, and investigate potential energetic consequences of the behavioral response to oxygen stress.

Don't you know you're toxic? Metals and metalloids in a *Callorhynchus callorhynchus* specimen

Ingrid Hyrycena, Amanda Pontes-Lopes, Isabel Quental-Willmer, Tatiana Dillenburg-SaintPierre, Natascha Wosnick, Rachel Ann Hauser-Davis

The Plownose chimaera (*Callorhynchus callorhynchus*), also known as the American elephantfish, is one of eight holocephalan species identified in Brazilian waters, representing 14% of all known Chimaeriformes. Holocephalans are rarely encountered in Brazil; if caught incidentally, they often go unreported by fishers or are swiftly sold at reduced prices, sometimes as shark meat. Despite their infrequent catch and limited commercial value, it is vital to examine their health status and potential risks, including human consumption hazards. In this study, samples from various organs and tissues of a female *C. callorhynchus* specimen, caught as bycatch off southern Brazil, were analyzed for the presence of the four most toxic metal(loid)s—arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg)—via ICP-MS. High levels of As and Cd were detected across all organs, while Hg was detected in all organs except the Ampullae of Lorenzini jelly (AoLJ), fin, brain, and uterus. Lead was detected in all organs except AoLJ, bile, muscle, spleen, uterus, gonads, and kidneys. Notably, muscle concentrations surpassed permissible consumption limits for both As (12.68 mg kg⁻¹ wet weight) and Hg (0.12 mg kg⁻¹ wet weight). These preliminary findings underscore significant concerns regarding species contamination by these toxic elements and the potential risks associated with their consumption in Brazil. Further investigations are imperative to fully comprehend the extent of these risks and their implications.

Researchers and fishers: how can this partnership benefit the conservation of elasmobranchs?

Ingrid Hyrycena

Elasmobranchs (sharks, skates, and rays) are frequently captured, both intentionally and accidentally, by different fisheries around the world. The overexploitation impacts observed throughout the years on this group have required new approaches to ensure the conservation of these populations. Technological devices, aiming to mitigate the incidental capture of non-target or prohibited species, have already been implemented in Global North commercial fishing fleets, showcasing successful results. However, such methodologies and equipment are neither accessible nor applicable in small-scale artisanal fisheries operating in low-income countries of the Global South. A commonly used strategy to mitigate bycatch effects on charismatic megafauna, such as marine mammals and sea turtles, consists of releasing live animals after interacting with fishing gear. In 2020, during the COVID-19

pandemic, a monitoring and compensatory release program was established in partnership with artisanal fishers in southern Brazil. From May 2020 to August 2023, more than 1000 cownose rays (*Rhinoptera* spp.) and other threatened species accidentally caught by beach seine, such as spiny butterfly ray (*Gymnura altavela*), longnose stingray (*Hypanus guttatus*), Brazilian electric ray (*Narcine brasiliensis*), scalloped hammerhead shark (*Sphyrna lewini*), were released. Thus, compensatory release holds a cost-effective promise to counteract population declines of sharks and rays, but its success is contingent on various factors including appropriate release protocols, post-release monitoring, as well as engagement of local traditional communities, and collaboration of policymakers.

Experimental Validation of the MVUE Model on the Refuge Tracking Behavior of Weakly Electric Fishes

Fatmagul Ibisoglu, Semanur Yalcin, Ceren Ozdemir, Ozlem Turan, Ismail Uyanik

In this work, we evaluate the practical validity of the minimum variance unbiased estimator (MVUE) model on the multisensory integration of weakly electric fish. The MVUE model suggests that each sensory cue is reciprocally weighted based on their variances during multisensory integration. A majority of the literature focuses on experimental validation of the MVUE model in relation to sensory conflict. However, animals continuously experience changes in sensory salience, which alter the quality of perception for the same stimulus. In this work, we experimented with two uniquely-suited species of weakly electric fishes, *Apteronotus albifrons* (N=5) and *Eigenmannia virescens* (N=5), during their refuge tracking behavior. We conducted five repetitions with a sum-of-sines-type refuge stimulus under different sensory conditions (illumination, conductivity, refuge length and structure). We transformed the tracking response of the fish into probability distribution diagrams using histograms. In dark, fish generates tracking response based on the electrosensory information only, while in the light and dim-light experiments, fish uses both visual and electrosensory inputs. We use the MVUE model to back-calculate the probability distribution for the visual input only and cross-validate the result under different sensory conditions. Our results show that the MVUE model captures the changes in variance but not the tracking gain, probably due to their super-additive nature. Supported by TUBITAK (120E198).

The effects of melatonin on seasonal behaviors and sex steroid hormones in green anole lizards

Bernadette Igo, Taylor Grossen, Nicholas Shankey, Rachel Cohen

Reproduction in seasonally breeding animals should occur at the appropriate time of year to ensure reproductive success. Most animals use environmental cues (i.e., photoperiod, and temperature) to regulate internal mechanisms, such as melatonin production, that result in appropriate physiology and behavior. Melatonin secretion is increased with the shorter photoperiods that occur during winter and has been shown to decrease reproduction in a variety of seasonally breeding vertebrates, although less is known about this role in reptiles. We examined the idea that melatonin regulates reproduction in the seasonally breeding green anole lizard (*Anolis carolinensis*). Lizards were treated with either a melatonin or blank capsule at the beginning of the breeding season. After five or ten weeks, reproductive and aggressive behavior assays were conducted. Blood was collected from the lizards to measure T or E2 levels using ELISAs. We found that males treated with melatonin for 5 weeks exhibited significantly more total displays than the blank group ($F(2,26) = 3.58, p = 0.042$) and melatonin significantly increased T plasma levels among males ($t(22.33) = 3.61, p = 0.002$). In females, there was no significant effect of treatment on total display behavior ($H1 = 0.78, p = 0.377$) or plasma E2 levels ($t(7.62) = 1.69, p = 0.132$). Surprisingly, our results revealed that chronic melatonin treatment appears to increase some aspects of reproduction in this species, but the mechanism for this effect remains unclear.

Modeling hindlimb muscle activation in walking alligators across limb posture and body size

Masaya Iijima, Richard Blob, John Hutchinson

Geometric and dynamic similarity predict that larger animals should experience increased biomechanical demands during terrestrial locomotion. These demands are partially mitigated by modulating gait and limb posture in mammals and birds—a trend that is also evident in American alligators with non-parasagittal limb posture. However, it remains unclear how limb posture and body size influence limb muscle recruitment in alligators. To answer this question, we estimated activations of 36 hindlimb muscles during high walking in alligators, employing inverse kinematics, inverse dynamics, and static optimization applied to 3D musculoskeletal models. The analyses tracked joint coordinates and external forces measured in seven juvenile to subadult alligators (0.2–5.6 kg). For anti-gravity muscles, activation during stance phase was generally greater for knee extensors and ankle plantarflexors compared to hip adductors across the full range of body sizes compared. Notably, some knee extensors and ankle plantarflexors exhibited greater activation in larger alligators. However, a few hip adductors exhibited greater activation in smaller alligators, reflecting their greater hip joints than in larger alligators. Hence proximal vs. distal limb muscle activations on land; and their influences on locomotor performance; may scale (intraspecifically if not interspecifically) in opposite directions with body mass in less parasagittally locomoting tetrapods.

Biological springs are most energy efficient when loaded and unloaded at equal rates

Mark Ilton, Lucien Tsai, Paco Navarro, Siqi Wu, Taylor Levinson, Elizabeth Mendoza, M. Janneke Schwaner, Monica Daley, Manny Azizi

The rate of mechanical loading and unloading of biological springs can play an important role in determining how the properties of these springs affect movement. We investigate the mechanical energy efficiency of biological springs (American bullfrog plantaris tendons and guinea fowl lateral gastrocnemius tendons). We perform mechanical experiments under symmetric rates (with equal loading and unloading durations) and asymmetric rates (with unequal loading and unloading durations) using novel dynamic mechanical analysis measurements. We find that mechanical efficiency is highest at symmetric rates and significantly decreases with a larger degree of asymmetry. Performing the same measurements on synthetic elastomers, we find the same effect—mechanical energy efficiency decreases as a function of asymmetry between the loading and unloading rates. A viscoelastic model with no fitting parameters captures the experimental results based on independently-characterized properties of the materials. The model further shows that a broader viscoelastic relaxation spectrum enhances the effect of rate-asymmetry on efficiency.

Effect of α -pinene on ectoparasite resistance in tree swallows

Sila Inanoglu, Sarah Knutie, Sydney Horan, Lorraine Perez, Hannah Brewer

Many bird species incorporate volatile plant material, such as pine needles, into their nests, which can correlate with reduced nest ectoparasite survival. However, it is unclear whether the material directly affects parasite survival or indirectly affects parasite survival through the effects of the plant material on the host's immune system. For our study, we disentangled the direct and indirect effect of α -pinene (the volatile compound in pine needles) on tree swallow (*Tachycineta bicolor*) nestlings and their nest ectoparasite community. We experimentally treated nests with an α -pinene or control solution then identified and quantified nest ectoparasite taxa (blow flies [*Protocalliphora* spp.] and mites [*Dermanyssus* spp. and *Ornithonyssus* spp.]) and antibody response of nestlings. Blow fly abundances did not differ significantly between treatments. Pinene-treated nests had fewer mites, which resulted in nestlings with larger feathers, compared to control nests. Preliminary evidence suggests that laboratory mites treated directly with α -pinene had lower survival than control mites. The antibody response of nestlings did not differ between treatments, nor did it correlate with mite abundance. These results suggest that the

relationship between nest pine needles and parasite abundance is mediated by the direct effect of α -pinene on parasitic mite survival rather than an indirect effect through the host.

Maintaining oxygen uptake and movement to the flight muscle during migratory flight in songbirds

Catherine Ivy, Kevin Shoemaker, Christopher Guglielmo

Migratory flight is an intensive exercise that requires birds to maintain high aerobic capacities for many hours or days. Maintaining oxygen supply to flight muscles is therefore important during migration, especially since tracking studies have shown that songbirds will ascend to altitudes of 6,000 m during migratory flight where oxygen is less readily available (hypoxia). Whether there are adaptations or seasonal plasticity along the oxygen cascade and hypoxic chemoreflex that allow songbirds to fly at such high altitudes during the migratory season is unknown. Research during my postdoc has shown that migratory songbirds exhibit seasonal plasticity along the oxygen cascade which enhance oxygen uptake and movement to the flight muscle during migration. This includes changes in breathing pattern, haemoglobin-oxygen binding affinity, and muscle fiber size and phenotype. We have also shown that the magnitude of seasonal plasticity may be dictated by migratory distance and/or whether the songbird species is migratory or resident. Additionally, in yellow-rumped warblers there appears to be a blunted hypoxic chemoreflex in response to flight at high altitude (~3,000 m above sea level). Measurements of adrenaline concentration increased with flight, but were not significantly higher when flight was conducted at high altitude. Together, these findings show that songbirds exhibit seasonal plasticity along the oxygen cascade which would be important for maintaining oxygen movement to the flight muscle during high altitude flight.

Diel and vertical patterns opsin expression from zooplankton metatranscriptomes at Station ALOHA

Tom Iwanicki, Arianna Krinos, Erica Goetze, Harriet Alexander, Mireille Steck, Megan Porter

The oceanic zooplankton community is ecologically diverse and vital in forming a key link between trophic levels. Many of these interactions are structured by light. Here we use zooplankton metatranscriptomes, containing mRNA from hundreds of individuals and species, to estimate phototransduction gene diversity and expression to investigate the role of light in these interactions at the community level. Zooplankton metatranscriptomes were produced from a light and depth gradient across the epipelagic (0–200m) and mesopelagic (700–1000m) zones in the North Pacific Subtropical Gyre (Station ALOHA, 22.75N, 158W; Day & Night). Using the EUKulele pipeline and custom databases, we found arthropod, mostly copepod, euphausiid, decapod, amphipod, and ostracod mRNAs were dominant throughout the water column, with a marked increase in fish mRNA abundance in large body-size, deep samples. Phototransduction genes were detected using a custom dataset of 1,780 opsin genes and KEGG orthology. Visual gene expression varied by taxa, opsin subtype, zooplankton size fraction, and depth. The relative proportions of phyla differed between whole community and opsin mRNA samples, suggesting that patterns in focal genes may offer functional insights independent of community structure. We briefly discuss trends in key crustacean migrators, Pleuromamma copepods and euphausiid shrimp. Metatranscriptomes are an emerging tool for functional analyses of genes from whole zooplankton assemblages and can be used to analyze patterns at multiple levels of biological organization.

Aquatic ecology, education, and regulators: Seeking meaningful long-term monitoring collaborations

Erika Iyengar, Karen Tuerk

Project-based learning has maximum student impact when the students learn something new alongside collecting useful data that benefits the community. As educators, we strive to find projects that can be conducted any time of year, works with both classes and personal research, and are sustainable in terms of funding, time and long-term need. How do we find the prized project

that meets all of these criteria? We are at the early stages of two long-term collaborations. In one, we have partnered with the Allentown Stormwater Bureau (local government) to examine the health and hoped-for restoration of a small urban stream that is deemed by state agencies as impaired. Our second project has partnered with a local high school to monitor the suite of invertebrate diversity within a number of ponds across seasons and years. We will describe the groups engaged in each of these collaborations, the current and future investigations involved, assess the benefits, challenges, and hurdles of both of these collaborations and outline the long-term intended goals (educational, scientific, and legislative). There is also an associated poster also at this conference that presents some of the data results to date from the stream collaboration.

Geographic Variation Patterns in Cranial Shape and Pelage Color of the least chipmunk (*T. minimus*)

Donavan Jackson, Sharlene Santana

One of the primary endeavors of evolutionary biology is to investigate the selective pressures that explain patterns of variation, adaptation, and diversity. Studies that focus on phenotypic variation within widely distributed species can be particularly informative of the factors and mechanisms leading to evolution and adaptation. Here, we used the least chipmunk, *Tamias minimus*, to test the hypothesis that intraspecific variation in cranial shape and pelage color are driven by environmental gradients. The least chipmunk has a vast geographic range – spanning as far north as Yukon and extending southward to its lower terminus in New Mexico. We photographed the skulls and skins of over 200 museum specimens to examine the extent and influence geography imparts on intraspecific cranial shape and pelage color over a broad environmental gradient. We employed geometric morphometrics and conducted principal component analyses to examine correlations between skull shape and size, and quantified color pattern variation in a multivariate context. We then examined the relationship between these morphological aspects and precipitation, temperature, and latitude as key environmental variables. Our findings suggest that cranial shape variation is not driven by environmental variables, but size is. Preliminary analyses suggest color variation is correlated with environmental variables (temperature and precipitation). These results highlight the functional characters that might be involved in ecological specialization, or lack thereof, in this species.

Life history traits vary on the basis of phylogeny across seven species of *Anolis* lizards

Kendall Jackson, Morgan Muell, Daniel Warner

Life history strategies vary considerably among taxa and have been shown to rapidly evolve in response to changing environmental pressures. Interspecific comparisons of life history traits among closely related taxa can provide insight into the patterns of life history evolution, as well as the ecological pressures that might shape its diversity. Life history strategies in lizards have been studied extensively and, in many cases, have been found to be conserved along phylogenies. *Anolis* lizards, for example, produce a single egg clutch and this life history trait is conserved within the approximately 400 species in this genus. Consequently, life history traits are often considered fixed within this group, but variation among species in other life history traits are understudied. We studied seven species of *Anolis* lizards occupying South Florida and compared life history traits among these species to explore potential differences among taxa and their potential drivers. We collected data on egg size, offspring size, offspring allometry, and hatchling growth rate and survival over 8 weeks from individuals produced from two different incubation treatments. We demonstrate that species differ considerably in all life history traits tested, and phylogeny often explains a large amount of variation in trait values. While *Anolis* diversity is typically discussed in the context of morphology, this study highlights the reproductive diversity of this genus in ways that have been previously underappreciated.

Hidden impacts of climate change on marine organisms

Juliette Jacquemont, Katharina Alter, Paolo Domenici

Conflicting results remain on how climate change affects the biological performance of different marine taxa, hindering our capacity to predict the future state of marine ecosystems. Using a novel meta-analytical approach, we tested for directional changes and deviations across biological responses of fish and invertebrates from exposure to warming (OW), acidification (OA), and their combination. In addition to the established effects of climate change on calcification, survival and metabolism, we found deviations in the physiology, reproduction, behavior, and development of fish and invertebrates, resulting in a doubling of responses significantly affected when compared to directional changes. Widespread deviations of responses were detected even under moderate (IPCC RCP6-level) OW and OA for 2100, while directional changes were mostly limited to more severe (RCP 8.5) exposures. Because such deviations may result in ecological shifts impacting ecosystem structure and processes, our results suggest that OW and OA will likely have stronger impacts than those previously predicted based on directional changes alone.

Analyzing relationships between the social environment and maternal hormone allocation

Anna James, Lynn Siefferman, Kendall Terry, Leigh Bailey, Alexandra Bentz

Across taxa, females allocate hormones to their offspring in response to their environment, but female birds are particularly good models for investigating hormone-mediated maternal effects. For example, the deposition of testosterone (T) into egg yolks can be impacted by the level of social competition females experience. Many females deposit more yolk T in response to heightened social competition, which can lead to potentially adaptive phenotypic changes in the offspring. However, some females display unexplained individual- and species-level variation in their yolk T responses to social competition, making the adaptive value of this maternal effect unclear. Here, we investigated the extent to which the timing of social competition, individual behavioral responsiveness, and species-specific sociality affect yolk T allocation in colonial and solitary songbird species. For each species, we conducted simulated territorial intrusions (STIs) with a conspecific decoy and vocalizations for several consecutive days prior to egg-laying (3 – 10 days), while control nests were undisturbed. We collected behavioral data during the trials to quantify each female's behavioral response to the STI treatment and quantified yolk T concentrations in eggs. These data help clarify the role individual- and species-level factors play in shaping variation in yolk T responses to the social environment and ultimately increase our understanding of both the adaptive value of this maternal effect and how hormone allocation is impacted by the maternal environment.

Salamander Fight Club: Heat-Induced Behavioral Plasticity in *Plethodon* Hybrids

Emmy James, Martha Munoz

Temperature mediates performance in ectotherms. It determines the range of behaviors that organisms are capable of completing and, in turn, their ability to grow, survive, and reproduce. Interspecific interactions like aggressive behavior are a key example of thermally-dependent behaviors that may impact fitness. Woodland salamanders (Genus: *Plethodon*) often exist in crowded communities along thermal gradients. These organisms tend to exhibit increased aggression at warmer temperatures. However, it is thus far unclear how salamander hybrids, which may encroach on their parent populations and drive competitive exclusion, respond behaviorally to temperature change. Here, I examined the effects of temperature on aggression and evasion in the *Plethodon shermani* and *Plethodon teyahalee* hybrid system. Upon encountering a salamander from a different population, parent individuals exhibited heightened evasion and some increased aggression at higher temperatures. In contrast, hybrid behavior appeared largely unaffected by temperature. Hybrids of the two populations exhibited no trend towards increased aggression or evasion with temperature. Future research is needed

to elucidate the relationship between this behavioral response and hybrid physiology - namely, how is this behavior related to energy usage or thermal preference of hybrid salamanders?

Bats Show Variation in Sugar Metabolism Corresponding with Dietary Guild

Jerrica Jamison, Kenneth Welch

Bats have evolved diverse specialized diets, including high-sugar diets such as frugivory and nectivory. Most mammals develop diseases such as diabetes when fed a high-sugar diet, however nectivorous and frugivorous bats must regularly process large amounts of sugar in their natural diets. We sought to characterize the ability of bats across dietary guild to metabolize glucose and fructose, the two most common simple sugars in nature. Nectivory and frugivory are both high in simple sugars, and both have evolved multiple times independently in bats, allowing for the comparison of sugar metabolism within and among dietary guilds. We used stable isotope respirometry to determine how bats across dietary guilds responded to a ^{13}C labelled sugar meal of either glucose or fructose over a one-hour period. We found significant differences among dietary guilds and within independent lineages of nectar-feeding for both glucose and fructose, though patterns were not consistent between sugars. The differences among dietary guilds, even those that ingest similarly high levels of sugar, indicate that meal type, not just sugar composition, influences metabolic evolution. Furthermore, the differences between independent lineages of nectar-feeding bats reveal that mammals modify different metabolic pathways to adapt to nectivory. Finally, the unique patterns of metabolism for glucose and fructose show that each sugar has a distinct influence on metabolic physiology when mammals specialize on high-sugar diets.

Optogenetic control of odor intensity and duration in free flying *Drosophila*

Kaylee Jamison, Stanley Stupski, Floris van-Breugel

Insects are among the most adept organisms at locating the source of chemical cues. While tracking odors they integrate sensory information from olfaction, the wind environment, and visual cues to make flight-based decisions. In natural environments, odor plumes are dispersed from a source in intermittent packets and carried by winds. How flying *Drosophila melanogaster* use sensory information from odor encounters of varying intensities and durations to make flight-based decisions is poorly understood. Here, we use an optogenetic paradigm to administer carefully controlled virtual olfactory experiences of varying intensities and durations. We then explore how these temporal aspects of the olfactory experience of freely flying flies influence two features of their search behaviors: the decision to turn upwind, and their decision to approach visually salient features in their environment. We expect these experiments to yield insights into how flies integrate sensory information to reliably find the source of a volatile chemical cue. Our findings could inform pest control and search algorithms for biomimetic robotics.

Visual ecology of tidal creeks: how two types of crab approach a challenging light environment

Madison Janakis, Daniel Speiser, Daniel Chappell

Different environments pose distinct challenges to animal visual systems. For instance, tidal creeks can be spectrally narrow, decreasing contrast between colors, and suspended particles scatter light, reducing sighting distances of objects. Further, the light environment is constantly shifting due to local conditions like weather and tides. To learn how visual systems function in tidal creeks, we investigated vision in two species of South Carolinian crab: the green porcelain crab, *Petrolisthes armatus*, and the Atlantic mud crab, *Panopeus herbstii*. For both species, we used behavioral optomotor assays to estimate visual acuity and test polarization sensitivity, and electroretinography to measure spectral responses. Using achromatic optomotor stimuli, we found both species have a visual acuity between 4 and 10° . Only *P. herbstii* responded to the polarized optomotor cues, which were perpendicularly arranged strips of linear polarized filter 10° wide. By fitting visual pigment

templates to spectral response curves, we found that *P. herbstii* has two classes of photoreceptors with peak sensitivities of about 470 nm and 540 nm. In contrast, *P. armatus* appears to have three classes of photoreceptors with peak sensitivities of about 375 nm, 480 nm and 560 nm. Our evidence suggests these two crabs address the visual challenges of tidal creeks differently: mud crabs use polarized light to increase visual contrast of scenes while porcelain crabs utilize UV wavelengths.

Transcriptomic changes in American lobster (*Homarus americanus*) developmental stages I through V

Aubrey Jane, Doug Rasher, Jessica Waller, Eric Annis, Markus Frederick

The American lobster (*Homarus americanus*) is arguably the most iconic invertebrate in the Gulf of Maine, and landings data have allowed for documentation of this species' climate-driven range shift up the eastern coast of the United States. While decades worth of research has been done on adults and post larvae of this species, especially in relation to thermal tolerance, there are few studies which target multiple early developmental stages. Further, the relatively recent development of RNA-seq technology provides an unparalleled ability to probe differential gene expression in an organism's entire transcriptome. We applied this technology to investigate transcriptional changes in American lobster developmental stages I through V. Investigating the most significantly enriched GO terms and KEGG pathways, our findings characterize changes in gene expression of cuticle development and energy metabolism. We found patterns in gene expression within GO terms related to heat response, such that stages I and V are more tolerant to cold stress, while stage IV post larvae more tolerant to heat stress, tracking with their known distributions throughout the water column. This corroborates previously collected evidence for ontogenetic shifts in this species. We also identified DnaJ as a potential molecular marker for thermal tolerance in *H. americanus*. These findings deepen our understanding of crustacean development and can be used to inform population distribution modeling efforts.

Muscle fiber composition, reproductive investment, and jumping performance in an amphibious fish

Yonathan Janka, Cooper Byers, Chase Counton, Ryan Earley

In moist environments, hermaphroditic mangrove rivulus fish can survive on land for at least two months where they escape from predators or relocate by jumping. We previously revealed consistent among-individual differences in both jump number and jump distance, but the underlying mechanism is unknown. We conducted 4 jumping trials for each hermaphrodite over 4 consecutive weeks using a kiddie pool lined with moist paper towels and an overhead camera. Each fish was tested individually for 2 minutes; in the first minute, fish could jump voluntarily while, in the second minute, fish were probed gently to induce a jumping response. There was some evidence for habituation, with probe jump number and distance decreasing significantly by the final trial. After the fourth trial, we harvested bodies and tails to test the hypotheses that jumping performance is associated with variation in: 1) glycolytic (easily fatigued but powerful) and non-glycolytic (endurance) muscle fibers; and 2) reproductive status (egg investment). We predicted that individuals with larger muscle cross-sectional areas, more glycolytic fibers, and fewer mature oocytes should jump greater distances, potentially indicating a trade-off between investment in survival (jumping) and reproduction. Data collection is ongoing but, results will provide key insights into the mechanisms underlying among-individual variation in jumping performance and will facilitate exploration of the fitness consequences of such variation (e.g., survival in habitats with tides or abundant predators).

Artificial light at night creates a network of habitat patches for an urban arthropod community

Katelynn Jankowiak, Zach Stahlschmidt

Artificial light at night (ALAN) is increasing, and it is linked to global biodiversity losses caused by alterations in animals' circadian rhythms, activity patterns, and phenology. To examine ALAN's effects on community dynamics, previous work has typically introduced ALAN into ALAN-naïve communities

and/or focused on large-scale spatial effects by comparing sites separated by >1 km. However, ALAN sources (e.g., street lamps) often create patches of light across a landscape, and animal assemblages can vary on a fine spatial scale. Therefore, we used pitfall trapping in the urban setting of the University of the Pacific in Stockton, CA to track the diurnal and nocturnal community composition of terrestrial invertebrates on a fine spatial scale (2-6 m) across which illuminance (lux) varied 10-fold to determine: (1) the effects of ALAN on a nocturnal animal community, and (2) whether the effects carryover into diurnal communities. Across 38 transects, we collected over 11,000 individuals from 39 families. We found that overall richness, diversity, and abundance in nocturnal communities was highest near ALAN sources. However, there were no carryover effects of ALAN on diurnal communities. Thus, for animals in areas subjected to longstanding ALAN (our study system is ~100 years old), the mosaic of nighttime lighting in urban ecosystems may serve as a network of habitat patches, rather an array of ecological traps.

Effects of flash drought during incubation on common snapping turtle phenotype and fitness

Fredric Janzen, Ayley Shortridge

The frequency and intensity of flash drought are increasing across the central United States, with peak occurrence in the summer months. The associated depletion of soil moisture could have broad ecological impacts. Many turtle species, including common snapping turtles (*Chelydra serpentina*), have flexible-shelled eggs that are highly sensitive to hydric conditions in the nest. Moreover, the high rate of predation on hatchling turtles means that phenotypic differences at this early life stage may be under strong selection. Here, we present the results of an experimental study on the effects of flash drought on eggs and hatchlings of *C. serpentina*. First, we manipulated soil water potential during egg incubation in the laboratory. Second, we conducted an experimental release in the field to determine the effects of flash drought on hatchling survivorship during dispersal from the nest. As predicted, we found that eggs decreased in mass during flash drought events. We also observed a significantly shorter incubation time and decreased mass in hatchlings that experienced flash drought during mid- to late incubation. Finally, hatchlings that experienced flash drought during late incubation had higher mortality rates during dispersal from the nest. These results suggest that turtle populations may be significantly impacted by increasing flash drought.

Megalodon's chipped tooth: What tooth damage can tell us about the behavior of megatooth sharks

Steven Jasinski, Sidney Hostetter

Damage present in the teeth of sharks shows consistent patterns. This damage can be categorized, and is present to varying degrees in both modern and fossil shark species. We collected data from over 2000 teeth of *Otodus megalodon* and other shark species to determine variation within these teeth and provide a basis for investigating tooth damage variation in shark (Selachimorpha) teeth. Additionally, these damage types can be hypothesized to represent various actions and forces as their causes. While some types of damage can be taphonomic, others are due to shark behavior, particularly during feeding. Different taxa show different types and percentages of damage, implying different feeding strategies. Teeth of megatooth sharks (*Otodus*), particularly those of *O. megalodon*, show higher rates of damage than those of other sharks, with the highest occurrence of damage occurring at the tip. Additionally, the higher percentage of damage in *O. megalodon* teeth may also signify their retention in the mouth for longer periods, allowing more damage to accumulate before teeth are shed. This damage also suggests *O. megalodon* would have attacked large-bodied prey, but does not explicitly mean it would have only eaten large-bodied prey. Other shark species were also attaining larger body sizes around the same time as *O. megalodon* (e.g., *Alopias grandis*, *Carcharomodus*, *Cetorhinus*, *Parotodus*), suggesting abundance in numerous resources allowing these predators to grow to large sizes.

Introduction to moving in an uncertain world adaptively in biological and bioinspired systems

Kaushik Jayaram, Jean-Michel Mongeau

To succeed in the natural world, locomoting organisms and robots need to be both robust and adaptive to maintain adequate performance in ever-changing conditions. Emerging research in this area indicates biological organisms use a variety of mechanisms including smart body mechanics, hierarchically integrated neural feedback, learning, etc., to mitigate the effect of perturbations. This knowledge has informed the development of novel bioinspired fault-tolerant strategies, intelligent control, and robust mechanical designs to advance robot capabilities. The goal of this symposium is to facilitate communication across various disciplines to promote cross-fertilization of ideas and techniques in this rapidly growing research theme and to enable training the next generation of integrative biologists capable of addressing grand challenges in this field that are often at the interface of biology, physics, and engineering. In this presentation, we will highlight some of the recent interdisciplinary advances on this topic such as biologically inspired reinforcement learning which has shifted the paradigm of robotics and AI, and neurogenetics which has enabled neuroscientists to unveil unprecedented details of neural mechanisms underlying robust and adaptive locomotion. We will present a few grand challenges related to this topic to set up a stage for nine experts in the fields of biomechanics, neuromechanics, motor control, control theory, robotics, and artificial intelligence to share their research and scientific approaches to addressing them this complex problem.

Bowhead whale reproductive cycles from the 1940's-1960's inferred from hormones and stable isotopes

Jen Jelincic, Danielle Dillon, C. Loren Buck, Matthew Rogers, Daniela Mello, Alyson Fleming, Kathleen Hunt

Longitudinal studies of individual mysticete whales are possible by utilizing the keratin matrix of baleen. Steroid and thyroid hormones are deposited and stored along the length of each baleen plate, resulting in up to 20 years of continuous hormone data per individual, which can reflect reproductive cycles and physiological responses to environmental stressors. We subsampled four ~2.5-m specimens of baleen (two males, two females), extracted and assayed corticosterone, cortisol, dehydroepiandrosterone (DHEA), progesterone, testosterone, and triiodothyronine (T3) from all subsamples and individuals to create multi-decade endocrine profiles. Hormone concentrations were quantified every 4 cm using validated enzyme immunoassays, while bulk isotopes of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were analyzed every 2 cm to confirm baleen growth rate and estimated year from collection date. Baleen of females contained widely spaced regions of high progesterone, interpreted as pregnancies, while male baleen had regular high-testosterone regions interpreted as annual breeding cycles. These data represent the first longitudinal endocrine profiles for these hormones in female bowheads, the first DHEA and T3 data for males, and add to our understanding of hormone patterns across multi-decade timespans in the longest-lived mammal. Our results underscore the importance of examining hormone concentrations with reference to reproductive state, the utility of historic specimens from natural history museums, and the value in evaluating several hormones concurrently in free-ranging wildlife studies.

Environmental impacts on freshwater turtle behavior across an urban-rural gradient

Justin Jenkins, Ivo Gross, Molly Folkerts-Caldwell, Matthew Wolak

Consistent among-individual differences in behavior often correlate with survival and reproductive success, thereby linking animal behavior to processes such as local adaptation and population persistence. A major question in animal behavior is whether optimal behaviors vary across populations that differ in their environment. Comparatively few studies focus on behavior in turtles despite their imperiled status and vulnerability to many aspects of global change. We studied yellow-bellied sliders (*Trachemys scripta*) in central Alabama, USA across populations that vary in the level of human presence and activity to associate differences in boldness behavior to levels of human activity. We developed a boldness assay for turtles based on seven anti-predator responses and applied this measurement to turtles in five ponds along an

urban to rural gradient. Our results suggest a negative correlation between bold behavior and several measures of human activity or interaction, such that turtles in ponds less impacted by humans tend to be bolder while turtles experiencing relatively increased levels of interactions with humans and human development are less bold. Overall, this study suggests turtles may use behavior to moderate the impacts of human-driven environmental change and highlights how animal personality may play a role in local adaptation.

Barnacles as a model for crustacean embryogenesis

Erin Jezuit, George von-Dassow

Barnacle embryogenesis was once interpreted as a modified form of spiral cleavage. This conclusion has not been revisited since the realization that arthropods do not group closely with annelids and other spiralian. Indeed, barnacle embryos have never been studied by direct lineage labeling. Using cell marking by photoactivation of caged FITC-dextran and 4D microscopy, we are evaluating barnacle embryonic cleavage and cell lineage. Barnacle zygotes undergo obvious cytoplasmic segregation and asymmetric, oriented cleavage divisions, with descendants of the yolk-rich posterior blastomere internalized at gastrulation to form endoderm. However, cell marking shows that identified blastomeres at the 4-cell stage give rise to varied ectodermal territories between individuals. Hence, lineage does not determine the patterning of ectodermal primordia. Based on time-lapse imaging and cell marking, we propose a new interpretation: for the first four cleavage divisions, yolk segregates posteriorly into an endoderm precursor (Y); successive anteriorly-directed divisions of Y give rise to yolk-free micromeres; micromere descendants undergo posteriorly-directed epiboly to internalize Y's great-great-granddaughters at gastrulation; initial divisions of each micromere occur orthogonal to the anteroposterior axis, but micromere descendants contacting Y divide along the anteroposterior axis. We are presently testing whether directed division and epiboly combine to yield mesoderm at the blastopore margin.

Investigating neural correlates of the winner-loser effect in poison frog tadpoles

Lucas Jimenez, Lisa Surber, Lauren Mobo, Eva Fischer

The winner-loser effect is a phenomenon in which previous fight outcomes affect future fight results: winners tend to keep winning and losers are more likely to keep losing. This phenomenon has been observed across taxa and life stages; however, the mechanisms underlying the effect are less clear, especially in juveniles where the neural basis of aggressive behavior is generally poorly understood. Our study organism, the dyeing poison frog (*Dendrobates tinctorius*), is an ideal system to fill this gap as tadpoles are aggressive and cannibalistic. To examine the effect of previous social experience on fight outcome, each tadpole in our study experienced two aggression trials one week apart. In the second trial, winners from the first fought individuals that lost their first. We found no evidence of a winner-loser effect, and instead found that current fight conditions (like size of partner) more strongly influenced fight outcome. These findings are of interest because they suggest resilience to winner-loser effects in *D. tinctorius* tadpoles. To explore this resilience, we quantified dopaminergic cell activity in the brains of tadpoles. We compared the number, activity, and location of tyrosine hydroxylase (the rate-limiting step in dopamine synthesis) positive cells in sectioned brain samples. Taken together our study sheds light on the critically understudied causes and consequences of juvenile aggression.

Tradeoffs between muscle output and body stiffness may help reveal the swimming strategy of scup

Yordano Jimenez, Erik Anderson, Gina Kim, Martha Sutter, Eric Tytell

Fish swim using deceptively simple undulations that emerge from complex interactions between internal body forces and external fluid forces. The same swimming muscles that generate bending torques for locomotion simultaneously change body stiffness, altering fluid-body interactions that could impact the acceleration, speed, and stability of swimming. Therefore, quantifying the ability of muscle to simultaneously bend and stiffen the body is key to understanding swimming performance. Using scup (*Stenotomus chrysops*),

we measured swimming kinematics at different swimming speeds in a flow tank. We euthanized the same individuals and mounted them in an oscillatory bending apparatus in which tail flexion was controlled with a servomotor on one end and bending forces were measured with a force transducer on the other end. We stimulated red muscle and bent the body using a range of observed and hypothetical kinematic and muscle activation patterns. Our data reveal a complex mechanical landscape with non-linear tradeoffs between muscle output (i.e., power and work) and active body stiffness. We examine the kinematics of scup in the context of these mechanical data to reveal how scup modulate steady swimming performance.

Chitinase from the gut of a small lizard and its potential efficacy as an antifungal agent

Alec John, Davida Smyth, G. Shelton, Charles Watson

Chitin is a major component of the exoskeleton of arthropods as well as the cell wall of fungi. This aminopolysaccharide polymer provides structure and protection to these organisms and represents a significant barrier to digestion of arthropod prey and fighting fungal infections. Chitinases are enzymes that break down chitin. Smaller insectivores, based upon the higher surface-to-volume ratio of their even smaller prey, ingest a larger amount of chitin per unit prey item. Therefore, they represent high-likelihood opportunities to bioprospect for novel chitinases that may be effective against pathogenic fungi. Fungal infections are notoriously difficult to treat, and they are the cause of ailments whose severity ranges from mild skin irritation to systemic infections that can lead to mortality. The purpose of this study is to document the activity and diversity of chitinase produced by a small vertebrate insectivore, the ground skink, and test the efficacy of these enzymes against potentially pathogenic fungi.

Putting color in context: backgrounds affect the perception and discrimination of color signals

Sonke Johnsen, Alexander Davis, Eleanor Caves, Matthew Zipple, Danae Diaz, Steve Nowicki, Susan Peters

The color signals of most organisms are surrounded by a background. As artists have known for centuries, the background surrounding a color influences both detection and discrimination of that color in opposing ways. Specifically, high color contrast between the background and two given color stimuli makes their detection easier but their discrimination from each other more difficult. However, it remains unclear whether achromatic background contrast affects signal discrimination in non-human animals. Here, we test whether the contrast between signal-relevant colors and an achromatic background affects the ability of zebra finches to discriminate between those colors. We found that higher achromatic contrast with the background decreases the ability of zebra finches to discriminate between target and non-target stimuli. This effect is particularly strong when color distances are small and achromatic contrast with the background is high. We suggest that researchers should consider focal color patches and their backgrounds as collectively comprising a signal, rather than focusing on solely the color patch itself.

Froude number of the crawl-bounce gait transition in sea stars

Amy Johnson, Olaf Ellers, Graham Lucas, Hank Marriott, Brady Nichols

Sea stars are underwater legged locomotors that use numerous podia to achieve two gaits: a slower crawl and a faster bounce. As has been ubiquitously found for terrestrial legged locomotors, gait transitions can be characterized by the Froude number, which is the ratio of kinetic to potential energy or the ratio of inertial to gravitational forces. But unlike terrestrial human locomotion where the transition between walking and running occurs at a Froude number of about 1, sea stars transition from crawling to bouncing at a Froude number of about 10-2 to 10-3. Similarly low Froude numbers have been observed in stepping flies. We filmed sea stars and analyzed gait kinematics. We identified the shift in gait dynamics and Froude numbers as sea stars transitioned between crawling and bouncing. Unlike the human walk-run

transition where the gait transition is clearly identified by both feet leaving the ground, sea stars always have some podia on the ground making identifying the moment of transition more elusive. Thus, we developed several metrics to detect the timing of the gait shift.

The effects of harmful algal blooms on isopods found in the Salish Sea

Dawson Johnson, Rylie Rogers, Ryan Kenton, Rosa León-Zayas, Christine Weillhoefer, Cecilia Brothers

Harmful algal blooms (HABs) are instances where algae, either marine or freshwater, can cause detrimental effects to humans and aquatic ecosystems. One organism that may be impacted by HABs is the eelgrass isopod *Pentidotea resecata*. These isopods are relevant intermediate food web links as they are consumed by numerous species of fish and are present from Alaska to Mexico. The purpose of this study is to observe the effects of HABs on the grazing, growth, and survival of *P. resecata*. A potential HAB-forming species (the diatom *Pseudo-nitzschia* sp.) and a mixture of non-HAB forming species (representing *in situ* conditions) were cultured, mixed with agar, and used to coat blades of the eelgrass *Zostera marina* as a food source. Preliminary grazing experiments determined isopods did not prefer consuming potential HAB versus non-HAB species ($n=6$). However, during a three week grazing experiment, HAB and non-HAB forming species were fed to the isopods in different ratios (100/0%, 66/33%, 33/66%, and 0/100%; $n=4$, respectively) to represent HAB conditions at varying severities. Isopods fed 100% *Pseudo-nitzschia* displayed the highest feeding rate (based on the first 12-hr grazing period) and had the highest survival rate of any treatment at the end of the experiment. Further work will quantify concentrations of the neurotoxin domoic acid in food and isopod samples to better understand the effects of HABs on marine invertebrate trophic interactions.

Nearing the Max: Thermal Stress Decreases Larval Survival in Tortoiseshell Limpets

Gabrielle Johnson, Terence Leach

With climate change rapidly affecting a multitude of environments, organisms are challenged to modify some aspect of their physiology under these short timescales or risk mortality. For marine environments, rising ocean temperatures - via global warming and heatwaves - threaten many coastal ecosystems. During early development, marine invertebrates are highly sensitive to environmental stressors, including elevated temperatures. Therefore, summer-spawning organisms may be particularly vulnerable in future oceans as their offspring already must survive the hottest temperatures of the year. This study evaluated the thermal limits within larvae of the summer-spawning tortoiseshell limpet, *Testudinalia testudinalis*, an important grazing mollusk that maintains different algal populations within intertidal ecosystems of the Northwestern Atlantic. *T. testudinalis* larvae were allowed to develop at ambient temperatures ($\sim 8^{\circ}\text{C}$) until the veliger stage, approximately 96 hours post fertilization. To test the thermal tolerance of the veligers, tubes containing larvae were placed in an aluminum block possessing a thermal gradient (24 - 35°C). As thermal stress increased, larval survival was negatively impacted. While temperatures above 34°C resulted in total mortality for larvae, in the short-term, larvae were resistant to temperatures significantly higher than the ambient temperature of their typical environment. Future studies could evaluate larvae during and after recovery period following thermal stress and into later juvenile and adult stages.

Extreme temperatures reduce strike success in smallmouth bass feeding

Grace Johnston, Emily Volpe, Katrina Whitlow

Successful feeding is critical to survival in fish. Particularly in fish that eat elusive prey, strike success is heavily dependent on the predator's kinematics. Temperature drastically impacts swimming and bioenergetics in ectothermic animals, as muscle performance both increases with temperature and is highest within a narrow homeostatic range, dropping off at extreme temperatures. Temperature also affects the behavior of fishes, though the mechanisms

behind the observed shifts are less clear. This study investigates how different temperature treatments affect striking kinematics and success in *Micropterus dolomieu* (Smallmouth Bass). Smallmouth bass have well-studied kinematics and bioenergetic requirements, preferentially occupy colder waters, and are predicted to be more vulnerable to increasing global temperatures, making them a prime candidate for this work. Bass were acclimated to temperature treatments ranging from 15°C - 30°C , fed live prey, and filmed. Preliminary findings suggest that extreme temperature treatments of 15°C and 30°C result in more missed strikes in bass, as well as colder treatments making the bass less prone to strike. The gape size and the velocity of mouth opening also appears to increase with warmer temperature treatments. Further analyses will examine the motion of the body and fins, striking distance, and role of prey response across temperatures. These performance shifts may negatively impact fish populations and aquatic ecosystems under future climate scenarios.

Functional Consequences of Fine Scale Morphology of the Aboral Surface of North American Sea Stars

Angela Jones, Brian Helmuth

Recent studies of Echinoderms have shown the importance of combining form, function, and behavior into our understanding of functional biology. Multiple studies have explored the biomechanical and fluid dynamics consequences of overall seastar gross morphology in driving processes such as lift and drag. But less is understood about how considerable differences in fine-scale surface morphology, such as spines and pedicellariae, may influence performance, survival, and ultimately ecological interactions. Many Asteriids, like *Asterias*, are known for their active wreath organs, a collar of pedicellariae that surround the aboral spines. In contrast, in Asteriids like *Pisaster ochraceus*, the spines are solitary, and presumably act to protect the surface. My work comparatively examines the spine morphology of the common Asteriid keystone predators, *P. ochraceus*, *A. rubens*, and *A. forbesi* by quantitatively and qualitatively examining both micro- and gross morphologies using fine-scale microscopy of field-collected specimens. Field surveys showed that *P. ochraceus* had more convex spines in exposed habitats compared to more protected environments, potentially correlated with wave exposure and sediment stress. Of particular interest in *Asterias* are the wreath organs that sit midshaft on the aboral spines of *A. rubens*, which contrast with *A. forbesi* that have more tubercle spines that have wreath organs at the base of the spine. Laboratory studies are being conducted to analyze oxygen consumption with activated wreath organs in the two species.

The Silverjaw Minnow: An Extraordinary Lateral Line System and Its Contribution to Prey Detection

Aubree Jones, Anabela Maia, Kevin Conway, Jacqueline Webb

The lateral line system (LL) of fishes, comprised of neuromast receptor organs on the skin (superficial neuromasts, SNs) and within bony canals (canal neuromasts, CNs), detects water flows and mediates prey detection in different behavioral contexts. Most fishes have one cranial LL canal phenotype, but *Ericymba buccata* has two - narrow canals dorsal and caudal to the eye (common among minnows) and widened canals ventral to the eye and on the mandible (a putative flow sensing adaptation). Multiple morphological methods revealed that the widened canals are larger in diameter with a mostly unossified canal roof. CNs in the widened canals are larger than those in the narrow canals, which are smaller in diameter with a well-ossified canal roof. Numerous SNs and taste buds were also found in the skin ventral to the eye and on the mandible. Feeding experiments (light/dark, intact/antibiotic-ablated LL) were used to test the hypothesis that the LL mediates prey detection. However, the behavioral results demonstrated that the LL system is not essential for benthic prey detection; when the LL system is ablated, chemoreception (presumably gustation) is sufficient to localize benthic prey in the dark. These results raise questions about the evolution of this extraordinary dimorphic LL canal system and suggest that *E. buccata* would be an excellent model for future comparative studies of LL evolution and sensory-mediated behavior and ecology.

Thermal ecology, activity patterns, and ecological niche modeling of *Gopherus* tortoises.

Julia Joos, Donald Miles

Almost 50% of modern tortoise species are endangered or already extinct. Niche modeling predicts extensive range contractions for many chelonian species due climate change induced habitat loss. For example, tortoise habitat in arid environments is expected to increase in temperatures and prolonged drought reducing habitat availability. Desert-dwelling tortoises dig burrows or seek refuge in rock crevices to avoid the heat and potential risk of overheating. There are six tortoise species in North America and Mexico (genus *Gopherus*) and they differ in genetics, distribution, morphology, body size, diet, habitat, and shelter site selection. Most *Gopherus* species have been assumed to have a similar physiology as *G. agassizii* despite differences in genetics and ecology and more recent evidence suggesting varying thermoregulatory strategies for different species. Therefore, understanding each species' specific habitat requirements and physiological properties are important to allow inferences about tortoises' potential to cope with temperature shifts and habitat alterations. Further, there is a lack of sufficient physiological data at an appropriate resolution of microhabitats to refine existing ecological niche models and improve predictions to guide conservation action for specific populations and species. Our study measures core physiological data, operative environmental temperatures, and movement and activity patterns of several species of *Gopherus* tortoises occupying differing habitats, ranging from desert habitat and arid grasslands to tropical deciduous forest habitat.

Multisensory integration of noisy cues in bumblebees

Katherine Jordan, Jordanna Sprayberry, Stacey Combes, Wilsaan Joiner

Multisensory integration can improve behavioral responses by enhancing the detection and localization of sensory cues and reducing response times. However, in real-world settings, sensory cues are often noisy; visual and olfactory cues can be deteriorated, masked, or mixed, making the target cue less clear to the receiver. Despite widespread interest in multimodal processing and perception of noisy cues in both human and animal studies, the majority of studies have involved combining a noisy cue in one modality with a clear cue in a different modality. Thus, the question of how multimodal processing affects response accuracy when both signals are noisy, as is likely the case in many natural settings, remains unresolved. As in human experiments, studies on bees have shown that multisensory integration of visual and olfactory cues can improve response accuracy. It is less clear, however, how noise in multimodal cues affects generalization. In this study, we show that not only does multisensory integration improve the accuracy of bumblebees responding to noisy cues, but also that bees trained with clear multimodal cues respond more accurately to the noisy versions of both learned cues than to versions with clear shape and novel odor cues. These results enhance our understanding of the conditions in which multisensory integration can be advantageous, showing that this process may be particularly critical in environments where sensory cues are unclear.

Reproductive state in females and hold time in males predict N:L ratios of brown bats but not hair

Marcus Jorgensen, Diana Hews

Animals experiencing social and environmental challenges show hormonal stress responses, i.e., short-term glucocorticoid (GC) elevations, that alter energy allocation strategies. Among many effects, GC elevations mobilize glucose and alter cell-mediated immunity, including increased neutrophil to lymphocyte ratios (NLRs). We examined associations of NLR with an acute stress measure (hold time prior to blood sampling) and a retrospective GC measure (hair cortisol). Studying *Eptesicus fuscus* captured during three summers in northeastern Missouri, we examined thin blood smears for 243 individuals, and hair cortisol, which likely represents circulating levels during the previous year's presumed August molt when cortisol enters the growing hair shaft. Using GLMs and ANOVAs we asked if NLR variation was explained by hold time, age, sex, reproductive status and, in a smaller subset of 118 bats, hair cortisol. Hold time was a significant predictor of NLR only for adult males. Lactating females had significantly higher NLRs than post-lactating bats; mean

NLR in pregnant bats was intermediate in value. Neither hold time nor sex predicted NLR in juveniles. Hair cortisol was never a strong predictor of NLR, suggesting GC levels from the previous summer do not influence current NLRs. Adult males may have stronger NLR responses to elevated GCs and/or adult females and juveniles may have muted responses, perhaps because of energy devoted to reproduction and to growth, respectively.

Symposium Introduction: An integrative look at whole-organism trade-offs in reproducing females

Chloe Josefson, Teri Orr

Trade-offs during reproduction have long been a central focus within biology and much of the foundational work within life-history evolution has focused on females, as fitness of females is more easily quantified for use in theoretical models. At the organismal level, reproduction is thought to trade-off with other simultaneously-occurring processes and recent papers have sought to outline the issues with our current understanding of whole-organism trade-offs, though the field as a whole has not come to a consensus on what trade-offs mean to a reproducing female. Additionally, much of the work investigating trade-offs within integrative organismal biology has focused on males or non-reproductive females. To rectify this important gap in how trade-offs are discussed in the area of organismal biology as well as confusion about what constitutes a trade-off, our overarching goal of this symposium is to discuss trade-offs from an integrative perspective that places female reproduction at the center. By answering what trade-offs are and what they mean to reproducing females, what has been neglected in the context of whole-organism physiology, and how maternal effects fit within this framework, our group of speakers and their associated papers will crystalize nuances of measuring and determining presence (if any) of trade-offs in reproducing females in a range of taxa and subfields.

Roundtable Discussion: Organismal trade-offs during reproduction from a female-centered perspective

Chloe Josefson, Teri Orr

To conclude our symposium on organismal trade-offs in female reproduction, we invite our participants as well as all SICB attendees to join in a roundtable discussion. The symposium itself featured work from a diverse group of scholars studying this topic from a range of perspectives and in different taxa. We hope to expand this diversity by including an additional set of voices and perspectives so that we can further the field by facilitating critical dialogue among attendees and participants. Several key questions will be addressed including: What are the key gaps in our knowledge regarding trade-offs in reproductive females? Where might ignoring the female-centered biological perspective result in misguided conclusions about when trade-offs occur? How might our methods be impacted by emphasizing female-centered biology? Are there taxon-specific considerations to make on this topic? How do maternal effects fit within this framework? What are the next big questions in organismal trade-offs as they relate to the female-centered perspective? Symposium organizers and participants will be encouraged to bring forth questions that have been posed throughout our sessions. Our goal is to make strides in refining terminology as well as outlining gaps in our knowledge that are of broad interest to integrative biologists.

The effect of temperature on metabolism and oxidative damage in a lizard, *Sceloporus consobrinus*

Nicole Joseph, Kayla Lichtner, Redeit Woldebirhan, Benjamin Haussmann, Tiffany Hegdahl, Travis Robbins, Mark Haussmann

Ectotherm metabolic rates positively scale with temperature. As metabolism increases, mitochondria produce more reactive oxygen species, consequently increasing oxidative damage to biomolecules such as DNA. While a recent study in ectotherms produced the expected positive relationship between temperature and metabolism, it also revealed a surprising inverse relationship between metabolic rate and oxidative damage. Our study investigates the effect of temperature manipulation on metabolism and oxidative damage in three geographically distinct populations of *Sceloporus consobrinus* across

a latitudinal thermal gradient. Adult female lizards from each population were collected and exposed to two separate 23.5 hour temperature treatments in the lab: 18°C and 37°C. Metabolic rates were measured at each temperature, and blood samples were collected before and after each temperature exposure. Following DNA extraction and digestion, the oxidative damage biomarker 8-hydroxy-2'-deoxyguanosine was quantified for each individual using liquid chromatography with tandem mass spectrometry. As expected, our findings show that metabolic rates are higher at warmer temperatures. We will also discuss how oxidative damage levels are affected by geographic location and temperature. As climate change continues to amplify temperature extremes, ectotherms may be disproportionately impacted, ultimately influencing cellular and organismal performance.

Balancing my human experience and collaborative research on fluid homeostasis in tropical frogs

Bryan Juarez, Madison Lacey, Isaac Quintanilla-Salinas, Lauren O'Connell

Balance is key for human society and biological homeostasis. I will first discuss the concepts of race and ethnicity used by descendants of the native people of North and South America, our sense of identity in academia, and the need to balance academic demographics. Then, I will present collaborative research on water balance in anuran amphibians. Frogs possess a range of skin phenotypes ranging from permeable in aquatic species to less permeable in terrestrial species. Thus, we might expect aquatic species to dehydrate faster than terrestrial species. In addition to skin permeability, other factors such as water absorption, evaporation, urination, and temperature determine net dehydration rates (mass loss). Since dehydration is typically associated with cold and dry temperate environments, relatively fewer studies have been conducted on tropical species. The goal of this study was to determine how net dehydration rates vary according to water availability and temperature (26°C, 36°C) among tropical frogs of various microhabitat types (aquatic *Xenopus*, semi-aquatic *Rhinella*, terrestrial *Phylllobates*). We analyzed these data using phylogenetic generalized linear mixed models. Preliminary results suggest the importance of both microhabitat type but also behavior where some frogs chose not to use available water resources in hot treatments. Our study highlights the importance of temperature, water, and behavior, which can act as a buffer or intensifier of environmental effects on fluid homeostasis.

Compensating for effects of autotomy on locomotion in stink bugs (*Halyomorpha halys*)

Lilian Jubb, Brandon Jackson

Autotomy is the voluntary release of an appendage, typically to avoid predation or entrapment. Autotomy of a single leg can have minimal effects on locomotion in arthropods with more than six legs. Hexapods, however, use a double-tripod gait which is completely disrupted with the loss of a single leg, thus locomotor performance should be greatly reduced leading to lower survival. Autotomy is so costly to locomotion that some insects regenerate a lost limb in a subsequent molt leading to reduced investment in other important functions (e.g. reproduction). Autotomy persists in adult hexapods with no opportunity for regeneration, which suggests some ability to mitigate the severe effects of autotomy on locomotor performance. We hypothesize that insects have neural plasticity that helps them improve stability and mobility after the loss of a leg. In order to test this hypothesis, we chose to record and measure the effects of autotomy in adult brown marmorated stink bugs (*Halyomorpha halys*). We used high-speed cameras (Edgertronic, 100 fps) to record the stink bugs walking pre-autotomy, and at three times post-autotomy: immediately, one hour, and one week. We tracked eight points on the body and four points per leg with Deeplabcut and reconstructed 3D motion with Anipose. Following autotomy, we observed walking speed initially decreases, and variation increases for most metrics of gait.

Evolutionary and Functional Characterization of a Chromatin Regulatory Gene Family in *Drosophila*

Jack Jurmu, Andrew Arsham, Riley Reed

Many vital eukaryotic developmental processes hinge on the formation and regulation of heterochromatin. Broadly, heterochromatin is gene poor and a tightly compressed structure of chromatin which restricts the transcription of local genes. While much genomic heterochromatin (for example centromeres and telomeres) is constitutive, it can also be induced as a genomic defense against invasive DNA, and facultative heterochromatin regulates developmental gene regulation. Recently, genes in the ZAD family have been identified as key regulators of heterochromatin in *Drosophila melanogaster*. The ZAD family is evolutionarily dynamic and has expanded sporadically across insect lineages via retrotransposition and duplication. Phylogenetic analysis of a subset of ZAD genes across the *Drosophila* genus revealed striking patterns, with one dynamic ZAD subfamily containing almost all observed gene birth and death, and another subfamily containing single-copy orthologs stable over 30 million years of evolution. This pattern resembles the so-called phylogenetic blooms found in cytochrome P450 evolution in a wide range of taxa. We screened ZAD genes for heterochromatin regulatory function using RNAi in *D. melanogaster* to investigate functional differences between stable and dynamic ZAD subfamilies.

Feeding biomechanics and physiology of infant pigs raised on standard and biomimetic bottle nipples

Elska Kaczmarek, Kendall Steer, Max Sarmet, Hannah Shideler, Alexane Fauveau, Ani Smith, Skyler Wallace, Maressa Kennedy, Alex-Ann Velasco, Thomas Stroud, Morgan Blilie, Christopher Mayerl

Many infants are bottle fed rather than breastfed for a variety of reasons, however, infants use different biomechanics when bottle feeding (expression) compared to breastfeeding (suction) and derive greater physiological benefits from breastfeeding. One potential mechanism that may drive these differences could lie in bottle design. Typical bottle nipples have open space inside them, like a cistern, while breast tissue in most mammals has ducts embedded in connective tissue. We designed a biomimetic ducted bottle nipple and used pigs (which have ducted breast tissue) as a model to evaluate if feeding biomechanics and physiology differs between infant pigs raised on our ducted nipples (n=4) and those raised on conventional cisternic nipples (n=4). In addition, after raising each group of pigs on their assigned bottle nipple type, we fed them on the opposite nipple type. We quantified synchronous tongue and jaw kinematics, intraoral pressure generation, muscle activity, suck and swallow rate, and bolus size. We found that, when feeding on cisternic nipples compared to ducted nipples, all pigs had larger boluses, which has been correlated with greater risk of aspiration. When pigs raised on cisternic nipples were exposed to ducted nipples, they generated the greatest intraoral suction but obtained less milk per suck. This suggests that being raised on cisternic nipples results in decreased coupling of effort and acquisition when exposed to ducted nipples.

DNA Barcoding Nemertean Diversity in the Red Sea

Ethan Kahn, Audrey Wong, Svetlana Maslakova

The Red Sea is a region with high biodiversity and endemism, threatened by overfishing, pollution, and climate change. Critically, much of its diversity remains undescribed, especially small, soft-bodied, and understudied invertebrates, such as the predatory marine ribbon worms (phylum Nemertea). To date, 16 species of nemerteans have been documented from the Red Sea, but most are insufficiently described to permit subsequent identification, and are therefore considered invalid. The modern standard for species identification and description includes triple-documenting specimens with live images, morphological vouchers, and tissue samples for DNA barcoding. Based on recent sampling, partial Cytochrome Oxidase I sequencing, and species delineation analysis, we document 44 nemertean operational taxonomic units (OTUs, i.e. putative species) from the Red Sea. Of those, only one can confidently be assigned to a described species (98% are undescribed). Twenty-seven (61%) are only known to occur in the Red Sea, 15 occur elsewhere in Arabian waters (Oman), and five — more broadly. Notably, 18 belong to 15

cryptic species complexes, and 39 are sequenced for the first time. Diversity estimators predict much higher species richness (130-153 OTUs). Species are the basic unit of biological knowledge, and DNA barcoding expedites their discovery, delineation, and description, creating a baseline to monitor and conserve biodiversity. An estimated 66-71% of Red Sea nemertean species are still unknown, emphasizing the need for further comprehensive biodiversity surveys.

Unlocking the Mysteries Behind Ant Morphology using Ant in Motion

Darmon Kahvazadeh, Kaushik Rahman, Dal Hyung Kim, Clint Penick

Ant colonies function as a superorganism with diverse roles and functions which allow them to accomplish monumental feats. In a manner similar to the diverse functional range of human cells, most colonies feature a large range of body size and morphologies, which result in different ants being more suited to certain tasks than others. However, little research has been conducted exploring which ant morphologies are better suited to tasks involving the movement of materials, a role that requires ants to carry immense weight while in motion. This study aims to provide insight into which morphological traits define which ants are suitable to carry weight. By calibrating an automatic tracking system to accommodate ants carrying various loads, we can record the velocity, running speed, and gait patterns of ants in motion. In a previous experiment, we determined that ants with traits such as a large head provided a handicap to its exhibited velocity but showcased momentum that increased at a faster rate than body size would predict. We also observed that the speed of the ant increased with the overall size of the ant and gait length. It is unknown if these trends in data apply to ants carrying various weights. By observing changes in body size, leg length, and head size, and how they affect the maximum velocity and momentum at various weights, we can further understand the complex roles in ant colonies while assisting with the development of ant-inspired robots that mimic the biomechanics of ant movement.

Aerodynamics and structural modes of a high-fidelity dragonfly wing: implications for mechanosensing

Haruhiro Kajiyama, Richard Bomphrey, Huai-Ti Lin, Masateru Maeda

Dragonflies have hundreds of mechanosensors distributed on each wing. Information from these sensors is thought to be used for controlling wing kinematics. Fine detail of the wing morphology determines both its structural properties, and hence its mechanical behaviour, as well as the local flow field around each sensor. Recent sensor distribution mapping combined with numerical simulations enables the relationship between wing morphology and sensor distribution to be explored. Many of the wing-mounted mechanosensors are short hairs located along the major longitudinal veins and are likely to combine to provide information regarding the flow field. Others include campaniform sensilla that are capable of encoding spatiotemporal strain distribution patterns under aeroelastic buckling.

We constructed a high-fidelity, three-dimensional dragonfly wing model of a Common Darter dragonfly (*Sympetrum striolatum*). The venation pattern was obtained by stereo reconstruction of digitised points on fresh wing images, while the cross-sectional geometry of each vein was extracted from micro-CT imaging. Using this detailed model, we were able to assess gliding aerodynamic performance using computational fluid dynamics, conduct modal analyses using computational structural dynamics, and discuss the impact of details of wing morphology on sensory input.

Perceptual salience is insufficient for auditory streaming in eastern gray treefrogs

Lata Kalra, Mark Bee

Auditory streaming is the process of organizing complex acoustic mixtures into distinct 'streams', corresponding to the perceptual representations of different sound sources. This process requires that sounds produced by the same source must be integrated into a single stream while those produced by different sources must be segregated into distinct streams. Human psychoacoustic research has led to a prominent hypothesis that any perceptually

salient acoustic difference can promote auditory streaming. Compared to humans, auditory streaming is relatively less understood in non-human animals. Here, we used *Hyla versicolor*, a treefrog that breeds in noisy choruses, to test the hypothesis that perceptually salient acoustic differences are sufficient to promote auditory streaming. Our experimental paradigm consisted of interleaved pulsatile sequences (ABAB...) exhibiting differences in pulse rise-time, a robust signal recognition cue. We used phonotaxis assays to test if a perceptually salient difference in pulse rise-time promotes auditory streaming. Our results do not support our hypothesis. We conclude that perceptual salience is insufficient for auditory streaming in at least one non-human animal and that animals may differ in the range of cues that promote auditory streaming.

Morphological Disparity in the Weberian Apparatus of Marine and Freshwater Catfishes

Khizur Kamran, Jennifer Hoefflich, Juan Liu

The Weberian apparatus, the conductive hearing system of Otophysan fishes including catfishes, comprises four ossicles: scaphium, claustrum, intercalarium, and tripus. We investigated whether habitat correlated with ossicle morphology in Plotosidae, one of only two catfish families containing marine and freshwater species. We obtained CT image stacks of *Anodontiglanis dahl*i, *Neosilurus hyrtli*i, and *Porochilus obessi* (freshwater), as well as *Euristhmus lepturus*, *Paraplotosus albilabris*, and *Plotosus lineatus* (marine-brackish) from the MorphoSource database. Selected scans were required to have a voxel size under 60 micrometers and include the Weberian ossicles. The ossicles were segmented out in the Avizo software. Qualitatively, the scaphium and tripus (the beginning and end of the ossicle chain) of marine and freshwater species showed apparent morphological differences, whereas the intercalarium and claustrum did not. The anterior process of the tripus in marine species is thin; in freshwater species it ranges from thick to thin. In marine species, the tripus's ossification to the complex vertebra is shorter and the concha of scaphium is more elongated and dorsal ventrally depressed than in freshwater species. Wave activity in marine environments produces a noisier soundscape than most freshwater environments, which may contribute to the differences between the two groups. Our future directions of this study include increasing sample size and quantifying shape variation using geometric morphometrics methods to statistically test the effects of soundscape on hearing apparatus.

Mutations in a FMN reductase gene drive 5-Nitroimidazole resistance in *Mycoplasma genitalium* strains

Abhi Kancherla, Alessandro Rizzi, Cameron Weller, Derek Wood, Gwendolyn Wood

Mycoplasma genitalium (MG) is a sexually transmitted pathogenic bacteria that causes urethritis in men and pelvic inflammatory disease (PID) in women. MG has developed resistance to first-line antibiotics like azithromycin, doxycycline, and moxifloxacin—making it one of three microorganisms added to the CDCs Watch List for Antibiotic Resistant Threats in the US. 5-Nitroimidazoles (5-Ni) are being evaluated as alternative treatment options based on two recent studies. A 2020 study by Weisenfeld found that adding metronidazole to a standard multidrug treatment with ceftriaxone and doxycycline reduced MG presence in patients with PID and we recently showed that MG was susceptible to 5-Nis. These drugs may provide alternative treatment options following clinical studies, however, MG can develop resistance to them. In this study, we isolated and sequenced the genomes of nine 5-Ni-resistant G37S mutants with the MinION Mk1B genome sequencer. We identified mutations in the MG_342 gene, which encodes for a putative NAD(P)H-dependent FMN reductase. Mutations in the FMN reductase may cause reduced expression or loss of function of the enzyme leading to 5-Ni resistance in these mutants.

Fishy friction: Microscopic features on sculpin fin rays and their potential role in station-holding

Emily Kane, Austin Garner, Lily Hume, Thomas Pesacreta

Habitats with strong flows, such as fast-flowing rivers or wave-battered coastlines, can produce forces that fatally dislodge organisms or other objects.

Therefore, animals that live in these areas display adaptations that take advantage of biomechanical interactions with the substrate to prevent being swept away. In fishes, this includes traits such as small size, negative buoyancy, and negative lift generation, as well as more specialized abilities to directly grip or attach to the substrate. Marine sculpins typify many of these adaptations and have been used as a model for understanding station-holding in flow. However, macroscopic features have primarily been considered and the potential for microscopic features of the fins to assist in maintaining position have been overlooked, despite such structures being nearly ubiquitous in aquatic attachment systems. We recently documented epithelial projections on the ventral surfaces of sculpin paired fins, which vary across habitat type, fin type, and fin ray position. Similar projections have been described in other fishes, where they are presumed to enhance friction with the substrate. Therefore, microscopic friction-enhancing mechanisms may be more pervasive in benthic fishes than previously thought. These observations suggest a convergent example of a functional mechanism for maintaining position in flow and stimulate new questions regarding how these features complement other adaptations for benthic lifestyles in fishes.

Combining pose estimation, rendering, and simulations to understand complex motions

Suzanne Amador Kane, Chengpei Li, Theodore Bien, Aaron Xu, Benjamin Alexander, Natalie Goeler-Slough, Eric Beery, S. Tonia Hsieh

The potential accuracy, complexity, and detail of mathematical models of animal motion have increased dramatically along with greater access to inexpensive, fast methods for tracking motion, creating 3D models of organisms, and performing computer simulations. We present here two studies that model complex motions of the invasive spotted lanternfly (SLF, *Lycorma deliculata*) during jumping and self-righting—two ecologically-important behaviors frequently used by this species to evade predators and to disperse. This work used, a three-fold combination of: 1. 3D pose estimation based on high-speed multi-angle video; 2. the creation of detailed, realistic, posable 3D models based on photogrammetry and Blender rendering software; and 3. physics-based computer simulations. We give specific examples of how this multi-pronged approach was well-suited to reveal likely mechanical consequences of the suite of stereotyped SLF postures and motions observed during jumping and self-righting, and discuss the advantages, limitations, and remaining challenges of coordinating these multiple resources in the specific context of these SLF behaviors.

How “toe” spacing and flexibility influence active and passive foot intrusions into granular media

Suzanne Amador Kane, S. Tonia Hsieh, Willow Kohn, Syalomee Pradhan

Animals have evolved a wide diversity of foot shapes to meet the challenging task of running on sand. For example, sand-dwelling lizards may have iguanid-shaped feet with different length toes, fringed toes, or radially-arranged toes as are found among geckos. Interpreting the influence of foot morphology on intrusion into sand and other granular media is complicated because much prior work has focused on passive intrusions by rigid objects with simple geometries. Here we present the results of experiments in which 3D printed models of simplified lizard feet were intruded into poppy seeds with particle diameter (pd) and packing density similar to that of desert sand. Model feet with different toe geometries (splayed vs parallel), spacings, and compliance were either passively dropped or actively forced into poppy seeds at speeds consistent with those typical of sand-running lizards. As reported earlier for pairs of parallel rods and plates, we found that the work performed during active intrusion significantly depended on foot geometry, and was greatest for a toe spread of 4–7 pd. We also report on the effect of toe compliance on the maximum force and intrusion depth.

Using simulations and unsupervised learning to explore gait patterns in spider autotomy data

Suzanne Amador Kane, Xuanyi Wu, S. Tonia Hsieh

Many animals use autotomy (voluntary appendage loss) to evade predators. When this involves the loss of a leg—as is common among spiders—the organism must adjust its locomotion in response. Much as insects run with an alternating tripod gait during which three alternate limbs move in synchrony, spiders often run with an alternating tetrapod gait during which groups of four alternating limbs move together. A previous study used relative limb phases to map the use of these predefined gaits after two-leg autotomy. Running spiders were found to either employ an alternating tripod gait or to limp while maintaining the original limb groupings. In this study, we determined how the gaits of running juvenile spiders varied with time after limb loss to explore the time course of recovery to a stable gait, and whether they respond differently to repeated autotomies. We recorded dorsal 500 frame/s video of juvenile tarantulas during running, first with all legs intact, then after they were induced to simultaneously autotomize two limbs, and again following a second autotomy after limb regeneration. To interpret these results, we simulated the gaits previously reported for spiders moving with legs intact or autotomized and used unsupervised learning to compare the tracked and simulated data vs time and search for the effect of limb loss on patterns of gait usage.

Parrot “Reach-to-Grasp” Behaviors Mimic Mammalian Coordination Patterns

Stratos Kantounis, Reuben Jacobson, James Virga, Noah Chernik, Melody Young, Edwin Dickinson, Michael Granatosky

The “reach-to-grasp” paradigm is a well-established neuroscience framework that investigates how different taxa plan and execute movements when reaching to grasp objects. It serves as a fundamental model for exploring motor control, sensorimotor integration, and coordination in object manipulation. Birds, with non-grasping forelimbs adapted for flight, present an intriguing case study within this paradigm. Parrots, in particular, have developed a range of anatomical adaptations in their beak and hindlimbs to compensate for their non-grasping forelimbs. However, there remains a paucity of empirical data regarding: 1) whether parrots preferentially use their prehensile feet or beaks to initiate object grasping; and 2) if parrots exhibit similar reach-to-grasp patterns to those seen in mammals. Pilot data collected from public-domain videos of feeding parrots indicated that, regardless of pedal dexterity, all parrots predominantly employed their beaks to grasp food. Subsequent detailed kinematic tracking of five focal taxa revealed that parrots consistently demonstrate similar patterns in position-velocity profiles to mammals, and employ effector pre-forming. The observed similarities between parrot reach-to-biting and primate reach-to-grasping suggest shared functional complexity and sensorimotor control mechanisms mediated by analogous neural structures. These parallels indicate that challenges faced by the parrot’s neck-jaw system in motor control resemble those of primates’ arm-hand systems. Despite the simpler control system of the avian jaw, studying parrot grasping behavior offers valuable insights into sensorimotor integration, potentially impacting neurobehavioral research significantly.

To Flex or Flee: Multimodal control of a chemical defense behavior in the rove beetle *Dalotia*

Jessleen Kanwal, David Miller, Mina Yousefalahiyeh, Jaison Omoto, Joe Parker

Animals interact with a multitude of other species in their natural environment. Such interactions rely on the nervous system’s ability to recognize complex multisensory profiles of other organisms and rapidly select the appropriate behavioral response. Despite the importance of interspecies interactions for survival, how animals integrate interspecies cues to enact ecologically relevant behaviors is largely understudied. Here, we use the rove beetle, *Dalotia coriaria*, a new genetically tractable system, to examine how multisensory representations of other species enable behavioral defense responses during predator-prey interactions. *Dalotia* defend themselves from predatory ants by flexing their abdomen, which leads to an aversive chemical secretion towards the threat. We reconstituted this chemical defense interaction with ants in both

freely moving beetles and tethered beetles walking on a spherical treadmill. We quantitatively deconstruct this behavior, resolving the spatial and temporal dynamics of the beetle's abdominal flexion and kinematic locomotor response upon ant approach. Our results indicate that *Dalotia* modulate the frequency and angle of abdominal flexion, and walking speed in a graded manner as ant proximity increases. By genetically ablating olfaction through CRISPR/CAS9 editing, our results suggest that volatile, contact-chemical, and tactile ant cues act combinatorially, eliciting separable behavioral components of the rove beetle chemical defense response. Overall, our study reveals how multisensory heterospecific stimuli release a behavioral response motif that defines the ecological relationship between two animal species.

The Processes Governing the Joining and Leaving of Social Groups in Clownfish, *Amphiprion percula*.

Natalia Karadimitriou, Lili Vizer, Peter Buston, Kian Thompson

Cooperatively breeding groups, where certain individuals sacrifice their own ability to reproduce in order to assist others in reproduction, exist in a delicate equilibrium between cooperation and conflict. Commonly, more powerful, dominant individuals maintain order using punishment and the threat of eviction. In response, less powerful, subordinate individuals employ counter-strategies including "pay-to-stay" and reproductive restraint. The role that the environment plays in influencing the behavior of dominants and subordinates has yet to be fully explored. Here, we investigate how anemone size affects dominant and subordinate behavior at the time of social group formation in the clown anemonefish *Amphiprion percula* in a laboratory setting. Specifically, we test the hypothesis that juveniles will exhibit higher survival, settlement, and recruitment in large anemones than in small anemones. To create natural variation in anemone size, we spent 8 months feeding anemones. We then reared larval clownfish and introduced them to breeding pairs living in anemones of different sizes. Our findings indicate a positive correlation between anemone size and the survival, settlement, and recruitment rate of juveniles. This could be due to dominants being more tolerant and/or less able to control subordinates in large anemones. This study provides new insights into the role of habitat characteristics in decisions governing the joining of social groups.

Coordinated gene expression in intestinal oligo-peptide and -saccharide breakdown and absorption

William Karasov, Enrique Caviedes-Vidal, Melisa Magallanes, Agustin Baricalla, Antonio Brun

Groups of genes whose expression are synchronized in order for them to work together have been referred to as correlated gene modules. For intestinal absorptive cells (enterocytes), the hypothesis of evolutionary economic design leads to the a priori prediction of synchronization along the pathway for substrate hydrolysis followed by apical brush border membrane (BBM) uptake followed by basolateral membrane (BLM) efflux towards blood. In house sparrow (*Passer domesticus*) nestlings raised on formulated diets we used RNA-seq data to test for predicted correlated gene modules involved in oligo-peptide and -saccharide breakdown and absorption. We also tested other predictions from the same hypothesis that expression of enzymes and transporters would correlate with dietary level of their respective substrates (protein or carbohydrate). Expression of BBM amino acid transporters (e.g., SLC6A19, SLC15A1, SLC7A9, SLC3A1) was correlated with expressed BBM hydrolases (ACE2, DPP4, FOLH1, MME, TMPRSS15, and XPNPEP1; $p < 0.0001$), and also with expression of BLM transporters (e.g., SLC43A2, SLC38A2; $p < 0.0001$), as predicted, and expression of all of these was higher in nestlings fed a higher protein diet ($p < 0.05$), as predicted. Likewise, SLC5A1 mRNA, which codes for the Na⁺-glucose cotransporter (SGLT1) correlated with the carbohydrase SI ($p < 0.0001$) and also was higher in nestlings fed a higher carbohydrate diet ($p < 0.05$). Supported by NSF, FONCYT, CONICET and UNSL.

Puncture Resistance of Spider Egg Sacs

Katherine Karkosiak, Abby Weber, Todd Blackledge

Some parasitoid wasps lay their eggs near spider eggs for the wasp larvae to consume upon hatching. Although spider eggs are surrounded by a silk barrier called an egg sac, many wasps still manage to insert their ovipositors through

the silk layer to place their young. Spider egg sacs vary in their structure based on the species, leading to differences in shape, thickness, materials, and the pore structure of the fibrous material that could affect their functional ability of preventing predator attack. We tested the puncture resistance of various spider species egg sacs using a Nanobionix tensile testing machine to measure the ultimate puncture force and work to puncture using three different sized needles. We used three-dimensional confocal image stacks to determine the thickness and porosity of different species egg sacs and used scanning electron microscopy post-puncture to view whether the puncture included breaking through fibers or slipping between fibers. We compared the porosity and thickness of egg sac types to their puncture resistance to test the hypothesis that thicker egg sacs with smaller pore sizes provide greater resistance to puncture, especially against smaller needle sizes.

Using Google Earth Engine (GEE) to Access Effect of Temperature on Sea Turtle Emergence Success Rate

Nattapat Karmniyanont, Jacob Lasala

Rising global temperatures are having an effect on sea turtle hatchling sex ratios and survival rates. While warmer beach temperatures lead to higher proportions of female hatchlings, shorter incubation periods, and higher hatching success rates, recent research suggests that excessive heat during incubation may also result in higher hatchling mortality rates. Unfortunately, obtaining nesting data to assess these trends can be challenging, leading to gaps in the literature. In this study, we aim to analyze nesting data from a large sea turtle rookery on the Gulf of Mexico to investigate trends in emergence success rates (ES) in conjunction with remotely sensed land surface temperature data using Google Earth Engine (GEE). We will evaluate the potential of LiDAR and satellite imaging technology for future sea turtle conservation efforts. These models will examine loggerhead sea turtle nests across five beaches monitored by Mote Marine Laboratory in Sarasota County, Florida: Longboat Key, Lido Key, Siesta Key, Casey Key, and Venice from 2013-2023. We propose that our methods and data can contribute to further integration of remote sensing technology for marine turtle conservation.

The effects of rain and cold temperature on thermogenic capacity and physiology in captive finches

Jessica Karr, Jalyn Devereaux, David Swanson, Jamie Cornelius

Short-duration, high-intensity precipitation events may increase with climate change, which could negatively impact the fitness of avian species if thermogenic capacity cannot counter sudden changes in heat loss. Long-term cold exposure increases metabolism in many species, but how avian thermogenic capacity and physiology are impacted by long-term exposure to precipitation in different thermal conditions is largely undescribed. We measured metabolic and physiological responses of 48 red crossbills (*Loxia curvirostra*) held in warm (21°C) or cold (6°C) temperatures and dry or rain precipitation treatments. Two additional cold treatment groups were assigned a short-day photoperiod. Birds experienced rain daily for 30 minutes, and we measured summit (Msum) and basal (BMR) metabolic rates on weeks 1, 8 and 14. Food intake, activity, and body size were measured, and tissues were collected three days post final metabolic measurements. We found no effect of daylength, however, Msum increased with both decreasing temperatures and rain exposure. Msum at week 14 correlated positively with heart and pectoralis muscle masses. Neither temperature nor precipitation were predictive of BMR. Temperature and precipitation impacted activity and food intake over time, but there was no correlation between food intake and activity among treatment groups. These findings suggest that cold rain induces expansion of thermogenic capacity in songbirds and may require individuals to invest more energy into self-maintenance under similar conditions in the wild.

Complementary dynamics of two central brain neuron populations support goal-directed odor navigation

Nicholas Kathman, Katherine Nagel, Hannah Gattuso

During plume navigation, insects use stochastic sensory cues to navigate towards the unknown location of an odor source. Recent genetic and

optogenetic perturbations have implicated the fan-shaped body (FB)— a part of the central complex— in this behavior. However, little is known about the dynamics of neural activity in this structure during ongoing odor-guided navigation. We developed a virtual olfactory navigation paradigm to investigate neural dynamics during this behavior using 2-photon imaging. Using odor pulses and naturalistic virtual plumes, we identified two populations of FB local neurons in flies that show complementary encoding of odor and self-movement during olfactory navigation. One population of dorsal local neurons (hACK) showed a slow and persistent bump of activity in response to odor and also encoded segments of straight running outside the odor period. A second population of ventral local neurons (FC1) showed transient responses to odor, and preferentially responded during turns. Despite these different encoding dynamics, both populations showed a relationship with task engagement. Silencing of both neural populations together, but not separately, profoundly impaired olfactory navigation in a wind tunnel paradigm. Together, our data suggest that olfactory navigation requires internal representations that integrate sensory odor encounters with self-movement on different timescales. Our work provides insight into the laminar organization of the central complex and the dynamics of neural representations required for goal-directed navigation in noisy and uncertain sensory environments.

Testing the developmental role of nuclear receptors regulated by a plasticity switch

Eleni Katsougia, Erik Ragsdale

Nuclear receptors (NRs) include important mediators between environmental signals and the outcomes of plastic development. In the case of polyphenism, whereby developmental plasticity results in categorically alternative forms, such factors are predicted to allow switching between alternative gene networks. In the nematode *Pristionchus pacificus*, external cues such as starvation promote the development of alternative mouthparts, allowing access to different food resources when induced. Thus far, two NRs (NHR-40 and NHR-1) that regulate the polyphenism have been uncovered through forward genetic screens. However, co-expression networks influenced by these and other known, polyphenism switch genes have revealed potentially subordinate NRs in this plastic developmental response. Whether and how such transcription factors affect the outcome of a polyphenism is unknown, although intermediate phenotypes in known polyphenism regulators suggest that downstream factors may influence the integrity of canalized forms. To test this idea, we performed a reverse genetic screen for the effects of NRs revealed by polyphenism-biased co-expression modules. Specifically, we used CRISPR to perform functional analyses for NRs whose expression was controlled by multiple morph-constitutive lines. In summary, our results give insight into how alternative gene-networks faithfully execute a polyphenism decision.

Locomotor and Musculoskeletal Adaptations to Growth-Period Load Stimulus

Kavya Katugam-Dechene, Talayah Johnson, Stephen Piazza, Jonas Rubenson

We previously demonstrated that guinea fowl that were chronically limb loaded (LL) during development carried their additional limb mass remarkably more economically than control animals (CON). However, the mechanical underpinnings of these adaptations are less clear. We examined whether adaptations to (1) gait mechanics, and/or (2) muscle architecture, can explain the improvement in load-carrying economy observed after growth-period loading. After the growth period, LL and CON animals were video recorded treadmill walking in both unloaded and unilaterally loaded conditions. We analyzed swing-phase joint work and power in comparison to the animals' metabolic data. Animals were euthanized for muscle architecture analyses, with specific focus on the tibialis cranialis, an ankle flexor active during limb swing. Results showed that both CON and LL animals increased mechanical power production when unilaterally limb loaded, but that joint mechanical power did not explain the improved load carrying economy in LL animals. Muscle analyses in LL animals revealed between-limb differences in mass, pennation, and sarcomere length, with multiple adaptive strategies for increasing mechanical work and power capacity. These findings indicate that adaptations to gait mechanics alone do not explain the observed developmental improvements

in locomotor economy. Rather, plasticity of muscle architecture more likely accounts for energetic plasticity. These data are important for understanding developmental locomotor biology and have practical implications for human childhood health. Funded by NIH Grants R21AR071588 and R01AR080711.

Elephants develop wrinkles through both form and function

Lena Kaufmann, Andrew Schulz, Noémie Reveyaz, Cindy Ritter, Thomas Hildebrandt, Michael Brecht

Elephant trunks have prominent folds and wrinkles from birth, but we have little information on how wrinkle patterns differ between elephant species and how elephant trunks and their wrinkles develop. We assessed wrinkle patterns in Asian (*Elephas maximus*) and African savanna (*Loxodonta africana*) elephants. We find that adult Asian elephants have more dorsal trunk wrinkles (~126) than African elephants (~83). In both species, we find more dorsal than ventral trunk wrinkles and a closer spacing of wrinkles in the distal than in the proximal trunk. We also observed slight (10%) differences in wrinkle numbers as a function of trunk-lateralization, suggesting the wrinkle-pattern is use-dependent. MicroCT imaging revealed that the outer elephant trunk skin has a relatively constant thickness, whereas the inner skin parts are thicker between folds than in folds. In both elephant species in early fetuses the trunk shows the greatest length growth of all body parts and the ventral trunk tip develops before the dorsal trunk finger. In development, trunk wrinkles are added in two distinct phases, an early exponential phase and a later, slower phase. We suggest that wrinkles improve the ability of trunk skin to bend, and that differential flexibility requirements might explain dorsoventral, proximal-distal, left-right side, and species differences in wrinkle distribution.

Asian elephant water hose tool use

Lena Kaufmann, Lea Urban, Rolf Becker, Andreas Ochs, Michael Brecht

Animal tool use can be found across different species, with the most famous examples ranging from groups as diverse as primates, corvids, dolphins, or cephalopods. Elephants have been described to not only use tools but even modify branches to use them as fly switches (Hart et al. 2001). Here, we investigate the use of a water hose as a tool by a female Asian elephant named Mary at the Berlin Zoo. Due to their length, flexibility, and the water flow, hoses can be seen as relatively complex tools. Elephants regularly shower themselves, spraying water with their trunk, and water is essential for them, not only for drinking but also for body care. Depending on which body part Mary is showering, she grasps the water hose differently. The hose grasping behavior is lateralized and on average she grasps about 15 cm further back from the tip when she showers her right side (54 cm) compared to showering her left side (40 cm). When showering her back she grasps the hose even further back (62 cm). When water flow is stopped she also stops showering. In ongoing experiments, we offer Mary water hoses differing in diameter and stiffness and we investigate, whether she is actively washing off mud. Further, we are looking at behavioral responses from other elephants to Mary's showering.

Molecular Effects of Yolk Hormones During Embryonic Development

Jasmeen Kaur, Ryan Paitz, Alexandra Bentz

Females provide non-genetic information to their offspring in response to the environment. For example, birds allocate numerous hormones to their egg yolks in response to competition, predators, and other stimuli. These hormones can generate long-lasting changes to offspring physiology and behavior and potentially an adaptive means by which females shapes phenotypic plasticity. Despite the potential benefits of hormone-mediated maternal effects, very little work has been done to identify the molecular mechanisms by which maternal hormones shape offspring traits. Here, we explore how maternally derived hormones correlate with patterns of gene expression during early embryonic development using house sparrows (*Passer domesticus*). Eggs were collected and incubated to three developmental timepoints that occur prior to endogenous hormone production, including embryonic day 0 (ED0), ED3, and ED5. We collected yolk at each timepoint to quantify the concentration of 31 hormones and measured genome-wide gene expression in embryonic brain (forebrain and midbrain) and extraembryonic tissues at ED3

and ED5. We determined how yolk hormones and gene expression patterns changes over time and the degree to which they relate to each other. This work sheds light on how the maternal social environment is communicated to the embryo by showing how maternal hormones may be shaping molecular mechanisms in the earliest stages of development.

MATREX VR: Bridging Land, Water, and Air through Virtual Reality MATREX

Pavan Kaushik, August Paula, Anna Stöckl, Kajal Kumari, Liang Li, Ruiheng Wu, Iain Couzin

Collective behavior emerges from the spontaneous interplay of individual choices and interactions, orchestrating a spectacle across Earth, Air, and Water that is as captivating as it is complex to decipher. To navigate this complexity, we built MATREX, inspired from 'Prakriti Maye', wherein the tangible and the illusory interlace, manifesting an environment that subtly disrupts the boundaries between actuality and artifice. It's not a replication of an ecosystem but an illusion of one, gently guiding senses through an intricately constructed journey. MATREX stands for MATREX Architecture Terraforming Realistic Environments in X; X is in {Earth, Air, Water}. At its core, the MATREX enables isolated individuals, each positioned within their own arenas, to perceive and interact with each other in real time. Thus, crafting virtual swarms composed of real individuals, behaving as though in the natural world. To achieve this, we generate panoramic naturalistic input using high refresh rate, commercial LED panels and off the shelf parts. We designed parametric 3D printable modules to accommodate a wide range of organisms from *Drosophila* to a full adult Locust, paving the way for modular, low cost, and scalable arenas for studying walking and flying collectives. Advancing beyond traditional yaw-focused setups, our flight system features a fully 3D printable, 6-axis force-torque sensor capturing pitch, yaw, roll and translational forces. Our open source system simplifies the intricate data capture process and democratizes access to high throughput VR research. With its unique capacity to probe the neural basis of individual and collective decision-making, MATREX VR takes us a step closer to deciphering the complex interplay of collective behavior and untangling the enigma it presents.

Intrinsic Embryo Loss, Robustness, and Environmental Risk Among Vertebrates

Kathryn Kavanagh, Swapan Mallick

Populations adapt through natural selection on available phenotypic variations. Variation facilitates adaptation, but increased deployment of variational mechanisms (mutations, recombination, aneuploidy, or developmental network instability) also increases the risk of embryonic deformity and death. This "intrinsic risk with benefits" can be estimated by non-predation embryo loss rates. Here we ask, if extrinsic risk to embryos (predation, environmental risks) is reduced, e.g. by parental care, then does the trade-off shift such that mechanisms producing additional variation are selected? Do clades with protected embryos evolve greater intrinsic risk - with the associated variational benefits? In a comparison of literature-derived data on intrinsic and extrinsic risk for embryos of all major vertebrate clades, we find preliminary evidence in support of this hypothesis. To further examine this idea, we ran simulations to see how robustness evolves under different risk scenarios as a way to understand more about this trade-off. We found robustness always goes to fixation in stable environments, even with high risk. In a scenario with periodic massive mortality, both robust and variable embryos exist in the population if they have a moderate chance of survival. Intrinsically variable embryogenesis becomes dominant only when they have very high 'luck' and very low predation/environmental risk, a scenario suggesting the mammal strategy and fitting with the high intrinsic mortality of mammal embryos.

Applying 3D models of giant salamanders to explore form-function relationships in early tetrapods

Sandy Kawano, Johnson Martin, Emma Hsiao, Joshua Medina, Matthew Evans, Riley Lima, Jonathan Huie, Esther Langan, Kevin de-Queiroz, Matthew Carrano, R. Pyron, Duncan Irschick

The water-to-land transition was a pivotal event in tetrapod evolution that involved major transformations to the musculoskeletal system. Extant salamanders are used as modern analogs of early tetrapods due to general similarities in morphology and ecology, but the earliest tetrapods were aquatic and an order of magnitude larger than the terrestrial salamanders that have been studied to date. To better understand the locomotor biomechanics of an aquatic early tetrapod, we created a 3D computational model of under-water walking in Japanese giant salamanders (*Andrias japonicus*) through an interdisciplinary collaboration of scientists and computer animators from academic, non-profit, and government organizations. We present our giant salamander model to demonstrate our open-access pipeline, which can be quickly implemented on any computer and by any user to test how changing morphology and movement affects key locomotor variables (e.g., body speed, stride length, elbow flexion). 3D models create avenues for exploring biological limits to form and function by allowing users to conduct manipulative experiments that are difficult to perform (in vivo) (e.g., lengthening bones) and expand the range of testable hypotheses (e.g., 'what if shoulder mobility was limited?'), but developing computational models is a complex process. Our user-friendly pipeline streamlines the process to enable a broader community to get started in 3D modeling, thereby improving accessibility and advancing discovery in research and education.

Tooth sockets drive molar cusp offset across Rodentia

David Kay, Paul Gignac, Haley O'Brien

Mammals exhibit complex, multi-cusped crowns, the phenotypes of which can be directly affected by aspects of alveolus morphology. For example, parallel or offset tooth cusps were induced to vary based on manipulated lateral alveolar thickness in experimental vole and murine models. Whether or not this causal relationship is unique to these groups or is a more common aspect of rodent crown complexity is unknown. Here we evaluate the phylogenetic breadth of this pattern by hypothesizing that relative jaw robusticity represents a clade-wide mechanism for offsetting molar cusps. If supported, then cusp offset angle will be correlated with relative jaw robusticity across a broad phylogenetic sample of rodents. To test this hypothesis, we sampled eight rodent species from seven rodent families for: 1) lingual and buccal alveolar bone width at the dorsoventral midpoint of the m1 socket, 2) m1 width for body-size standardization, and 3) cusp offset angle between the protoconid and metaconid. We used phylogenetic generalized least squares regression to examine the linear relationship between alveolar thickness measures and cusp offset angle while accounting for relatedness. Results show a significant but poorly correlated relationship between the amount of alveolar bone and cusp offset angle. This pattern implicates the role of alveoli in shaping cusp complexity in the most speciose order of living mammals, indicating a previously unappreciated, broad contributor to tooth diversity within living thecodonts. Mammals exhibit complex, multi-cusped crowns, the phenotypes of which can be directly affected by aspects of alveolus morphology. For example, parallel or offset tooth cusps were induced to vary based on manipulated lateral alveolar thickness in experimental vole and murine models. Whether or not this causal relationship is unique to these groups or is a more common

Uncovering the odor transformation from antennal lobe neurons to descending neurons in the hawkmoth

Pramod KC, Jeff Riffell

Insects have evolved a remarkably sensitive olfactory system to locate relevant floral odors. However, how the flower odors are encoded in the primary olfactory system, the antennal lobe (AL), and the architecture of downstream descending neurons (DNs) that mediate sensorimotor information processing is unclear. Using the *Manduca sexta* moth, and floral odors from its hostplant, *Datura wrightii*, we combined neural ensemble recording in the AL, and a

sharp glass electrode for intracellular recording and staining of DN from one of the cell bodies clusters (DNA: Liu et al., 2023) to measure the odor-evoked response to the synthetic odor mixture of 7 components and its diluted ratios of *Datura wrightii*. As a first step, we retrogradely stained neurons in the neck connectives to characterize the distribution and organization of DNs in the brain. Five DN cell body clusters were identified and located in different regions in the central brain and these neurons primarily arborised in the ventral part of the brain. From the AL recordings, we found that the neural ensemble produced different patterns of response in the AL to the floral mixture and its diluted concentration. Furthermore, there is odor-evoked synchrony between AL and DN neurons to the floral mixture and its diluted concentrations. These findings will help to contribute to understanding the neural processes mediating an important moth-flower mutualism.

Thermal preference of heterothermic bumble bees across species and life stage

Ellen Keaveny, Travis Rusch, Eleanor Holloway, Michael Dillon

Temperature influences nearly every aspect of ectotherm life cycles. Therefore, many ectotherms have strong thermal preferences, seeking out environments to optimize body temperatures for key fitness-related traits. Heterotherms sometimes allow body temperatures to track ambient temperatures but can also expend energy to endogenously regulate body temperatures. They could save energy by selecting ambient temperatures that minimize the cost of regulating optimal body temperatures. For heterothermic bumble bees, temperature effects on physiological and life history traits are well-documented but thermal preference has not been studied. Bumble bees are annual with spring queens initiating colonies which ultimately produce fall queens that overwinter. We hypothesized that preferred temperatures would vary with species and life stage: spring queens select microhabitats with temperatures that facilitate colony growth whereas fall queens select underground hibernacula with temperatures that facilitate maintenance of depressed metabolic rates until spring emergence, the timing of which can vary by species. To test this hypothesis, we tracked selected temperatures and body temperatures of queens on a thermal gradient. Mean preferred gradient temperatures did not vary across life stage but minimum and maximum preferred gradient temperatures and body temperatures did. Spring queens maintained higher, less variable body temperatures. Similarly, across species, *B. huntii* preferred a narrower range of gradient temperatures and maintained higher, less variable body temperatures than *B. vancoverensis*, suggesting species-specific differences in preferred ambient and body temperatures.

Larval dietary restriction makes butterfly wings less colorful

Chloe Keck, Carol Boggs, Daniel Speiser

Resource allocation, the process by which consumed nutrients are directed toward growth and reproduction, is key to understanding an organism's life history. Organisms acquire a set amount of nutrients throughout their lifespan, so allocating those nutrients to effectively increase fitness is necessary. Resource allocation strategies balance the distribution of these nutrients, resulting in trade-offs. Holometabolous insects, such as butterflies, allocate larval nutritional energy during the pupal stage of development toward various adult traits, such as wing color. In many species, wing appearance is involved in mate choice and recognition. In *Speyeria mormonia* (Lepidoptera: Nymphalidae) butterflies, male adults preferentially approach females with more orange wings. Therefore, wing color is a key component in individual fitness. Here, I examine the effects of decreased larval food resources on adult *S. mormonia* wing color. I performed a larval dietary restriction experiment in which there were two experimental groups. The control group received *Viola* larval hostplant ad libitum, and the treatment group received half the quantity of *Viola* hostplant as the control group. After pupation, adult wing color was measured using reflectance spectroscopy and pigment quantity was measured using thin-layer chromatography. Preliminary results suggest that larval dietary stress affects wing color, in that the wings of adults with a complete larval diet are a more saturated orange in color and contain more pigment.

Evolutionary Morphology of Snake Hemipene Spines Informed by Puncture Mechanics

Rachel Keeffe, Bingyang Zhang, Philip Anderson, Patricia Brennan

The genitalia of squamate reptiles differ from other amniotes in that they develop paired intromittent organs called hemipenes. Hemipenes are morphologically diverse, varying in number of lobes, relative size, and the presence or absence of complex features like calyces, flounces, and calcified spines. Hemipene spines differ in size, shape, density, and position along the length of the hemipene. Despite this diversity, no studies have tested the functional properties of different spine morphologies. It is assumed that spines puncture into the vaginal tissue during copulation to secure the hemipene in place and aid in sperm transfer success, but this assumption has not been investigated in detail. Here we describe for the first time the puncture mechanics of different hemipene spine shapes. From CT scans of 7 snake species representing 4 families, we isolated 12 spines of different morphologies and produced 3D mesh files that were then 3D printed in resin. Using the 3D models of the spines, we performed serial compression tests into polydimethylsiloxane silicone polymer at increments of 10 degrees until puncture was impossible. We found that the range of successful puncture angles differed based on spine shape. Our results will help us understand the evolutionary forces driving snake genital evolution and will provide a broader context for the role of genital spines in other animals.

Bio-Inspired Magnetoreceptive Navigation In Space

Catherine Kehl

Established methods of navigating away from Earth are computationally expensive and not good at compensating for deviations from the expected path without relying on long distance, slow communications. While these methods are mature and reliable, a complementary approach which relies only on local data and which can be run on less powerful systems can fill in some of these shortcomings. Stars and many large planets have strong magnetic fields, which can provide locational data. Uranus, one of the ice giants, has such a magnetic field, though it is notably different from that of earth, as the magnetic pole does not pass through the center of the planet, and is also not parallel to the axis of spin. Here we are using magnetoreceptive navigation algorithms drawn from animals on earth to attempt to navigate over a model of the navigational field of Uranus. These algorithms successfully can navigate between starting and end points a) along a path towards and away from the magnetic poles b) around the planet perpendicular to the poles, and c) arbitrary starting and end points, though there are some navigation failures with points too close to the magnetic poles.

Reducing corticosterone may affect resistance and tolerance during acute avian malaria infection

Tosha Kelly, Keegan Stansberry, Melanie Kimball, Kaitlin Couvillion, Allison Cannon, Christine Lattin

The hypothalamic-pituitary-adrenal axis is ideally positioned to mediate resistance and tolerance to disease because it secretes corticosterone, which shifts immune profiles toward a more anti-inflammatory state. Exogenous corticosterone supplementation reduces host resistance (increases parasite loads) during avian malaria infection and has mixed effects on tolerance. We tested whether this relationship is linear by reducing corticosterone secretion using the drug mitotane before and during acute malaria infection (*Plasmodium relictum*) in house sparrows (*Passer domesticus*, $n=36$). We predicted higher resistance (lower parasite loads and fewer individuals infected) and lower tolerance (lower physiological condition for a given parasite load; higher fatality) in mitotane-exposed sparrows compared to control sparrows. Mitotane reduced restraint-induced circulating corticosterone, but not baseline corticosterone. Sixteen of the 18 sparrows receiving mitotane became infected with malaria compared to 11 of the 18 control sparrows ($X^2 = 3.7$, $p=0.054$). Although infected mitotane and infected placebo sparrows had similar circulating peak parasite loads 13 days post infection ($z=0.4$, $p=0.7$), only infected mitotane-treated sparrows exhibited mortality ($n=2$). However, infected mitotane-treated sparrows and infected controls did not differ in hematological measures of tolerance (residuals of hemoglobin and hematocrit

scaled against parasite load). These results suggest a non-linear relationship between corticosterone and host resistance and tolerance to disease, and a role for stress-induced corticosterone with some measures of avian malaria resistance (inoculation outcome) and tolerance (death).

Impact of individual plasticity and supergene genotype on *C. maenas* heat wave response

Julia Kelso, Yaamini Venkataraman, Carolyn Tepolt

The European green crab (*Carcinus maenas*) is a high-impact invasive species due in part to its broad thermal tolerance. A multi-gene region in the *C. maenas* genome, called a supergene, has been linked to thermal tolerance on a population level. As marine heat waves intensify in the Pacific Northwest, it is important to understand how these conditions impact green crab spread and survival. This project investigated how prior heat wave exposure and supergene genotype affected crab response to a secondary heat stress. Thirty-six crabs from Grays Harbor, WA were genotyped at the supergene, predominantly displaying the putative cold allele (62.5%). Crabs were distributed into two experimental groups which were acclimated and maintained at 15°C. The treatment group experienced two 20-24 hour heat waves (30 °C) during days 1 and 5 of the experiment. The control group only experienced heat wave conditions on day 5. On day 5, the treatment group showed a significantly smaller mean difference in time-to-right (time for a crab to right itself) between days than the control. Treatment crabs consumed oxygen at a lower rate than the control group on day 5, but this trend was not statistically significant. While genotype and physiology showed no significant association, future work should investigate this with larger sample sizes. These results show how individual plasticity may facilitate green crab spread in a warming climate.

A Comparison of Reproductive Metrics in Captive and Wild Dark-eyed Juncos

Anna Kelson, Ellen Ketterson, Sarah Wanamaker

Biodiversity is declining and the loss of birds is well documented. Three billion fewer birds are found in North America than 50 years ago owing to declines in productivity. Captive rearing is a conservation tool that might be used to combat losses, but it is notoriously difficult to breed songbirds in captivity due to the complexity of recreating natural reproductive cues in captive settings. However, few studies have directly compared breeding metrics between captive and wild passerines. We compared clutch size, hatch rate, and fledging success between captive and wild dark-eyed juncos (*Junco hyemalis*). Data was collected from captive dark-eyed juncos housed at Kent Farm aviary (Bloomington, IN) and from their wild counterparts at Mountain Lake (Pembroke, VA). Captive birds had significantly smaller clutches than wild birds, and the hatch rate of eggs laid in captivity was significantly lower than the hatch rate of eggs in wild nests. In captivity, nests that reached fledgling stage also fledged a smaller proportion of nestlings. Captive birds fared worse at each stage of the nesting process. We suggest that high population density in captivity may trigger increased territorial aggression and nest interference. Real or perceived dietary insufficiencies in captivity may also contribute to poor egg quality and low hatch rates, and could lead to partial or full brood loss due to brood reduction and starvation.

Variation in pectoral fin shape relates to swimming performance in the chimaeras

Duncan Kennedy, Kelsey Lucas

Chimaeras – an ancient group of cartilaginous fishes closely related to sharks, skates, and rays – inhabit the coast to the deep sea. By flapping their pectoral fins in a manner resembling bird flight, these fishes induce an undulatory wave on their pectoral fins which travels from leading to trailing edge. A trade-off exists between manoeuvrability and efficiency for fishes that swim by undulating their pectoral fins. Here, we investigate how fin shape influences swimming performance among chimaera species. A geometric morphometric analysis of the pectoral fins of 17 chimaera species reveals that the three chimaera families occupy distinct sections of the morphospace: Rhinochimaerids have broad fins, Chimaerids have slender fins, and

Callorhynchids have more moderately shaped fins. Additionally, the pectoral fin shape of Chimaerids is further split into genera based on convex versus concave trailing edges. We also built a fin-flapping robot and measured forces exerted by artificial chimaera pectoral fins. Preliminary results indicate swimming performance, and thus the trade-off between manoeuvrability and efficiency, is affected by morphology. Studying how variation in pectoral fin shape affects swimming performance will help explain how swimming demands among habitats may have contributed to chimaera diversification and could lead to the development of novel underwater propulsors.

Impact of Chronic Use of Viscous Milk on Infant Feeding Physiology: Swallowing

Maressa Kennedy, Elska Kaczmarek, Max Sarmet, Kendall Steer, Thomas Stroud, Alexane Fauveau, Ani Smith, Hannah Shideler, Alex-Ann Velasco, Skyler Wallace, Morgan Blilie, Christopher Mayerl

Infant mammals acquire food by swallowing liquids during suckling. While we know that variation in oral sensation can impact suckling physiology and performance, less is known about the downstream impacts on swallowing. For example, different food textures, such as increased milk viscosity, impact oral function and may also impact swallowing physiology and performance. We used an animal model, infant pigs, to investigate how the chronic consumption of thickened milk impacts swallowing function. We raised three infant pigs on standard formula and three on thickened formula. At the end of infancy, we collected videofluoroscopic data of pigs feeding on standard formula and thickened formula to evaluate if swallowing kinematics and performance differed between groups and milk type. In response to acute exposure to thickened milk, control pigs reduced their swallow rate and had increased pharyngeal transit time, while maintaining similar bolus size. In contrast, pigs raised on thickened milk swallowed smaller boluses than control pigs, especially when feeding on standard formula. While pigs raised on standard milk exhibited decreased swallow rate and lower swallow efficiency when exposed to thickened fluids, pigs raised on thickened milk did not exhibit changes in these responses when exposed to standard formula. These results suggest that being raised on thickened milk reduces the ability to respond to variation in milk thickness and hinders oropharyngeal function.

Humming Along: Sound Production in the American lobster

Renske Kerkhofs, Olaf Ellers, Amy Johnson

American lobsters (*Homarus americanus*) produce humming sounds by vibrating their carapace using the second antennal promotor and remotor muscles. These sounds have a fundamental frequency on the order of 100 Hz, with multiple higher harmonics. Lobsters seem to hum most readily when threatened, but many studies have found it difficult to reliably find soniferous lobsters. To find a way to reliably evoke sound production in American lobsters without contributing to the sound environment, several techniques were tested. Based on the results of those trials, lobsters were exposed to overhead abstract visual stimuli on a screen, after which their behavioral reactions were recorded, as well as any sound production in response to the stimulus. Lobsters responded to the screen stimulus with the same types of behaviors with which they responded to general overhead physical stimuli. During experiments, I observed sounds that are similar in structure to the known hums, as well as some similarly structured sounds with frequencies an order of magnitude higher, suggesting that lobsters may use a wider range of sounds than previously thought. This study demonstrates that American lobsters may produce high-pitched sounds and that abstract visual cues can be used as a silent tool to elicit lobster behaviors and hums.

My, Those Head-Teeth Are Long!: Morphology of the Cephalic Tenaculum Through the Lens of Ecology

Kaitlyn Kern, EW Misty Paig-Tran

Chimaeras are chondrichthyan fishes that are the only extant animal to possess accessory copulatory structures known as cephalic and pre-pelvic tenacula. These denticle-covered structures are found on the head and pelvic girdle of male chimaeras which aid in grasping females during mating events. This structure has widely varying morphology across taxa. Additionally,

chimaeras occupy a variety of depth and temperature profiles. Because of these differences across taxa, we wanted to investigate the influence of ecology on morphology. In this study, we collected morphometric data (length, width, density) of the denticles on the cephalic tenaculum across taxa and ecological data (depth, temperature) to find if ecology influences morphology. We used scanning electron microscopy (SEM), micro-CT, and macrophotography to collect morphometrics for each tenaculum across the three families of chimaeras: Rhinichimaeridae, Chimaeridae, and Callorhincidae. Stereopairs were created to visualize the dimensionality of the cephalic tenaculum cup that holds the denticulated bulb. We found that the denticles of the cephalic tenaculum vary in density, shape, and length across taxa. We found that ecology affects the morphology of the denticles; as depth increases, the density and length of denticles increase whereas width decreases. Deep-water chimaeras have similar denticular density, whereas shallow-water chimaeras have similar denticular density. These data are critical for understanding the functional performance of the cephalic tenaculum during mating events in all families of chimaeras.

Using video, accelerometry and machine learning to infer behavior of free-ranging northern fur seals

Sarah Kerr, Cory Williams, Carey Kuhn

The Eastern Pacific stock of northern fur seals (*Callorhinus ursinus*) has declined drastically over the past half century, with pup production consistently declining on St. Paul Island, AK (SPI), the largest fur seal colony in the US. Changes in ocean conditions that altered the availability of key prey is one of the leading hypotheses for this decline. Previous studies found that lactating females on SPI are traveling long distances from the colony to obtain prey resources. However, continued efforts are needed to link foraging strategies to energy transfer between mother and pup, and modern technologies may help us examine this relationship. Using data from female fur seals equipped with animal-borne video cameras, we are developing supervised machine learning models to quantify foraging success based on accelerometer data. This is the first step in a larger project that will also develop and validate supervised machine learning models to measure northern fur seal pup suckling frequency based on accelerometer data. By coupling accelerometry measurements with stable isotope analyses and satellite-telemetry, we plan to examine how maternal foraging location influences foraging success and patterns of energy allocation to pups. The development of machine learning tools to quantify behavior can improve our understanding of both intrinsic and extrinsic factors related to female foraging and reproductive success in this declining population.

Speciation in the holopelagic ctenophore *Mnemiopsis*

Remi Ketchum, Edward Smith, Whitney Leach, Leandra Toledo, Adam Reitzel, Joseph Ryan

Little is known about how holopelagic species arise and are maintained in the open ocean without clear barriers to gene flow. *Mnemiopsis* is a holopelagic ctenophore that plays an important ecological role in its native habitat along the east coast of the Americas. Although historical literature described three species of *Mnemiopsis*, the lack of stable morphological characters has led to the collapse of this group into a single species, *Mnemiopsis leidyi*. Here, we collected and performed whole-genome sequencing of 120 *Mnemiopsis* individuals from 13 populations along the Atlantic and Gulf coast of the United States. *Mnemiopsis* sampled across 3000 km of coastline revealed two major genetic lineages ($F_{st} = 0.32$ across 4.5 million variants): a “northern US Atlantic lineage” and a “southern US Atlantic lineage” with the Outer Banks, North Carolina being the major genetic breakpoint between the two lineages. Interestingly, we find potential hybridization between the two lineages at the Outer Banks collection site, mitonuclear discordance, and 266,000 SNPs exhibiting high fixation values ($F_{st} > 0.7$) potentially involved in speciation and adaptation. Our study suggests that *Mnemiopsis* found north and south of the Outer Banks are distinct species, provides insight into how holopelagic species arise without clear dispersal barriers, and highlights mechanisms involved in adaptation along a very large environmental gradient.

The Lizard’s “Third Eye” and Its Impact on Reproductive Physiology

Mia Kholy, Michele Johnson

In addition to two lateral eyes, lizards also have a third, parietal, eye. The parietal eye is more than a translucent scale on the top of the head; it is a true eye with a lens, lumen, and photoreceptors, and the pineal nerve connects it to the pineal gland in the brain. The parietal eye is thought to be involved in regulating light-sensitive behavior and physiology, such as daily sleep-wake cycles and seasonal reproduction. In this study, we altered the exposure of the parietal eye of *Anolis sagrei* (brown anole) lizards by adhering “scale caps” onto their heads. Nine males had a scale cap covering the interparietal scale, while 10 had a scale cap at the back of the head, allowing light to enter the parietal eye. Males were housed with a female for five weeks, and testes were collected at the end of the experiment. Covering the parietal eye altered reproductive physiology, as the average testis mass was significantly smaller in experimental than control lizards, suggesting that reduced exposure of the parietal eye to light initiated the process of seasonal testis regression early. Yet, the architecture of the seminiferous tubules within the testes did not differ between groups. We will next examine testis cell type distribution and density to determine how light exposure through the parietal eye impacts lizard reproductive potential.

microRNA correlates of behavior in poison frog tadpoles

Neil Khosla, Lauren O’Connell

Dendrobatidae is a family of poison frogs that display a diverse spectrum of tadpole social behaviors, ranging from tolerant group coexistence to fierce cannibalistic tendencies exhibited by those in isolation. Some behaviorally-plastic dendrobatids even tend to be more aggressive when raised in isolation, but remain gregarious when developing in groups. MicroRNAs are a class of epigenetic modifiers that are particularly suited for enabling behavioral adaptation – instigating gene network-wide expression changes in a context-specific fashion. This study is a comparative analysis of microRNA expression across Dendrobatidae species and tissues. Preliminary data suggests conservation of neural regulatory function and shared mechanisms among behaviorally-similar taxa. Pilot experiments suggest potential for in vivo manipulation validation experiments in lab.

Blubber proteome response to prolonged fasting during postnatal development in seals

Jane Khudyakov, Anthony Chuang, Andrew Nguyen, Kari Tanji, Richard Zhao, Dan Crocker

Most mammals decrease energy expenditure while fasting. Northern elephant seals (NES), on the other hand, fast for up to 4 months while undergoing energy-intensive activities such as postnatal development, molting, and breeding. These activities are fueled by large energy stores in blubber, which is the main energy depot in marine mammals. We examined changes in the blubber proteome over eight weeks of fasting concomitant with postnatal development in NES pups ($n = 4$). We isolated proteins from blubber collected from pups at weaning and after 2, 5, and 8 weeks of fasting and analyzed them using label-free quantification by LC-MS/MS. Abundance of 95 proteins changed over the first 2 weeks of fasting, while 18 and 6 proteins were differentially abundant between 2 and 5 weeks and between 5 and 8 weeks, respectively. Proteins that increased in abundance over fasting were involved in lipolysis, lipid transport, glycolysis, adipogenesis, and protein ubiquitination and degradation, while those that decreased in abundance were associated with protein synthesis and processing, electron transport chain, fat synthesis and storage, and response to oxidative stress. These findings were surprising as previous studies did not detect evidence of oxidative damage or catabolism of protein stores during fasting in NES pups. These data highlight the complexity of cellular and metabolic adjustments of blubber tissue to prolonged fasting during development in seals.

Behavioral Variation of Juvenile Humpback Whales (*Megaptera novaeangliae*) in the Gulf of Maine

Eman Khwaja, Kathryn Kavanagh

The lives of Humpback Whales (*Megaptera novaeangliae*) are broadly characterized by three stages: calf (Birth – Year 1), juvenile (Year 1 – Year 5), and adult (Year 5 – death). This study aims to determine the change in foraging and surface active behaviors throughout the 4-year juvenile stage. Using long-term behavioral data collected opportunistically in the Southern Gulf of Maine, the behavior of juveniles was quantified during the feeding season from March to November annually. Foraging behaviors are split based on complexity and tool-use into two categories: complex (bubble & kick feeding) and simple (lunge & subsurface feeding). Surface active behaviors are categorized based on long-range impact (breaching, lobtailing, etc) and short-range impact (flipper slapping, trumpet blows, etc). It was found that most foraging behaviors decreased from Year 1 to Year 4, suggesting an increased competence in foraging. It appears that simple foraging behaviors were favored in early years. Surface behaviors peaked during Year 2, and showed the lowest frequency during the last year of the juvenile stage. Close-range communication appears to diminish throughout the four years, while long-range communication peaks during Years 2 and 3. The significance of this research is to better inform the conservation and management plans of a biologically important species, as well as better understand the ontogeny of behavior in baleen whales.

Green Fluorescent Proteins: Examining the Underlying Factors of Brightness Using Machine Learning

Lillian Kidd, John Koberstein, Srinivas Turaga, Alison Tebo

Green fluorescent protein (GFP), originally discovered in the jellyfish *Aequorea Victoria*, is a protein that emits green fluorescence when its internal fluorophore absorbs blue light. GFPs have high utility in scientific research as fluorescent markers that are used to visualize biological processes, structures, and interactions. Engineering of fluorescent proteins (FPs) generated color variants through mutation of the wildtype sequence that subsequently shifted excitation wavelength. However, it is poorly understood how sequence mutations influence fluorescence at 405 nm versus 488 nm, which represent the two predominant excitation peaks of GFP and related proteins. To elucidate how sequence mutations shape the GFP fluorescence spectra, we developed a novel hybrid neural network model that combines a black-box deep network with a biochemical model of fluorescence, including parameters for protein folding, quantum yield, and fluorophore pKa to predict fluorescence intensity from the amino acid sequence. We trained the model on a published dataset consisting of paired sequence-function measurements for thousands of FP variants which critically included excitation at both 405 and 488 nm. The model accurately predicted fluorescence measurements at each excitation wavelength from sequence alone. The interpretability of the model parameters allows for inference and assessment of the biochemical factors underlying shifts in excitation wavelength. Further improvements can be made by extending the model to also fit *in vitro* measurements.

Feather corticosterone is lower in translocated populations of the endangered Laysan Duck

Denyelle Kilgour, Michael Reed, L. Michael Romero

Identifying reliable bioindicators of population health and habitat quality is a central goal of conservation physiology. Measures of physiological stress, especially glucocorticoids, are often applied and can assist in managing endangered species if linked to fitness traits. We investigated feather corticosterone, a cumulative measure of physiological stress, as a bioindicator for the critically endangered endemic Hawaiian bird, the Laysan Duck (*Anas laysanensis*). We hypothesized that feather corticosterone would reflect the improved reproduction and survival rates observed following the translocation of this species to Midway and Kure Atolls from Laysan Island. We found that feather corticosterone concentrations are lower in the translocated populations than in the source population on Laysan Island, suggesting that the translocated individuals are experiencing less physiological stress. Additionally, samples taken over the last 45 years from Laysan Island show higher feather corticosterone levels than those collected in the early 1900s.

Feather corticosterone appears to be a valuable indicator of population status and could be used as a non-invasive physiological monitoring tool for this species. Furthermore, these results, combined with published demographic data, suggest that current Laysan conditions are not an optimal habitat for this species.

Conspecific alarm calls prevent the attenuation of neophobia behavior in wild-caught house sparrows

Melanie Kimball, Danna Masri, Eve Gautreaux, Keegan Stansberry, Tosha Kelly, Christine Lattin

Some individuals respond to new objects, foods, or environments with wariness (neophobia), whereas others are willing to approach and explore. The effect of alarm cues on neophobia has been well-studied in an olfactory context but is poorly understood in an auditory context. To understand how conspecific calls influence neophobia, we exposed individual house sparrows (*Passer domesticus*) to either conspecific alarm calls ($n=12$), conspecific contact calls ($n=12$), or no playback ($n=12$) and measured latency to feed in the presence of novel objects. We also measured novelty responses with no sound the week before and after the sound treatment week for all individuals. We predicted that sparrows exposed to conspecific alarm calls would increase neophobia behavior, that conspecific contact calls would decrease neophobia, and that these effects would persist the week after exposure. Instead, we found that individuals in the contact call and no playback groups became less neophobic as weeks progressed, and that the alarm call group showed no attenuation of neophobia. Additionally, there was a significant week*treatment effect, where neophobia responses over the three weeks were significantly different for individuals exposed to alarm calls compared to the contact and no playback groups combined. These results suggest that house sparrows may learn social information about potentially threatening stimuli from alarm calls; here, that novel objects may be dangerous.

Bird nest construction as cognitive material science

Hunter King

When a bird forages for nesting material, it may poke, lift, or shake a candidate item before deciding whether to incorporate it into the growing collection of like objects gradually forming a firm, cohesive cup. In so doing, it somehow relates the mechanical properties of that item to those which emerge from their combination. This apparent foresight is particularly surprising given the non-trivial self-assembly of packing grains, the complex mechanics of the resulting nest, and its crucial role in the safety of the bird's unhatched offspring. Building is not entirely instinctual and birds adapt their strategies for varying circumstances. How do they navigate decisions of material choice and manipulation within this complex design space? We think bird brains internalize

certain rules for tuning complex media that elude corresponding efforts in physics and engineering. This talk will summarize a developing interdisciplinary effort on the interface between avian cognition and material science to reveal those rules where they reside, integrating live-bird behavioral experiments with rigorous mechanical analysis, in an iterative process that parallels natural nest construction.

Orbital hoods protect snapping shrimp from shock waves without impairing their visual abilities

Alexandra Kingston, Rebekah Hansen, Rebecca Lucia, Daniel Chappell, Daniel Speiser

Shock waves are supersonic high-amplitude pressure waves that cause blast-induced neurotrauma. Snapping shrimp (Decapoda: Alpheidae) are protected from the shock waves they create with their snapping claws by their helmet-like orbital hoods. We previously learned that shock waves have the potential to harm snapping shrimp, but do not do so under natural conditions because orbital hoods dampen shock waves, at least in part by redirecting and releasing their kinetic energy. Armed with this information, we next asked if the orbital hoods of the bigclaw snapping shrimp *Alpheus heterochaelis* 1) have structural properties that contribute to shock wave dampening and 2) impair vision by covering the eyes. We found that orbital hoods are thinner

and less dense than the carapace. Though the epicuticle and exocuticle layers do not differ in thickness between these regions of exoskeleton, the endocuticle layer of the orbital hoods has lamellae that are thinner, more numerous, and more tightly packed than those of the carapace. This may contribute to differences in the material properties of these tissues. Despite orbital hoods covering the eyes of snapping shrimp, we found that *A. heterochaelis* without orbital hoods demonstrated equivalent visual acuity as those with hoods. Our results suggest that the transparent helmet-like orbital hoods of snapping shrimp have structural properties that enhance their shock wave dampening abilities without impairing vision.

Jumping ability of aquatic frogs in a terrestrial habitat

Chase Kinsey, Olivia McNelly, Richard Blob

Locomotor performance can vary greatly across habitats of differing complexity, with a particular potential for such variation among organisms that only encounter specific habitat features infrequently. For example, *Xenopus laevis* is an aquatic frog that exits the water in search of new pools. They must be able to effectively move across land despite rarely interacting with terrestrial substrates, but data are lacking on how *Xenopus* locomotion is impacted by features like slopes on pool edges. We measured ground reaction forces produced by a size range of *Xenopus laevis* jumping from horizontal and inclined substrates, simulating the land-water interface of pool margins. The right hindlimb of each frog was placed on either a horizontal or inclined (30°) platform set on a force plate and filmed with high-speed video. Component and resultant forces were calculated, and size normalized and jump angles at take-off were calculated from video. There was no difference in size normalized peak forces between horizontal and inclined platforms. Vertical forces increased with jump angle on horizontal, but not inclined platforms. Furthermore, there was greater variability in jump angles at smaller body sizes and resultant forces increased as body size increased, though the magnitude of component forces varied across treatments. These results indicate that *Xenopus* can maintain consistent performance in at least some aspects of locomotion, despite encountering some habitat variations only rarely.

An evaluation of the relationship between energy state and protein synthesis in a nematode worm

Stephen Kinsey, Sarah Fausett, Dylan Orcutt

In recent years it has become generally accepted that energy state exerts control on rates of protein turnover. However, this conclusion has largely been reached by evaluating the ATP concentrations in extracts from tissues or organisms. The ATP concentration alone is not a good reflection of energy state because ATP-generating pathways are activated during energy stress, resulting in nearly constant ATP levels. Further, high-energy phosphate groups on molecules like ATP are labile and often are hydrolyzed during the extraction process, giving an underestimate of concentration. To gain a better understanding of the relationship between energy state and rates of protein synthesis, we developed a flow-through system that allows us to monitor energy state *in vivo* in the nematode worm, *C. elegans*, using ³¹P-nuclear magnetic resonance (NMR), which is a well-established method for gaining a more complete view of energy status. We have coupled this approach with measurements of protein synthesis by following incorporation of ¹³C-labeled amino acids into protein using ¹³C-NMR. These techniques provide a means of examining the linkage between energy state and protein synthesis during treatments that induce energy stress. In addition, *C. elegans* is a highly tractable genetic model organism that allows the evaluation of mutant strains with different capacities for energy maintenance and strategies for coping with energy stress.

Development of Reproductive Organs in the Brown Anole

Bonnie Kircher, Richard Behringer

Reproduction modes across vertebrates are diverse. Lizards and snakes have particularly diverse reproductive strategies yet the reproductive organs of squamates have a similar overall architecture. Here, we describe the development of the reproductive organs in one species of lizard, the brown anole (*Anolis sagrei*). Though the embryonic structures that develop into adult

reproductive tract organs have been described in reptiles, a comprehensive understanding of morphological changes in these tissues across developmental time is lacking. The brown anole is popular as a research model and CRISPR genome editing techniques that rely on manipulation of the reproductive tract have been developed. Using histology and 3D imaging, we describe morphological changes to the embryonic precursors of the adult reproductive anatomy, the paired Müllerian and Wolffian ducts. In mammals, the reproductive organs develop from these ducts which are present in both sexes; however, Anti-Müllerian Hormone secreted by the male testis causes regression of the Müllerian Ducts, while testosterone facilitates differentiation of the Wolffian ducts into male reproductive tract organs. In females, the Wolffian duct degrades and the Müllerian ducts differentiate into female reproductive tract organs. We also describe preliminary results of the role of Anti-Müllerian Hormone in female reproductive tract development. Our data highlight morphological changes in the brown anole embryo that are unique to this species compared to mammals. Supported by NIH T32 HD098068 and NSF PRFB 2209150.

Ambient lighting effects on sea turtle nesting behavior on the Gulf of Mexico

Jasmine Kirchner, Jacob Lasala

Lighting can affect many nocturnal animals' behavior by disrupting natural cues necessary for survival. Sea turtle behavior can be affected by artificial lights and repel nesting females from nesting beaches. To determine if ambient light affects normal nesting behavior, ambient light readings were taken at loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtle nests or false crawls after the turtle had left the beach. Data were collected from a sea turtle rookery on the Gulf of Mexico in 2022 and 2023, and cloud coverage, moon phase, and moon presence were documented. Ambient light was significantly affected by the Julian date and the corresponding visible moon phases, as well as cloud coverage. There was no significant difference in ambient light averages between nests and false crawls, and ambient light increase did not significantly affect nesting success. Nesting behavior between species was significantly different in relation to ambient light. Understanding how sea turtles are affected by lighting is essential to determine the best conservation methods for these threatened marine animals.

Parental care shapes offspring growth and is influenced by environmental temperature in two passerines

William Kirkpatrick, Sarah DuRant

To define thermal drivers of behavior, we must assess relationships between thermal conditions and behavior at multiple timescales to explore responses to long-term trends in thermal conditions and acute exposure to suboptimal thermal conditions. There is limited knowledge of the interaction of avian behavior and external temperature during nesting, especially from multiple temporal and thermal perspectives. We predicted that natural thermal variation and mean thermal conditions would significantly alter time parents spent away from nestlings, and the effects of increased temperature and altered behavior would negatively influence nestling growth. In nest-boxes, we examined how natural thermal variation and mean temperature impacts nesting behavior in Eastern Bluebirds and Tree Swallows. We quantified adult breeding behavior using NestIQ, a machine learning software which recognizes off-bouts by comparing multiple nesting temperatures. As thermal variation increased, individual and daily measurements of off-bout duration decreased while off-bouts lengthened as mean temperatures increased. Also, nestling growth rates are moderately influenced by adult behavior, but not environmental temperature. As off-bout duration increased, growth rates decreased regardless of the thermal environment, suggesting that behavioral adjustments have more direct control than natural thermal conditions. We conclude that thermal variation and average temperatures have opposing, equally important effects on breeding behavior. Parents may exhibit altered behavior in response to the thermal environment, which can influence nestling physiological development. To define thermal drivers of behavior, we must assess relationships between thermal conditions and behavior at multiple timescales to explore responses to long-term trends in thermal conditions and acute exposure to suboptimal thermal conditions. There is limited knowledge of the interaction

of avian behavior and external temperature during nesting, especially from multiple temporal and thermal perspectives. We predicted that natural thermal variation and mean thermal conditions would significantly alter time parents spent away from nestlings, and the effects of increased temperature and altered behavior would negatively influence nestling growth. In nest-boxes, we examined how natural thermal variation and mean temperature impacts nesting behavior in Eastern Bluebirds and Tree Swallows. We quantified adult breeding behavior using NestIQ, a machine learning software which recognizes off-bouts by comparing multiple nesting temperatures. As thermal variation increased, individual and daily measurements of off-bout duration decreased while off-bouts lengthened as mean temperatures increased. Also, nestling growth rates are moderately influenced by adult behavior, but not environmental temperature. As off-bout duration increased, growth rates decreased regardless of the thermal environment, suggesting that behavioral adjustments have more direct control than natural thermal conditions. We conclude that thermal variation and average temperatures have opposing, equally important effects on breeding behavior. Parents may exhibit altered behavior in response to the thermal environment, which can influence nestling physiological development.

Inhibition of sphingosine kinase disrupts symbiosis of two cnidarian model systems

Sheila Kitchen, Angela Poole, Alexa Bilsky, Mary Rowland, Samuel Piorkowski, Kira Turnham, Mónica Medina, Aki Ohdera

Bioactive lipids play a pivotal role in determining cell fate and mediating host-microbe interactions. Modulation of intracellular sphingolipids by the enzyme sphingosine kinase (SPHK) creates a pro-survival environment for the host, microbe or both partners. In the symbiosis between cnidarians and dinoflagellates of the family Symbiodiniaceae, sphingolipid metabolism mediates partner interactions at various stages, from the onset to the establishment of long-term associations. In this study, we examined the role of SPHK in the early stages of symbiosis by disrupting its function through pharmacological inhibition and RNA interference in two symbiotic cnidarians, the jellyfish *Cassiopea* and sea anemone *Exaiptasia*. In both species, the inhibitor-treated animals significantly reduced uptake of symbionts in a dose-dependent manner. At the highest concentrations, symbiont numbers were comparable to those found when either animal was provided heat-killed symbionts, suggesting a failed symbiosis. SPHK inhibitor also prompted nearly complete symbiont loss in fully colonized *Cassiopea* but not *Exaiptasia* after three days of exposure. To identify the conservation between *Cassiopea* and *Exaiptasia* SPHK-mediated lipid signaling pathways, we compared orthologous gene expression of treated and non-treated animals during the onset of symbiosis within and between species. Overall, these findings support the conserved regulatory role of SPHK during symbiont uptake in distantly related symbiotic cnidarians.

Differential gene expression between growth plate and non-growth plate forming ends of bones

Kelsey Kjosness, Sungdae Park, Sherrie Wallace, Sarah Doelp, Maria Biancaniello, Douglas Menke, Philip Reno

Several bones within the developing mammalian autopod possess a growth plate on only one end. This includes the metatarsals (MT), the pisiform in the wrist, and calcaneus in the ankle. While regulation of growth plate cartilage is well understood, little is known about which genes specify where growth plates do and do not form in the skeleton. We used RNA-seq to identify differentially expressed genes (DEGs) between four tissues with a growth plate forming and non-forming region in postnatal day 4 (P4) and P9 mice: 1) proximal versus distal MT1, 2) distal versus proximal MT3, 3) pisiform versus all other carpals, and 4) proximal versus distal calcaneus. We compared DEGs that had a positive fold change and a significance value of $p < 0.05$ for all four datasets within each age group to identify common genes with higher expression in the growth plate forming region. We identified 4 genes at P4 and 8 genes at P9 that met these criteria. *Stra6*, a member of the retinoic acid signaling pathway, was the only gene to be shared between both age groups. In situ hybridization shows *Stra6* to be strongly expressed in the perichondrium adjacent to

proliferative chondrocytes in growth plate and minimally expressed in the non-growth plate forming cartilage. This analysis demonstrates the utility of using variation in growth plate location to identify growth plate specific genes.

Examining cuttlefish suckers using FEM and biomimetics: An open-source workflow for studying suction

Brett Klaassen-van-Oorschot, Timo van-Leeuwen, Lara-Marie Jess, Guillermo Amador

Cuttlefish suction cups contain a hardened proteinaceous ring. The function of this ring is unknown. We hypothesized that in common cuttlefish (*Sepia officinalis*) this ring provides structural support to resist collapse under the low pressures exerted during suction. Alternatively, the ring may provide a sealing surface in concert with papillae that grow from the ring's edge. We tested the ring's function using a finite element model in FEBio and validated this model empirically using a biomimetic suction cup. Our findings demonstrate that sucker rings resist buckling of the suction cup rim and dramatically improve attachment. Moreover, we present 1) a novel biomimetic suction cup that is reversible, strong, and capable of adhering to a variety of surfaces; and 2) an open-source workflow for exploring suction cup biomechanics across morphologies.

Mistaken synapomorphy: The evolutionary developmental origins of the arachnid patella

Benjamin Klementz, Grace Hareid, Hugh Steiner, Guilherme Gainett, Emily Setton, Sophie Neu, Ethan Laumer, Charlotte Wood, Isaac Hinne, Monika Gulia-Nuss, Austen Barnett, Georg Brenneis, Prashant Sharma

The patella is the leg segment that confers a “double-bend” architecture to the pedipalps and walking legs of Euchelicerata (arachnids and horseshoe crabs). It was postulated that the patella was a synapomorphy of Arachnida and resulted from neofunctionalization of a new copy of the gene *dachshund-2* (*dachshund-2*). Two aspects of this reconstruction are difficult to reconcile across the literature. First, some arachnid orders are thought to lack patellae (e.g., Solifugae, Pseudoscorpiones, some acariform mites). Second, *dachshund-2* is restricted to a subset of six arachnid orders (Arachnopulmonata); various arachnid groups outside of the arachnopulmonates possess a true patella, but not *dachshund-2* (e.g., Opiliones), suggesting that *dachshund-2* evolved after the patella. Thus, neither the developmental genetic basis for patellar formation, nor when the patella evolved, are clearly understood. Here, we show that a novel expression domain of the gene *extradenticle* is associated with the patellar segment in embryos of the harvestman Phalangium opilio. Gene silencing of *extradenticle* results in the loss of the patella, suggesting that this transcription factor underlies the origin of the patellar segment. We tested whether this novel *extradenticle* expression domain was regulated by Notch-Delta signaling, which is responsible for leg segmentation. Knockdown of Notch resulted in unsegmented appendages, in addition to diminution of the median *extradenticle* domain. With this developmental genetic definition of the patella, we surveyed chelicerate orders to pinpoint patellar origin.

Foraging Behavior of Fruit Flies (*Drosophila melanogaster*) in a 3D Arena

Michael Knabe, Jimena Aracena

Fruit flies (*Drosophila melanogaster*) exhibit negative geotactic behavior, which has many advantages including the ability to escape from predators. We tested the effect of negative geotactic behaviors on foraging. We presented the flies with a feeding cube (2.5 cm side length) to test their feeding preference in a 3D arena (10 x 10 x 10 cm) to determine their preference to feed from the top, sides, or bottom of a cube with 9 wells on each side filled with colored sucrose solution. The top, bottom, or sides had either a red, yellow, or blue solution. The flies were deprived of food for 24 hours and tested in the dark for one hour. They were then frozen, and the contents of their abdomens were evaluated for color. There was a preference for feeding on the top over the sides and/or the bottom of the cube. The flies also preferred the yellow solution, while avoiding the red solution regardless of position (top, bottom,

sides). The decision about where to feed on the cube seems to be influenced by several variables, including resource quality, possible ability to escape or hide from predators, and difference in energy cost caused by feeding against gravity.

Speedy, stiff, sharks: Vertebral morphology and 3D microstructure of lamniform sharks

Jamie Knaub, Madisan Biordi, Delaney Frazier, Maria Uribe-Mejia, Michelle Passerotti, Lisa Natanson, Tricia Meredith, Marianne Porter

The mackerel sharks (order Lamniformes) are an ecologically diverse group of sharks, including some of the fastest swimming species. During swimming, lateral oscillations travel along the body, bending the cartilaginous vertebral column over successive tailbeats. In lamniform sharks, the lateral oscillations are restricted to the posterior body and caudal fin for high-speed swimming, subjecting vertebrae to greater compression and tension forces. Previous research showed that lamniform vertebral stiffness increased in the posterior body and varied among species. Here, we quantified lamniform vertebral microstructure along the body. We hypothesized that the stiffer posterior vertebrae would have increased mineral structures to support fast swimming, and that the fastest species, the shortfin mako, would have the most microstructure. We micro-CT scanned vertebrae from common thresher (N=9), shortfin mako (N=14), porbeagle (N=9), and white (N=5) sharks, and quantified vertebral mineral architecture: lamellae and node counts, intermedialia angles, double cone angles, and arch insertion angles. We found that mineral architecture varied across body regions; posterior centra had greater lamellae counts and anterior vertebrae had greater node counts. Across species, common thresher and white shark vertebrae had the most lamellae, and thresher sharks had the greatest number of nodes. Our results suggest that variation in mineral architecture along the vertebral column may support a functional need; more lamellae in posterior centra may be an adaptation to withstand loading during high-speed swimming.

Designing a miniature fish robot – an aquatic analog for hexbugs

Hungtang Ko, Brian Mmari, Di Ni, Radhika Nagpal

Fish school is a complex system that requires coordination from multiple sensing agents. One challenging and often neglected aspect in studies of fish schools is the embedded fluid environment. How does the wake of a swimming fish affect the movement of other fish? What self-organizing features would we observe if fish were deprived of all sensory organs and could only swim open-loop? A simplified fluid model posed by applied mathematicians Weihs and Lighthill predicted that a diamond formation would emerge from purely hydrodynamic interactions. However, careful examination of the complex system calls for robotic models. In this project, we designed a miniature, low-cost, and autonomous fish robot to study the hydrodynamic interactions of fish schools. We explored actuation strategies including the use of a DC motor and magnetic coils. In addition, we conducted tests and improved designs for waterproofing and balance. The final design of the mini fish robot will be used for future studies of underwater collective behavior.

Testosterone Regulation of Synaptic Ribbons in the Inner Ear of the Vocal Plainfin Midshipman Fish

Kobi Kobi, Yassir Azzam, Jonathan Perelmuter, Joseph Sisneros, Paul Forlano

Developmental and reproductive state-dependent changes in hearing are documented in several vertebrates; however, the mechanisms behind these changes remain unclear. The plainfin midshipman is a seasonally reproducing, vocalizing fish with robust hormone-driven changes in peripheral auditory sensitivity that enhance the detection of social acoustic signals including advertisement calls— a great model to study the mechanisms underlying auditory plasticity. Testosterone levels peak during the pre-nesting spring months and experimental treatment of testosterone in winter, non-reproductive females mimic seasonal increases in high frequency encoding and overall sensitivity of the inner ear found in summer reproductive females. Using

electron microscopy, we previously revealed a seasonal increase in hair cell synaptic ribbons in summer females. These specialized structures are necessary for fast transmission of auditory information to the brain. Here, we tested if testosterone mediates this seasonal change in the number of synaptic ribbons. Testosterone or control silastic capsules were implanted into ovariectomized, non-reproductive winter females for 4 weeks. Sacculles, the main end organs of hearing, were collected and processed for immunohistochemistry to label for the ribeye protein— a marker for synaptic ribbons. Images were taken on an epifluorescence microscope and synaptic ribbon number, size, and intensity were quantified. We observed an inverted U-shape relationship between plasma testosterone levels and synaptic ribbon number, supporting a role for testosterone in regulating hair cell afferent synapses and thus peripheral auditory sensitivity.

A Novel Experimental Assay To Study Multisensory Integration for Zebrafish During Rheotaxis

Orhun Koc, Sumeyye Anilmak, Azra Nur Sert, Ismail Uyanik

Animals experience a variety of sensory signals via different sensory organs. The CNS filters, weights, and processes these signals for faster information processing, optimal accuracy, and minimal noise. This work focuses on understanding the behavioral mechanisms adopted by freely-swimming adult Danio rerio (zebrafish) during rheotaxis. We built a novel experimental assay, specifically a speed-controlled flow tunnel. As a natural behavior, the zebrafish orient their bodies toward the flow for station keeping. We then placed a D-shaped semi-cylindrical obstacle inside the water to obscure the flow, creating a low-gradient regime for the fish. This obstacle is attached to a linear actuator, which moves laterally to change the location of the low-gradient region. Zebrafish tracks the movement of this tube laterally to remain within this energetically advantageous region by combining visual and mechanosensory information generated by the tube. A crux of our design is that the D-shaped tube is a transparent shell, and it contains a visually transparent LED light strip. The light strip is attached to another lateral linear actuator, enabling a suit of sensory conflicts. We experimented with N=5 zebrafish under different sensory conflict scenarios. Our results showed that mechanosensory stimulation is fundamental to initiate the tracking response, while visual stimulus alone does not trigger reference tracking. Nonetheless, visual and mechanosensory cues improved the state estimation performance when presented synchronously. Supported by TUBITAK (120E054).

Effects of captivity on mitochondrial respiration and production of red pigments in the House Finch

Rebecca Koch, Chidimma Okegbe, Chidambaram Ramanathan, Xinyu Zhu, Matthew Toomey, Yufeng Zhang, Geoffrey Hill

Red carotenoid coloration often serves as a signal of quality, but the physiological basis for associations between organism function and red coloration remains uncertain. We held wild-caught male House Finches in small cages and large flight cages during feather molt to create a stressful environment during the production of ornamental coloration. We assumed that small cages would present a more stressful environment than large flight cages. We measured mitochondrial respiration and circulating carotenoid pigments of captive as well as free-living wild males. Contrary to our assumptions, we found no evidence that living in small cages imposes greater stress on wild-caught House Finches than living in large cages—we found no significant differences in mitochondrial respiratory function or production of red pigments by males in the cages of different size. However, mitochondria were more uncoupled for captive birds, resulting in a lower respiratory control ratio, indicating captivity had a significant impact on mitochondrial respiratory function. In addition, male House Finches growing feathers in cages produced less ketocarotenoid from yellow dietary precursor than males that were free-living. Within captive males, effects on mitochondrial function were highly variable, and the males that produced the most ketocarotenoids had the highest mitochondrial function for complex II substrates. These data provide support for the hypothesis that the production of red carotenoid pigments via ketolation reactions is tied to mitochondrial respiratory function.

Investigating the effects of compliant substrates on Cane toad jump take-off

Cooper Kocon, Caitrin Eaton, Crystal Reynaga

Cane toads (*Rhinella marina*) are a well-studied model for controlled landings. Previous research has shown cane toads can adjust body position and muscle recruitment while in mid-air. In contrast, faster jumping frog species, shown in the Cuban tree frog, are limited in their ability to modulate muscle recruitment once a jump is initiated. We predict the feedback mechanisms utilized by hopping cane toads may allow for greater energy recovery from a variety of compliant substrates, in contrast to Cuban tree frog species. To test this hypothesis, we created four substrates ranging in compliance resembling a cantilevered beam using a Prusa MK4 filament 3D printer. A strain gauge was attached to the fixed portion of the cantilevered beam to measure jump force. The design and selection of substrates were based on adjustments to substrate length and thickness, effectively changing the substrate spring constant. After substrate calibration, we will film five cane toads using 3D highspeed videography. The videos will be digitized using DLTdv8 deep learning network in MATLAB. Analyzing jump performance on these variable substrates will provide insights into understanding feedback mechanisms and control systems used in hindlimb movement. By gaining a deeper understanding of how diverse organisms use muscle recruitment strategies to navigate complex environments, we can gain insights to advance rehabilitation technologies and design of synthetic systems, by mimicking biological control mechanisms.

Marine larvae respond to hydrodynamic signals they encounter near surfaces in turbulent wavy flow

Mimi Koehl, Rachel Pepper

Microscopic organisms swimming in the ocean are carried by turbulent water currents and waves. We study behavioral responses of microscopic organisms to the chemical and hydrodynamic signals they experience in such flow to learn how their responses can bias where they are transported by ambient water motion. Larvae of sea slugs, *Phestilla sibogae*, which recruit onto coral reefs, were used to study the temporal and spatial patterns of hydrodynamic signals they encounter as they near rugose benthic surfaces in turbulent wave-driven flow. Water motion measured in the field over coral reefs was mimicked in a wave tank where fine-scale instantaneous velocity vector fields were measured using PIV. Agent-based models of swimming larvae carried and tumbled by this flow revealed that they encounter brief pulses of shear, acceleration, and vorticity that increase in magnitude as larvae near the substratum. Pulses of flow in a fluidic device mimicking shears and accelerations larvae encounter near reef surfaces caused larvae to stop swimming. Experiments using shaking or spinning tanks mimicking pulses of acceleration or vorticity experienced by larvae revealed that they do not respond to their own linear acceleration or rotation. Thus, pulses of shear indicating that a surface is nearby are the hydrodynamic signals used by larvae of *P. sibogae* to sink, a behavior enhancing their settlement onto a reef as they are carried by ambient, wavy flow.

Convergence in the feeding apparatus of freshwater characiform fishes

Matthew Kolmann, Jack Rosen, Devya Hemraj-Naraine

Convergent evolution offers a potent model system for understanding trait evolution using a robust, comparative phylogenetic context. The sheer diversity of teleost fishes similarly offers a multitude of lineages adapted for similar ecological roles, from piscivores to durophages. What has shaped our understanding of fish feeding systems, with the monolithic exception of cichlids, is a focus on marine over freshwater taxa. Here we use characiforms (tetras, hatchetfishes, piranhas, etc.) to explore the evolution of different feeding ecologies and their morphological proxies in freshwater environments. Using osteological and contrast-enhanced, micro-computed tomographic imaging (microCT and diceCT), we describe the major axes of morphological variation in serrasalmids (pacus & piranhas), in regards to their diet and feeding behaviors. We compare these axes of phenotypic disparity to other freshwater characiforms, in an effort to identify convergent features independent of phylogenetic

and developmental contingency. Next, we evaluate whether constraints on ecological niche lability are mirrored by constraints on morphology. Finally, we discuss our phenotypic findings alongside on-going ecological observations during fieldwork in South and Central America. We document little overt morphological similarity among ectoparasites, for example, except generally smaller body sizes. We also note the importance of body size for frugivorous and herbivorous fishes. We discuss the mosaic means by which convergence can act on phenotypic and ecological traits.

Predicting skeletal muscle energetics in vivo using physiologically based models

Ryan Konno, Glen Lichtwark, Taylor Dick

Skeletal muscle energy usage is important for understanding the principles behind locomotion and muscle design. Physiologically based muscle models have been developed to predict energy consumption and bridge the gap between computationally expensive biophysical models and high level phenomenological models; however, the ability of these models to capture in vivo muscle energetics across a range of contractile conditions has not been tested. In this study, we implemented a model based on physiological data obtained through single muscle preparations. Further, we considered the role of motor unit recruitment on the energetic cost by scaling energetic parameters to use fibre-type specific values. This model was then applied to experimental data for single joint in vivo tasks involving varying contraction frequencies (van der Zee and Kuo, 2021), duty cycles (Beck et al. 2020), and fascicle lengths (Beck et al. 2022). Our model captured the general trends in the experimental data, with increasing energetic rates associated with increasing shortening rates; however, the model often failed to predict the exact magnitude of energetic cost. To capture high energetic rates during high frequency contractions, incorporating motor unit recruitment was required. We demonstrate the ability of physiologically based models to capture skeletal muscle energetics in vivo. Our results demonstrate further improvements to these models, such as including motor unit contractile dynamics, could improve their predictions of energetic cost across varied movement tasks.

Impacts of dietary protein quality on reproduction and lifespan of lubber grasshoppers

Emma Kordek, Amaya Yip, Alicia Horton, Hope Sohn, John Hatle

The reproduction vs. longevity trade-off can be broken in *Drosophila* by editing dietary protein quality to provide sufficient levels of all essential amino acids but not excessive levels of any (as methionine is harmful in excess). Few other studies have explored the role of dietary protein quality in the trade-off. Dietary amino acids can be matched to the composition of the precursor to egg yolk protein (vitellogenin; Vg). Previously adult female lubber grasshoppers were fed isonitrogenous agar-based diets with amino acid compositions matched 100% to Vg. They exhibited marginally increased lifetime reproduction and no difference in lifespan compared to grasshoppers on diets matched 50% to Vg. Here we evaluated reproduction and longevity of grasshoppers using lower quality diets with approximately one-third less protein and more carbohydrate, matched varying to Vg (100%, and three low-quality groups all 25% or less). To date, there were no significant differences in feeding rates for six of seven weeks, indicating that protein quality, not protein quantity, is largely responsible for reproductive differences. The 100% group laid 24% younger with 50% more eggs than all three lower quality groups (MANOVA; $p < 0.05$) for clutch 1. The 100% group laid 2.5-fold more eggs over the total lifespan ($p < 0.05$). While the lifespan data is currently inconclusive, the 100% Vg (high-quality) diet is leading to better reproductive performance compared to the three lower quality groups.

Slow as it goes: Mechanical energetics of turtles and tortoises

David Kramer, Frank Fish, Anthony Nicastro, Matt Wileyto, Rebecca Bottiglio-Kramer

The mechanical energetics of walking is an important parameter to determine the overall effort in moving the body. Because of their body being enclosed

in a bony shell, turtles and tortoises cannot use mechanisms associated with flexion of the spine to aid in reducing the energetic cost of terrestrial locomotion. The walking motions of red-eared slider, Russian tortoise, Hermann's tortoise, Aldabra tortoise, and Galapagos tortoise were video recorded walking over level ground. The mechanical power output expended by turtles and tortoises over a range of body sizes (0.4-186 kg) was accessed by examining the frequency, amplitude, and velocity of the center of mass. The speed of the animals relative to carapace length (CL) varied from 0.1-0.5 CL/s and the peak-to-peak amplitude was 0.03-0.14 CL. The frequency and walking speed declined with increasing size of the animals. Based on a biomechanical model, the power output was found to increase with body size, whereas the mechanical cost of transport decreased. Possession of a body with a shell constrains the locomotion of turtle and tortoises to a stable, but slow walking gait that reduces the mechanical energy cost of locomotion.

The microbiome: an underappreciated factor in parasite transmission and heterogeneity

Rachael Kramp, Faith Rovenolt, Jason Walsman, Jessica Stephenson, Catherine Wynne, Devin Henry

The host associated-microbiome, which consists of trillions of microorganisms that inhabit the host, has been shown to play a crucial role in regulating the immune system and protecting against infectious diseases. However, the microbiome is still an understudied factor in parasite transmission, and its role in shaping the transmission dynamics of infectious diseases is not well understood.

To understand how the microbiome could influence parasite transmission, we use a uniquely experimentally tractable fish-parasite system (guppy *Poecilia reticulata*-ectoparasitic flatworm *Gyrodactylus turnbulli*). We conducted extensive, manipulative experiments testing how microbiomes affect the behavior and contact rate of infected and uninfected hosts, their *G. turnbulli* infection intensity, and the transmission competence of infected individuals.

We found striking differences in host behavior, infection intensity, and competence between our microbial treatment groups. Fish reared in *Lactobacillus acidophilus*-enriched water were highly competent at transmitting *G. turnbulli*. Those raised in autoclaved water had lower infection intensities. Furthermore, fish appeared to be more social with conspecifics from the same microbial treatment. Overall, these results demonstrate that the host-associated microbiome plays a crucial role in determining the risk of parasitic infections and transmission, highlighting the importance of considering the microbiome when studying parasitic diseases and developing interventions to prevent their spread.

Temperature modulates the sensitivity of oogenesis pathways to E2 stimulation in a temperate fish

Kseniya Krayeva, Zoey Dale, Teresa Guerre, Sean Lema

Warmer temperatures from climate change threaten to impact fish reproduction. For female fishes, changes in HPG axis endocrine signaling under high temperatures often leads to inhibited oocyte development, ultimately resulting in lower ovary mass, fewer or smaller eggs, and decreased egg viability. Some of those oogenesis effects materialize from diminished liver synthesis of vitellogenin egg yolk and choriogenin egg envelope proteins. However, it is unclear if changes in vitellogenin and choriogenin production arise solely from lower blood 17 β -estradiol (E2), or if the liver itself becomes less sensitive to E2 at elevated temperature. Here, adult female Amargosa pupfish (*Cyprinodon nevadensis*) maintained at 20°C, 28°C or 36°C for 14 d were either collected directly from their tanks (baseline control), administered a single intraperitoneal injection of E2 (5 μ g/g body mass), or given vehicle solution only (injection control). Both ovarian mass and plasma E2 were lower in females at 36°C compared to those from lower temperatures. Plasma E2 concentrations increased in females given exogenous E2 at all temperatures. However, E2 only upregulated liver vitellogenin and choriogenin gene transcripts in females at 28°C and 36°C, and not in females at 20°C. Those findings provide evidence that insufficient E2 stimulation of liver egg yolk and

envelope protein synthesis contributes to impaired reproduction at elevated temperatures, but that diminished liver sensitivity to E2 may limit reproductive performance at low temperatures.

A network-based comparative method to study reptile scalation—and more!

Isaac Krone

Herpetologists often rely on the relative sizes, positions, and numbers of scales to distinguish reptile taxa, but have paid less attention to how these important characters evolve. Here, I present a comparative framework for scalation patterns and other two-dimensional patterns made up of units with clear homology relationships. Units are modeled as vertices in a network, connected via edges representing adjacency relationships. Edges and nodes can encode biological information such as suture completion, scale size, color, and other properties. In concert with this representation, I present a graph-edit-distance-based framework for measuring differences between these networks, allowing them to be compared systematically. I apply these techniques to investigate the evolution of scalation and fossoriality in a poorly-understood family of lizards, the dibamids (Dibamidae), presenting scalation-network based phylogenetic hypotheses for the family and demonstrating a relationship between tail length (a fossoriality proxy) and head scalation. The methods presented are available as an R package, pholidosis.

On the origin of patterns of temperature-dependent sex determination

Caleb Krueger, Fredric Janzen

Evolutionary explanations for temperature-dependent sex determination (TSD), particularly in relation to the more well-known and intuitive genotypic sex determination (GSD), have long puzzled and occupied biologists. However, evolutionary explanations for the variety of patterns of TSD have received much less attention. Here we probe the macro- and microevolutionary dynamics of TSD patterns in turtles to better understand their origin and potential adaptive significance. Our ancestral state reconstructions suggest that producing females at cool incubation temperatures (i.e., pattern II TSD) is derived and adaptive. However, the ecological irrelevance of these cool temperatures and a strong genetic correlation across the sex-ratio reaction norm in common snapping turtles (*Chelydra serpentina*) both contradict this interpretation. Furthermore, we find the phenotypic consequence of this genetic correlation reflected across all turtle species with TSD, suggesting it is underlain by a single genetic architecture. This common genetic underpinning can explain the macroevolutionary origin of discrete TSD patterns without assigning cool-temperature female production an adaptive value. However, this architecture may also constrain adaptive microevolutionary responses to ongoing climate change.

Genomic variation of the pearl oyster *Pinctada radiata* from the Arabian Peninsula

Quinton Krueger, Remi Ketchum, Edward Smith, John Burt, Adam Reitzel

The genetic relationships of coastal marine species can be difficult to predict and interpret due to unknown dispersal dynamics, physical barriers, and organismal behavior. The Persian/Arabian Gulf (PAG) experiences extreme gradients in temperature and salinity and is young, having originated approximately 6,000 years ago. Species inhabiting the PAG are also present in the nearby Gulf of Oman (GO) and are the likely source populations. The PAG has higher temperatures and salinity (>37°C and 42 PSU, GO < 32°C and 37 PSU). The PAG provides us with the unique opportunity to study genetic relationships of marine species that have recently colonized an extreme environment. In this study, we describe the population genomics of *Pinctada radiata*, an introduced pearl oyster with cultural and economic importance. We sampled 15 *P. radiata* from eight locations between Abu Dhabi (PAG) and Dibba Al Fujairah (GO). We performed short-read whole genome sequencing on 120 individuals and compared these genetic relationships within and between locations using genetic markers (e.g. SNPs). We will report on the evaluation of our hypotheses that the genetic diversity of *P. radiata* in the PAG will be lower

and distinct from sampled populations in the GO due to the constrained environment. These results are important for understanding the genomic variation of benthic marine invertebrates that reside in stressful environments and to help identify mechanisms for adaptation.

Squirrel Paw Pad Stabilization of High-Impact Branch Landings Tested Using a Physical Model

Duyi Kuang, Stanley Wang, Sebastian Lee, Hannah Stuart, Robert Full

Many arboreal animals rely on grasping when locomoting on narrow branches. Previously, we observed dynamic and static trends in paw adaptation mechanisms of fox squirrels (*Sciurus niger*) during landing on curved substrates, suggesting a spectrum of geometry-dependent landing strategies. Here we tested the embodied control of the prominent foot or volar pads to provide effective energy management and substrate engagement. Energy management is dominated by the inherent capability of the structure to absorb impact embodied by both the damping of foot pads and compliance of the joints. We designed a two-digit front loaded lander as a robo-physical model to parametrize key mechanical attributes of paw function of the squirrel foot. We conducted vertical drop tests to simulate rapid, high-impact landing. By varying touchdown position and drop heights, we determined stable, successful landing positions. For the lander model with no pads, stable landing location was limited to the lowest drop height at a single position where the lander's center of gravity was aligned with the center of the rod. Attaching foam pads below the digits increased stable landing regions for both landing position and drop height tolerance. In the absence of prehension mechanisms, such as opposing thumbs, foot pads appear essential for stable, non-prehensile grip actions during dynamic impacts with a substrate.

Continued population growth of a fur seal colony results in increased localized resource depletion

Carey Kuhn, Rodney Towell, Jeremy Sterling, Rolf Ream

For central place foragers, forming colonies can lead to extensive competition for prey around breeding areas and a zone of local prey depletion. A previous study of northern fur seals (*Callorhinus ursinus*) that recently colonized Bogoslof Island in Alaska (USA) found that as the population grew adult female fur seals were forced to increase their foraging effort to obtain the resources necessary to raise a pup (1997-2011). Rapid population growth on Bogoslof has continued with the number of pups born increasing 57% between 2011 and 2019. In 2015, 2019, and 2023 (preliminary data), we equipped adult female northern fur seals with satellite-linked dive records ($n=27$) to re-examine their foraging behavior in response to the further increase in intra-specific competition. Using measures of at-sea movements and dive behavior, we found that the fur seals continued to increase foraging effort by expanding their foraging range and making longer foraging trips. Despite the increase, foraging effort of fur seals on Bogoslof remains significantly lower than that of fur seals on the nearby St. Paul Island which has been experiencing an unexplained population decline. The continued population growth and change in foraging behavior on Bogoslof contradicts the previous study that suggested the population could be approaching carrying capacity. Our study offers insight into the dynamics of population growth and impacts of increasing population density on a large marine predator.

Neglected no longer: Phylogenomic resolution of higher-level relationships in Solifugae

Siddharth Kulkarni, Hugh Steiner, Erika Garcia, Hernán Iuri, Ryan Jones, Jesús Ballesteros, Guilherme Gainett, Matthew Graham, Danilo Harms, Robin Lyle, Andrés Ojanguren-Affilastró, Carlos Santibañez-Lopez, Gustavo Silva-de-Miranda, Paula Cushing, Efrat Gavish-Regev, Prashant Sharma

Advanced sequencing technologies have expedited resolving higher-level arthropod relationships. Yet, dark branches persist, principally among groups occurring in cryptic habitats. Among chelicerates, Solifugae ("camel spiders") is the last order lacking a higher-level phylogeny and thus, historically characterized as "neglected [arachnid] cousins". Though renowned for aggression,

remarkable running speed, and xeric adaptation, inferring solifuge relationships has been hindered by inaccessibility of diagnostic morphological characters, whereas molecular investigations have been limited to one of 12 recognized families. Our phylogenomic dataset via capture of ultraconserved elements sampling all extant families recovered a well-resolved phylogeny, with two distinct groups of New World taxa nested within a broader Paleotropical radiation. Divergence times using fossil calibrations inferred Solifugae radiated by the Permian, and most families diverged pre-Paleogene-Cretaceous extinction, largely driven by continental breakup. We establish Boreosolifugae new suborder uniting five Laurasian families, and Australosolifugae new suborder uniting seven Gondwanan families using morphological and biogeographic signal.

Buckling instability in jumping nematode and inspired soft model

Sunny Kumar, Victor Ortega-Jimenez, Ishant Tiwari, Adler Dillman, Saad Bhamla

Nematodes are abundant in most ecosystem, where they exhibit swimming and crawling locomotion. A few nematodes parasites of insect in the genus *Steinernema* possess the ability to leap toward the host. During the jumping process, these entomopathogenic nematodes (*S. carpocapsae*) form an alpha shape with capillary latch and upon reaching their buckling limit, a kink configuration enables them to achieve high-velocity jumps (~2 m/s). In this study, we designed a soft jumping model by drawing inspiration from nematode leaping using buckling, which showed similar jumping behavior and performance. We explored various parameters such as aspect ratio, kink instability, and different modulus effects on jumping capabilities. By combining these biological insights with engineering principles, a soft jumping mechanism is devised, holding the potential to offer opportunities for soft limbless locomotion and actuators.

Impacts of paleoclimatic river flow change on the diversification and gene flow of lower Congo river

Naoko Kurata, Liz Alter, Melanie Stiassny, Michael Hickerson

Climatic and geomorphological changes during the Quaternary period impacted global patterns of speciation and diversification across various taxa, but few studies have examined these effects on African riverine fishes. The lower Congo River (LCR) is a natural laboratory for understanding complex speciation and population diversification processes. A previous study using genome-wide SNP data highlighted the importance of dynamic hydrological regimes to the diversification and speciation in LCR cichlids. However, the impacts of long-term extrinsic drivers, such as historical climatic and hydrological changes (e.g., reduced river discharge during extended dry periods), have not yet been investigated. Here, we examine the impacts of paleoclimatic factors on ichthyofaunal diversification and patterns of gene flow in four riverine lamprologine cichlids endemic to the LCR, including the blind cichlid, *Lamprologus lethops*. We used data from reduced representation sequencing (2RADseq) to infer demographic history and test different gene flow models. Our study indicates that the LCR lamprologine species emerged during the Early-Middle Pleistocene transition, characterized as one of the earth's major climatic transformation periods. Modeling results also suggest gene flow across populations and species has occurred in temporally constrained pulses. The possible correlations between estimated divergence times and the timing of gene flow events with glacial-interglacial fluctuations suggest that the Quaternary climate changes associated with river discharge fluctuations may have impacted the evolutionary processes of riverine species along the LCR.

Quest for the crest: mapping the origins of the zebrafish neural crest during gastrulation

Elaine Kushkowsky, Adam Kuuspalu, Victoria Prince

The neural crest (NC) is a transient, multipotent cell population that is important for the development and evolution of vertebrate morphological novelties. While much is known about NC specification, migration, and differentiation

in several vertebrate lineages, the spatial origin of this cell type in the early zebrafish gastrula is less clear. We have developed a photoconvertible lineage labeling system to produce regional and single-cell fate maps of NC precursors that describe how precursor cell position in the gastrula correlates with regional NC identity in the zebrafish larva. Our data demonstrate that NC precursors are generally located between neural and epidermal precursors; however, we find that the NC domain is surprisingly compact and located closer to the animal pole than predicted by previous zebrafish fate maps. Further, our results show AP regionalization of NC precursors at the onset of gastrulation and indicate extensive cell movements during gastrulation that distribute the NC along broad swaths of the embryo. We are also investigating how gastrula-stage FGF signaling impacts the establishment of the NC. Using pharmacological manipulations and analysis of regional gene expression, we describe how FGF signaling affects the prospective NC field throughout gastrulation, underlies cell movements that contribute to the posterior body, and ultimately influences NC fate. Our results highlight the complexity of early cell type specification events in the context of signaling environments and cell movements.

Inter-arm pathways of the oral intramuscular nerve cords in coleoid cephalopods

Adam Kuuspalu, Ishaan Ghosh, Melina Hale

The nervous systems of coleoid cephalopod include a series of nerve cords that extend the full length of each arm. Previously, we found that one set of these cords, the oral intramuscular nerve cords (INCs), connects between arms that are two arms apart creating a uniform pattern of anatomical neural pathways around all eight arms in *Octopus bimaculoides*. To examine the presence and variation of this feature among cephalopods, we identified and compared INC interarm pathways in juvenile squid (*Euprymna scolopes*) and cuttlefish (*Sepia bandensis*) using immunohistochemical markers, cryo-sectioning and confocal imaging. We found that squid and cuttlefish INC patterns are not consistent with *O. bimaculoides*. Rather, the oral INCs display a different pattern of pathways between arms. INCs are bilaterally symmetric but connections between arms differ based on arm position. For example, the adjacent oral INCs from the front (position 1) arms are found to converge. Other INCs bypass the immediate adjoining arm with a directionality as in the octopus. The interarm segment of the INC at the arms' base is wrapped in muscle, as in *O. bimaculoides*, but is not tightly embedded into robust muscle layers as seen in that species. These findings show increased interspecies variability among pathways of the oral INCs and contribute to our functional and behavioral understanding of cephalopod nervous systems. US Office of Naval Research N00014-22-1-2208.

Osteology of the autopodium of Tropidurinae lizards: modularity and phenotypic integration

Stella Kyomen, Monique Simon, Tiana Kohlsdorf

Diversification of the tetrapod limb involves multidimensional processes that integrate several levels and relate to developmental pathways and also to functional demands in specific environments. Here, we evaluated the modular architecture of the autopodium in Tropidurinae lizards (Squamata: Iguania) to test for evidence of functional and developmental modules. We measured the lengths of phalanges, metacarpals and metatarsals of digits I-V in x-rays of the manus and pes in eight Tropidurinae species, and used covariance and correlation matrices to estimate the strength of covariation between osteological elements and test modular hypotheses of development and function. Results suggest distinct modular patterns among Tropidurinae species, and show disparities in morphological integration and modularity in each autopodium, suggesting that manus and pes may differ regarding their modular units. For the developmental hypotheses, most species exhibit signs of modularity in the pes for the hypotheses of full integration and metapodial module. This result provides evidence that metapodia and phalanges may be part of different developmental modules, as previously suggested in the literature. Regarding the functional hypotheses, the distal module was identified in both autopodia for most species and seems associated with ecological divergence in Tropidurinae. Our study provides novel data to evaluate phenotypic

integration patterns in Tropidurinae and discuss how developmental mechanisms and functional demands may affect relationships between eventual autopodial modules during lizard evolution.

Dynamic similarity and the unusual scaling of maximum running speed

David Labonte, Peter Bishop, Taylor Dick, Christofer Clemente

The variation of maximum running speed with animal size is of substantial ecological importance, and has thus long been a topic of interest in animal physiology and biomechanics. Remarkably, empirical data has it that running speed increases up to a critical body mass, and then decreases; the fastest animals are those of some intermediate size. This pattern is a noteworthy outlier among scaling relationships, which are typically monotonous, and can be described by simple power laws. Here, we show that the initial increase and subsequent flattening of maximum running speed with size arises from size-dependent physiological limits to muscle work output, set by the force-velocity and force-length properties of muscle. Beyond a critical size, maximum running speed decreases, because the gravitational force approaches the maximum muscle force, and so truncates the accessible muscle strain rate, and redirects muscle work from kinetic into gravitational potential energy. Both effects can be accounted for in classic dynamic similarity arguments, yielding two dimensionless numbers that can inform comparative work on both extant and extinct animals.

Changes in *Gasterosteus aculeatus* bone density and morphology in response to calcium concentration

Joseph Labun

Evolutionary mutant models have provided us with a unique window into human disease. In particular, threespine stickleback (*Gasterosteus aculeatus*) show rapid reduction in ectodermal plates when introduced to freshwater systems. In these low calcium concentration environments, stickleback struggle to mineralize their highly calcified plates and thus use the *Eda* pathway to downregulate plate growth. In humans, the same *Eda* pathway causes ectodermal dysplasia and impacts many body systems including reduced nails, malformed teeth, and facial malformations. However, this pathway and the traits involved remain understudied. Understanding how the pathway is modulated by other genes and environmental factors is crucial for developing novel treatments for ectodermal dysplasia patients. Here we investigate the impact of environmental calcium concentration on the bone material density (BMD) and morphology of the stickleback lateral plates. We captured 120 stickleback from freshwater and marine populations, analyzing their plate structure using μ CT scans and geometric morphometrics. We found a reduction in freshwater stickleback BMD compared to their marine counterparts, which agrees with our current understanding that low environmental calcium concentration leads to a reduction of bone mineralization. In the future, we need to closely examine the relationship between calcium concentration, lateral plate development, and the *Eda* pathway by identifying up- and down-stream components of this pathway and quantifying the effect of modulating them on lateral plate morphology.

Measuring β -HSD activity in microdissected songbird brain using mass spectrometry

Emma Lam, Minseon Jung, Melody Salehzadeh, Kiran Soma

Steroids synthesized locally within the brain ("neurosteroids") can modulate behaviours. Neurosteroids can be synthesized de novo from cholesterol or from circulating precursor steroids. β -hydroxysteroid dehydrogenase/ Δ^5 -4 isomerase (β -HSD) converts inactive dehydroepiandrosterone (DHEA) to androstenedione (AE), and AE can be converted to testosterone. β -HSD is present in the brain of many vertebrate species in a region-specific manner. Thus, β -HSD activity can alter the availability of active steroids within the brain and modulate behaviour. It was not possible to measure β -HSD activity in discrete avian brain regions (< 2 mg tissue) with previous techniques that lacked sensitivity and specificity. We developed an assay to examine β -HSD activity within microdissected regions of the song sparrow brain using liquid

chromatography-tandem mass spectrometry (LC-MS/MS), an ultrasensitive and specific method for steroid quantification. We incubated 1.5 mg of song sparrow brain tissue with DHEA, and then measured AE produced using LC-MS/MS. Timecourse and saturation curve analyses were conducted to determine the optimal incubation time and substrate concentration, respectively. We will use this method to investigate 3 β -HSD activity across discrete regions of the social decision-making network and seasonal changes. This novel method enables us to measure steroidogenic enzyme activity in small tissue amounts with high sensitivity and specificity, providing vital insights into neurosteroid regulation of behaviour.

Plasticity in *Ipomopsis* floral traits over space and time in response to water availability

Kristal Lam, John Powers, Diane Campbell

Climate change is altering water availability in subalpine ecosystems. Shifts in snowmelt timing and summer monsoonal precipitation, which alter soil moisture, have the capacity to elicit plastic responses in plants. Plasticity in floral traits such as nectar production and flower color can impact pollen transfer and thus fitness in *Ipomopsis aggregata*, since these traits have been shown to play an important role in facilitating pollinator visitation. Understanding how these traits vary spatially and temporally in response to changes in water availability will better inform how *I. aggregata* will persist and be distributed in the future. Two studies were conducted to assess variation in nectar production and flower color: a long-term study analyzed temporal variation and a short-term study assessed spatial variation within populations. Snowmelt date and total summer precipitation were used as a proxy for water availability in the long-term study while soil moisture was used in the short term study. The short-term study found no significant effect of soil moisture on flower color or nectar production in 2023, but nectar concentration declined with higher moisture. The long-term study indicates that both measures of water availability interacted to impact nectar production and flower color. These results suggest that floral traits respond to variation in water availability across years.

Is wheel running a stereotypic behavior in mice selectively bred for voluntary wheel running?

William Lampman, Theodore Garland, Thomas Nguyen

Behaviors classified as stereotypic are exhibited by animals in captivity, considered abnormal or unusual, and not displayed in their natural habitat. Laboratory mice exhibit several common stereotypic behaviors, including biting cage bars, “backflipping” from the lid of a cage, and running in circles. Environmental enrichment typically reduces stereotypes in captive animals – such as when a running wheel is offered. However, some mice run excessively, at the expense of other activities and behaviors. As a result, wheel running is controversially considered a stereotypic behavior when conducted to excess. The 4 replicate High Runner (HR) lines of mice have been selectively bred to run over 200% more revolutions/day on a wheel than 4 non-selected Control lines. If this excessive running is stereotypic behavior, then it should be reduced when they are provided with the alternate enrichment of cardboard toilet paper tube rolls. At the same time, as compared with C mice, the HR can be expected to display a higher rate of other stereotypic behaviors in the home cage when not provided with wheels. HR and control mice were given cardboard tubes while having wheel access, and other behaviors were scored by observations to compare the rate of stereotypic behavior.

Chasing Squishy and Crunchy Invaders: eDNA for Dynamic Invasive Species Surveillance in Tide Pools

Emily Lancaster, Markus Frederich, Erin Grey

Rising ocean temperatures are compelling both native and invasive species to migrate towards cooler waters, whether at higher latitudes or deeper depths. The timely identification of shifting invasive species is important for effective invasive species management, as early detection is required for a successful eradication. Environmental DNA (eDNA) has been used to identify invasive species even in very low concentrations, showing promise as a tool for comprehensive surveillance. We collected monthly eDNA samples

over two years in a tidepool in Maine, USA, in the rapidly warming Gulf of Maine. We also assessed biomass using photography and visual methodologies. We analyzed the eDNA by metabarcoding and quantitative real-time PCR (qPCR) for several invasive invertebrate species, and compared the effectiveness of the different approaches. The analysis revealed fluctuations in species abundance in both visual data and qPCR results for species with a soft exterior surface (“squishy,” i.e. *Botrylloides violaceus* and *Botryllus schlosseri*), with certain patterns persisting in the metabarcoding analysis. For species with a hard exterior surface (“crunchy,” i.e. *Hemigrapsus sanguineus* and *Membranipora membranacea*), seasonal trends are less well defined. Our findings suggest that eDNA-based monitoring efforts for invasive species need to be carefully evaluated by body plan, but can be successful for some species.

Do testosterone and parental behavior relationships vary across latitude in house sparrows?

Samuel Lane, Holland Galante, Lindsey Chiesl, Timothy Greives, Britt Heidinger

Across species of birds, paternal investment tends to vary with latitude. Males at lower latitudes often express higher investment in current reproductive attempts compared to males at higher latitudes who invest more in breeding attempts. However, the mechanisms that govern this relationship remain unclear. Testosterone, a sex steroid hormone that modulates male behavior throughout the breeding season, has also been positively associated with latitude and may be one of the mechanisms governing latitudinal variation in paternal investment. Though these patterns are generally observed, few studies have directly examined the correlation between testosterone and paternal incubation and provisioning behaviors across latitudes, and even fewer have done so within species. Additionally, gonadotropin-releasing hormone (GnRH) induced testosterone levels may be more informative than daytime basal levels of testosterone. Previous research has shown that GnRH induced levels reflect endogenous daily peak testosterone levels, which may better predict if an individual will invest in paternal behaviors over other breeding behaviors (i.e. extra-pair mating, territorial defense). In this study, we examined the relationship between baseline and GnRH induced levels of testosterone and the paternal incubation and nestling provisioning behavior of male house sparrows (*Passer domesticus*) at 3 sites between Fargo ND, and Stephenville TX. We predicted that both baseline and GnRH induced T would be positively associated with latitude and negatively associated with incubation and nestling provisioning behaviors.

Species physiology, distribution limits, and population-level abundances

Zachary Lange, Brooke Bodensteiner, Daniel Nicholson, D Mahler, Martha Munoz, Luke Frishkoff

Lab-measured physiological limits have long been used to predict species distribution limits. Yet little is known about how tightly linked physiological traits are to population-level abundances across species' distributions. In essence, do individual limits and preferences correspond to the limits and “preferences” (peak abundances) of populations? We address this question using lab-measured thermal traits (CTmin, CTmax, Tpref) and abundance data of 21 species collected from extensive mark-resight surveys of *Anolis* lizard communities across Puerto Rico and Hispaniola. Our findings suggest that thermal limits do map to distribution limits, such that CTmax and CTmin are significant predictors of maximum (Tocmax) and minimum (Tocmin) environmental temperatures, albeit with substantial error. Counter intuitively peaks in abundance, rather than distributional limits, are best predicted by thermal limits—even better than by thermal preferences. Curiously, physiological niche breadth size (CTmax – CTmin) does not correlate with distributional niche breadth (Tocmax – Tocmin), meaning species that can tolerate a wide range of temperatures do not always occur across a broad range of climates, limiting our ability to make clear cut statements about thermal generalists and specialists. Together, our findings suggest that the way individuals respond to temperature does not always scale up to predict the environmental temperatures that support population growth and persistence.

Intense female-female aggression in the Gila monster (*Heloderma suspectum*)

A Kristopher Lappin, Karl H. Peterson, Anthony R. Powell, John D. Taylor, Jennifer Alexander, Gordon Schuett

Since Darwin, research on aggression in animals has been dominated by studies of male-male interactions, though female aggression is receiving increasing attention, particularly in lizards. We documented female-female aggression in the Gila monster (*Heloderma suspectum*), a large venomous lizard of the American Southwest and Mexico. Based on four unique dyadic trials, we developed a qualitative ethogram of aggressive behaviors. Unexpected were the prevalence and intensity of aggressive acts, including sustained biting, envenomation, and lateral rotation (rolling body while holding opponent with jaws). Given the prominent role of biting during interactions, we conducted bite-force experiments on a separate group of captive subjects. Based on these data, we postulate that osteoderms (bony deposits in the skin) offer some degree of protection during intense aggressive interactions, thus mitigating serious injury during female-female fights. Male-male contests in *H. suspectum*, in contrast, are far less violent and highly ritualized, with biting rarely reported. Female-female aggression in other lizards has a role in territoriality, courtship tactics, and nest and offspring guarding. The fact that *H. suspectum* is a nest-raiding specialist raises the possibility that aggression by nesting females towards marauding conspecifics, including other females, may function to prevent nest cannibalism. Future research on aggression in Gila monsters (both sexes), as well as bite-force experiments on wild subjects, are warranted to test these and other hypotheses in the laboratory and field.

Unlocking the Visual World of Free-Flying Pigeons: Insights from Head-Mounted Cameras

Anthony Lapsansky, Douglas Wylie, Douglas Altshuler

Vision is crucial for diurnal animals to control flight. Studies in birds, insects, mammals, and reptiles corroborate that visual information is essential for flying animals to negotiate tight and obstructed spaces, regulate speed, manage contact with surfaces, capture prey, navigate long distances, and maintain position. Yet our understanding of how animals discern critical visual cues from a myriad of available options and encode that information to control behavior remains limited. A significant obstacle in advancing our understanding of this process is the scarcity of empirical data describing the visual stimuli that flying animals encounter in their natural habitats. To address this knowledge gap, we developed a head-mounted camera system to record the visual environment experienced by free-flying homing pigeons. We have recorded videos for 35 flights by 12 birds, released 2 km from their home loft. Paired with 30 minutes of data on pupil position during flight from the same animals, we find that pigeons stabilize their visual surroundings through slow eye movements. We hypothesize that these movements serve to enhance the detectability of obstacles, predators, and conspecifics.

Age and growth characteristics of Spotted Bass exhibit only one response to a thermal gradient

Gisele Lara, Michael Newbrey, Ashley Desensi, Jennifer Newbrey

The effects of a thermal gradient on the age and growth characteristics of the Spotted Bass (*Micropterus punctulatus*) species complex are unknown. Therefore, our goals were to quantify the relationships between age and growth characteristics and minimum, maximum, and 24-hr thermal gradients and identify differences in variability suggesting a multispecies response. We examined relationships among mean annual temperature (MAT) at 24-hr, maximum, and minimum MAT's with total length at ages 3 and 5 years old, maximum recorded total length (MTL), and longevity for 17 populations of Spotted Bass from published literature. Spotted Bass are native to the drainages from Ohio/Oklahoma and south to Texas/Florida, USA, so our thermal gradient samples ranged from 11–15.8°C MAT_{24hr}. We also examined ultimate total length (L_{∞}) for 19 state records of Spotted Bass from 9.2–20.0°C MAT_{24hr}. We found significant negative relationships among two of the three thermal gradients (24-hr and minimum MAT) and longevity, suggesting that the species complex has a similar and shared response to a thermal gradient. No other significant relationships were identified suggesting two competing

hypotheses: a) there was no response to a thermal gradient for the other age and growth characteristics examined, or b) that each species has evolved its own response to a thermal gradient, making differentiation impossible when all species are analyzed together. Initial analyses lend support for the former hypothesis.

Hypoxia impacts maternal reproductive physiology, influencing early gestational events

Ashley Larson, Kylie Jewett, Lizzie Brisnehan, Kathryn Wilsterman

Highland-adapted deer mice display reproductive adaptations to altitude that allow improved pregnancy management as compared to lowland individuals under simulated altitude. Our recent research showed that placental function shapes fetal growth trajectories, however hypoxia exposure prior to and during early gestation may also establish fetal growth trajectories by altering ovarian and uterine function. We hypothesize that maternal hypoxia impacts both the maturation of ovarian follicles and decidualization of the endometrium, and that adaptation to high elevations mitigates the negative effects of hypoxia on these tissues. To test this hypothesis, we first isolated endometrial stromal cells from lowland and highland female deer mice and cultured them under normoxic or hypoxic conditions. We then evaluated cell proliferation. We also isolated ovarian follicles from lowland deer mice and cultured them under hypoxic or normoxic conditions. We then assessed growth and survival of the isolated ovarian follicles. We found that hypoxia altered cell proliferation and growth in both reproductive tissues. Collectively, these experiments suggest that multiple processes involved in early gestation are impacted by hypoxia and could therefore shape reproductive success at high elevations. To understand the functional basis of these differences in growth and proliferation, we are evaluating transcriptional responses to hypoxia in cells and follicles from these experiments. In vivo follow-up work will also be necessary to understand how these effects carry across to the whole animal.

Principles of cellular behavior: integrating cellular structure, dynamics, and decision making

Ben Larson

Although it may be easy to think of cells as the simple building blocks of more complex organisms such as animals, single cells are capable of remarkably sophisticated behaviors such as navigating dynamic environments, hunting prey, and evading predation. These behaviors emerge from the interactions among myriad molecular components in conjunction with physical constraints and mechanisms that dictate interactions between the cell and its environment. The ciliate *Euplotes*, a cell that walks across surfaces using motile appendages (cirri) composed of bundles of cilia, is an ideal system for navigating this mechanistic complexity due to its extensive behavioral repertoire that is amenable to rigorous analysis. Analyses drawing on ideas from nonequilibrium physics and computer science revealed finite state machine-like processing embodied in walking *Euplotes* *eurytomus* cells. Cellular walking entails regulated transitions between a discrete set of gait states with stereotypy in sequential patterns of state transitions. Simulations and experiments suggest that the sequential logic of the gait is functionally important. Cirri are connected by microtubule bundles (fibers), and the dynamics of cirri involved in different state transitions are associated with the structure of the fiber system. Perturbative experiments revealed that the fibers mediate gait coordination, suggesting a mechanical basis of gait control. Comparisons among *Euplotes* species show a complex scaling relationship between cell structure and movement patterns. These results highlight the role of physical and developmental constraints in the evolution of cellular behavior. Ultimately, we aim to elucidate general principles of the regulation and evolution of cellular behavior by integrating understanding across scales of biological organization, linking cellular structure and physiology to patterns of behavior in environmental contexts.

Vocalization comparison within nests of sea turtles on the Gulf of Mexico

Jacob Lasala, Beth Brady

Vocalizations between individuals within nests of some reptiles and birds provide an avenue for synchronous behavior. Unique vocalizations have been observed in freshwater turtles and sea turtles both in captivity and in the wild. For some sea turtle species, embryos produce sounds within the eggs, hatchlings vocalize leading up to emergence, and post-hatching individuals create distinctive sounds when in the water, suggesting intraspecies communication. However, sea turtle vocal repertoire differs between species and has predominantly been studied in controlled settings - less is known about in situ behavior. The primary goal of this project was to classify and compare the vocalizations of two species of sea turtles within the nest prior to emergence. Loggerhead and green sea turtle nests laid on Gulf of Mexico beaches, were recorded up to a week prior to emergence and sounds were categorized and analyzed. Hatchling emergence was modeled in relation to specific sounds and behavior. Hatch and emergence success were compared to the quantity of individual sounds and provide a non-invasive resource for beach managers in the future to monitor nests.

Hot parents in your area: Ambient temperature affects parental behavior in a biparental rodent

Rikki Laser, Alexander Ophir, Laura Lee

Variations in the thermal environment during offspring rearing influence parental behavior, which is important for offspring development. Although this has been explored in egg-laying species, few studies have investigated the impacts of thermal environment on mammalian parental care. Moreover, in-depth examination of how ambient temperature (AT) affects maternal behavior is limited, with even less on paternal behavior. Here, we use biparental prairie voles (*Microtus ochrogaster*) to examine the effects of increasing AT on offspring-relevant parental behaviors. Our results show that when both parents are present during high AT, fathers spent less total time in contact with pups and mothers increased their percent of lateral contact time with pups. These results show AT is a source of variation in parental behavior, and AT affects mothers and fathers differently. Our study indicates that the interaction between abiotic and behavioral factors can serve as a putative mechanism to promote behavioral variation and phenotypic plasticity in mammals.

Uncovering the 3D anatomy of the primate clitoris

Daniel Latorre, Magdalena Muchlinski, William Sellers, Georg Hantke, Andrew Kitchener, Charlotte Brassey

The 3D anatomy of the human clitoris has only been documented in the last ~20 years following the application of medical imaging. This has revealed an extensive subsurface clitoral body, crura, and bulbs, compared to the small externally visible clitoral glans (the proverbial 'tip of the iceberg'). And whilst the morphology of the external clitoris of non-human primates has known interspecific variation, we know almost nothing of its comparative internal anatomy, nor any relationship to mating or social systems.

We report the first broad comparative anatomical study of the primate clitoris. We dissected 39 female primates from 21 species, all deceased zoo individuals. We applied Magnetic Resonance Imaging (MRI) and diffusible iodine contrast-enhanced Computer Tomography (diceCT) to segment the 3D architecture of the clitoral complex, alongside dissection and histology for tissue identification.

Our results show previously undocumented variation within the primate clitoris. Whilst the chimpanzee shares several features in common with humans, most primates deviate considerably from this condition. Excepting the apes, clitoral bulbs are small and difficult to differentiate. In contrast, the clitoral body and crura are always present and distinct from surrounding tissues. The spider monkey's pendulous clitoris is found to possess paired suspensory ligaments, presumably to support its enlarged size. Future work will quantify 3D shape and size of clitoral components and explore correlations to life history metrics.

A possible role for neophobia in invasion success: A tale of two sparrows

Christine Lattin, Kevin Krajcir, Tosha Kelly, Melanie Kimball, Ella Cochran, Keegan Stansberry, Blake Dusang, Ayushi Patel, Danna Masri, Sara Lipshutz

Invasive species are one of the main threats to biodiversity globally. However, most research has focused on environmental and ecological factors that allow for introduced species to succeed and become invasive, with relatively few studies assessing the role of behavioral and cognitive traits. To help fill this knowledge gap, we studied neophobia, an aversive response towards novelty, in non-native Eurasian tree sparrows (*Passer montanus*), and compared results to previous work in the Eurasian tree sparrows' more successful invasive congener, the house sparrow (*Passer domesticus*). We assessed the neophobia of wild-caught Eurasian tree sparrows in the lab using a standardized protocol measuring their response to novel objects and novel foods and their ability to habituate to the same initially novel object. We predicted that Eurasian tree sparrows, as less successful invaders, would overall be more neophobic than house sparrows. We did not observe differences in neophobia towards novel objects in the two species, but we did find that Eurasian tree sparrows were significantly less willing to try novel foods than house sparrows. Eurasian tree sparrows were also slower to habituate to repeated presentations of the same initially novel object compared to house sparrows. Although there are certainly multiple factors that influence invasion success, our results suggest that neophobia could be one trait that might make an introduced species less successful in a novel environment.

Uncovering the molecular basis of state coordination in polymorphic crickets

Meghan Laturney, Caroline Williams

Organisms must allocate their limited energy and nutritional resources into various physiological processes. In polymorphic species, morphs differ in allocation patterning as they prioritize different life history traits. For instance, we often observe one morph investing immediately into reproduction, while the other morph delays offspring production in favor of a temporary, reproduction-incompatible dispersal phase. In field crickets, *Gryllus lineaticeps*, conspecifics exhibit a wing-polymorphism that differ in reproduction strategy and flight capability. Short wing (SW) females immediately invest into reproduction and do not fly. Long wing (LW) females, on the other hand, initially invest into dispersal, made possible by their functional flight muscles and underdeveloped ovaries. After 3-5 days of adulthood, LW females undergo flight muscle histolysis and simultaneous ovary growth, transitioning into the reproductive phase. Recently, there has been large advances in our understanding of the genetic pathways that regulate histolysis of the flight muscles in LW crickets. However, how reproduction and flight capability are negatively coordinated remains completely unknown. My research aims to identify the molecular regulators of reproduction in *G. lineaticeps* and determine the critical differences contributing to ovary development between the morphs (SW vs LW) and reproductive states (early LW and late LW). Together, this system provides a convenient opportunity to study the biological basis of physiological tradeoffs and coordinated state transitions within a polymorphic species.

Horizontally transferred genes are recruited in the evolution of lensed light-interacting organs

Emily Lau, Rebecca Varney, Todd Oakley

Mutations, such as gene duplication and horizontal gene transfer, provide raw genetic material for evolution. While gene duplication is considered a major mechanism for generating evolutionary novelty, the role of horizontal gene transfer in the evolution of novel traits, especially in metazoans, is contested. Here we show that in multiple instances, the lenses of eyes and light-producing organs convergently evolved by recruiting horizontally transferred genes. Lenses, which are comprised of structural proteins called crystallins, convergently evolved by recruiting members of non-homologous gene families. We perform genome sequencing, phylogenetics, and comparative genomics to identify and investigate the evolution of a novel crystallin gene from the light-producing organs of toadfishes. Genes homologous to the

toadfish crystallin are found in other bony fishes of Actinopteri, and separately in non-metazoans such as bacteria and fungi. Our findings suggest that, after an ancient horizontal gene transfer, the gene was later co-opted as a crystallin during the evolution of bioluminescence in toadfishes. Similarly, the lenses of jellyfish and vertebrate eyes are comprised of non-homologous crystallins, some of which exhibit phylogenetic incongruence between their gene trees and a metazoan species tree. Altogether, this suggests that horizontally transferred genes have been repeatedly recruited in the convergent evolution of lensed light-interacting organs. Broadly speaking, our work underscores the importance of horizontal gene transfer in providing raw genetic material for the evolution of novel traits.

Integrating convergent evolution across levels of biological organization, organisms, and time

Emily Lau, Jessica Goodheart, Rebecca Varney

The extent to which evolution is predictable is a long-standing question in biology with implications for urgent biological issues such as the evolution of viruses, emergence of antibiotic resistance in bacteria, and organismal responses to climate change. Convergent evolution, the independent evolution of similar phenotypes, provides biological replicates useful for exploring patterns of predictability in evolution. However, to understand when and why evolution is predictable, we must go beyond simply recognizing patterns of convergent evolution to determining the evolutionary mechanisms that produce these patterns. Thanks to the development and integration of new experimental methods in diverse fields of biology — such as cell biology, genetics, physiology, development, and bioinformatics — researchers now have the means to investigate how, when, and why phenotypes convergently evolve across multiple biological levels. In this symposium, speakers will share their research on convergent evolution, which transcends levels of biological organization. By uniting findings and perspectives of researchers who study convergent evolution in diverse study systems and across levels of biological organization, our symposium will provide a space to identify and discuss tools and fresh perspectives to tackle long standing questions in the field of evolutionary convergence.

Don't Go Changing: Stability in the Mill Creek Ecosystem Pre-Pipeline Construction, Virginia

Jamie Lau, Gerald Smith, Andrea Beverley, Kristina Stefaniak, Tara Pelletier

Headwater streams account for approximately three-fourths of stream ecosystems and are consistent sources of high biodiversity. Unfortunately, headwater streams are disproportionately affected by human-induced modifications because they have little regulatory protections under the Clean Water Act. The Mountain Valley Pipeline is planned to cross several headwater streams in Southwest Virginia. Our team designed a before-after control-impact (BACI) study to determine the effects of the installation process on Mill Creek and one of its tributaries, Roanoke County, Virginia. Since 2019 and in March and August, we have conducted a systematic physical habitat assessment and collected aquatic insects in 4 control and 6 impact sites, pre-pipeline construction. We also recorded the microclimate and measured the water chemistry, including basic chemistry, metals, anions, and cations. A principal components analysis will be used to explore how our sites are structured in regards to the abiotic factors. Nonmetric multidimensional scaling (NMDS) will be used to explore the differences in the aquatic insect assemblage structure. We found that the physical habitat is stable across years and seasons, but the control and impact sites did not logically group together based on the water chemistry as expected. We are still identifying insects and will report on the NMDS results. The pipeline completion is expected in 2023; our BACI design will allow us to effectively capture any changes produced by the installation process.

The hydrodynamic function of shark skin

George Lauder, Tess Avery, Dakota Law, Molly Gabler-Smith

The skin of sharks is composed of numerous individual denticles (scales) that cover the body surface. Although recent research has documented the morphological diversity of shark denticles, the function of this diversity is

poorly understood. One common view is that the roughened skin of sharks alters water flow patterns over the body reducing drag and enhancing thrust, but quantifying the hydrodynamic function of shark skin has proven to be challenging. Here we use a custom flow tank and knife-edge plate to visualize water flow over small samples of shark skin from a variety of species compared to flow over a smooth control. We used high-speed video and particle image velocimetry to visualize flow in the boundary layer, and measured velocity profiles in both the free-stream and perpendicular directions at three speeds with five replicates per speed. From these data we calculated shear stress on the surface using the velocity profile in the viscous sublayer, and measured boundary layer thickness. We also used 3D profilometry to quantify the surface texture of each tested sample. Shark skin samples show altered shear stress compared to smooth controls and the shape of the near-surface velocity profile varies among species. Testing a variety of samples that differ in denticle morphology and orientation as well as 3D-printed denticles will help clarify the relationship between denticle morphology and hydrodynamic flow patterns.

Singing Cichlids: How to Serenade a Fish

Isabelle Laun, Suzy Renn, Andrew Anderson

Acoustic communication in fish is a growing field, and many African cichlids originating in Lake Victoria and Lake Malawi have been found to produce noises during courtship and territorial disputes. Despite the body of literature on cichlids originating from Lake Malawi and Lake Victoria, there is little known about how acoustic communication may have changed and evolved in species from Lake Tanganyika, potentially playing a role in interspecific mate recognition and subsequently speciation of visually similar species. Here, we investigate how acoustic communication may play a role in intra-specific communication in two species of African cichlids, *J. transcriptus* and *J. marlieri*, which originate from Lake Tanganyika. To investigate how these two visually similar species may produce and utilize different acoustic signals, we will characterize acoustic noises and the associated behaviors produced by courting and established pairs. Using simultaneous behavioral observations and acoustic monitoring, we can investigate how and when acoustic signaling is present during courtship, and acoustic analysis can reveal key differences in pulse rate and pulse period, among other acoustic qualities, that may play a role in multimodal communication during mate recognition and courtship.

Malleable mouths: upper and lower beak kinematics of shrikes in relation to bite force and velocity

Mason Laurin, Diego Sustaita

Loggerhead Shrikes (*Lanius ludovicianus*) are medium-sized (~50 g) passerines that feed on arthropods and vertebrates. Differences in the physical and behavioral attributes of these prey types are likely to impose disparate demands of force and speed on their jaws. However, because of the biomechanical trade-off between force and speed, it is not clear how shrikes generate both fast and strong bites. We analyzed high-speed videos of shrikes biting a force transducer in lateral view to obtain corresponding measurements of bite force, upper and lower bill linear and angular displacements, and jaw-closing velocities. We found no clear trade-off between bite force and jaw-closing speed among individuals. However, we found that the angular range of upper bill movement (i.e., depression and elevation about the craniofacial hinge) is correlated more with bite force, whereas the angular range of lower bill movement is correlated more with bite velocity, suggesting a potential way around the force-velocity trade-off. Further investigation reveals that shrikes bite at intermediate levels of both force and velocity, which consequently optimizes power. Biting power is more closely related to jaw-closing velocity than bite force, suggesting that shrikes generate greater biting power primarily by altering jaw-closing speed.

Uncovering the mosaic evolution of carnivoran skeletal systems

Chris Law, Leslea Hlusko, Jack Tseng

The diversity of vertebrate skeletons is often attributed to adaptations to distinct ecological factors such as diet, locomotion, and sensory environment. Although the adaptive evolution of cranial, appendicular, and vertebral

skeletal systems is well studied in vertebrates, how modes of evolution differ among skeletal components are unknown because comprehensive investigations of all skeletal components are rarely performed simultaneously. Here, we tested for distinct modes of evolution among all the major components of the cranial, appendicular, and vertebral skeletal systems of carnivoran mammals. We found mosaic evolution of the carnivoran skeleton in which only the mandible, hindlimb, and posterior region of the vertebral column showed evidence of adaptation towards ecological regimes whereas the remaining skeletal components followed clade-specific evolutionary shifts. We hypothesize that the decoupled evolution of individual skeletal components may have led to the origination of distinct adaptive zones and morphologies among extant carnivoran families that reflect phylogenetic hierarchies. Overall, our work highlights the importance of examining multiple skeletal components in ecomorphological analyses. Ongoing work integrating the fossil and paleoenvironmental record will further elucidate the carnivoran diversity we see today and reveal the complexity of evolutionary processes in multicomponent systems.

Effect of skeletal torsion on human locomotor biomechanics: Support for hominin locomotor diversity?

Austin Lawrence, Jamie Hall, Kevin Middleton, Jacob Thomas, Abdullah Alsharafi, Sam Weiss, Trent Guess, Carol Ward

Bipedal locomotion is a defining feature of the human lineage. However, the form of bipedality in different hominin taxa and the timing of emergence of a human-like striding bipedal gait have long been a subject of debate. Morphological features of the hip and pelvis have been used to argue for kinematically different gaits in *Australopithecus*, early *Homo*, Neanderthals, and modern humans. However, limited comparison of morphology and locomotor performance in living humans has left a weak base upon which to build hypotheses of locomotor diversity in fossil hominins. In this study, we address these problems by quantifying skeletal torsion features in the pelvis and lower limb and directly evaluating their relationships with transverse plane kinematics during walking in a sample of adult humans. Individually, femoral version, tibial torsion, acetabular version, and iliac blade orientation are not correlated with transverse plane walking kinematics in our sample. However, correlations between femoral version and tibial torsion and between femoral version and iliac blade orientation suggest that there may be anatomical compensatory mechanisms for lower limb skeletal torsion to maintain a forward-facing foot and relative lengths of hip abduction and internal rotation moment arms during walking. These results suggest that caution is warranted in interpretations of musculoskeletal function and performance based on features of skeletal morphology alone, especially in anatomical regions that are highly variable or functionally integrated.

Investigating Parental Investment and Reproductive Behavior in Northern Pipefish (*Syngnathus fuscus*)

Xylo Lazrinth, Evelyn Papalimberis, Kerstin Musolf, Anthony Wilson

Parental investment is highly variable across species and ranges from nutrient supplementation of developing embryos to offspring care. Members of the family Syngnathidae have a unique mode of reproduction in which males carry a brood pouch, allowing for male pregnancy. While previous research suggests that brood pouches allow for the transfer of nutrients and oxygen, relative parental energy investment in this group has not been confidently quantified. Here, we used controlled matings to quantify relative parental investment in the Northern pipefish (*Syngnathus fuscus*), measuring dry weight and concentration of three major macromolecules (carbohydrates, lipids, and proteins) in unfertilized eggs and released fry. Mating triads of one female and two males were placed into a three-chambered choice apparatus to track individual preferences and establish ethograms of courtship behavior. We observed polyandry in 50% of successful mating triads, suggesting the physiological potential for multiple mating in this species despite the dominance of monogamy in wild populations of *S. fuscus*. Nutrient analyses indicate that the nutrient content of unfertilized eggs is significantly higher than that of newly released fry, suggesting that male nutrient investment is negligible in this species.

Triglycerides in seabird chicks, Black-legged kittiwakes (*Rissa tridactyla*)

Alex Le, Sierra Pete, Shannon Whelan, Morgan Benowitz-Fredericks

Triglycerides are involved in energy storage and mobilization and may serve as a useful indicator of the physiological state of seabirds. In adult kittiwakes, higher triglycerides are associated with better nutritional state. However, triglycerides have not been well-studied in chicks, where interpretation may be challenging because elevated triglycerides can indicate either higher energy availability/storage, or reduced energy availability that requires energy mobilization. We tested factors that may affect triglyceride measurements in young seabird chicks, including, methodology, age, sex, chick ranking (A or B), and nutritional status (via experimental supplemental feeding). Methodologically, triglycerides in fresh whole blood (using a point-of-care meter) and thawed plasma (via an EIA) were positively correlated, while repeated bleeding (day 5 and day 10) decreased triglycerides. At 5 days old, supplemental feeding tended to reduce triglycerides in A chicks but increased them in B chicks. However, by day 10, the growth rate was the best predictor of triglyceride levels, regardless of feeding status or rank; this relationship was stronger in female than in male chicks for which the reasons remain unclear. The inconsistent effects of nutritional status at day 5 suggest triglycerides dynamics of very young chicks may be influenced by a complex interplay of age- and rank-related dietary and metabolic differences, with growth as a primary driver by day 10.

The genome of *Anoplarchus purpureus* (Stichaeidae) reflects its carnivorous diet

Ninh Le, Joseph Heras, Michelle Herrera, Donovan German, Lisa Crummett

Comparative genomics of digestive enzymes can help us better understand the genomic underpinnings of dietary specialization. The "Adaptive Modulation Hypothesis" (AMH) proposes that digestive enzyme activity, which increases with increased gene copy number, should correlate with substrate quantity in the diet. To test the AMH and reveal genomic underpinnings of herbivory vs carnivory, we sequenced, assembled, and annotated the genome of *Anoplarchus purpureus*, a carnivorous pricklyback fish in the family Stichaeidae; then we compared the gene copy number for key digestive enzymes to that of *Cebidichthys violaceus*, a herbivorous fish from the same family. A highly contiguous genome assembly of high quality (N50 = 10.6 Mb) was produced for *A. purpureus*, using combined long-read and short-read technology, with an estimated 33,842 protein-coding genes. The digestive enzymes that we examined include pancreatic α -amylase, carboxyl ester lipase, alanyl aminopeptidase, trypsin, and chymotrypsin. *A. purpureus* had fewer copies of pancreatic α -amylase (carbohydrate digestion) than *C. violaceus* (1 vs. 3 copies). *A. purpureus* also had one fewer copy of carboxyl ester lipase (plant lipid digestion) than *C. violaceus* (4 vs. 5). We observed an expansion in copy number for several protein digestion genes in *A. purpureus* compared to *C. violaceus*, including trypsin (5 vs. 3) and total aminopeptidases (6 vs. 5). These findings support the AMH and coincide with observed digestive enzyme activities for these two species.

Introduction to the symposium: the scale of resilience

Emily Le-Sage, Corinne Richards-Zawacki, Jamie Voyles, Cheryl Briggs

Despite decades of research advancing the theoretical and conceptual frameworks of biological resilience, many uncertainties concerning the mechanisms of resilience remain. This includes questions such as: How do we measure resilience and compare the relevant variables and properties across systems? What does resilience mean in different contexts and at various biological scales of organization? How does a perturbation at one biological scale impact processes or functionality at other scales? Progress toward understanding resilience is hindered by the lack of a common currency for measuring resilience, empirical data that span systems and levels of biological organization, and a flexible, quantitative framework in which to interpret these values. By bringing together leaders studying resilience in a variety of systems, responding to

various stressors, and across multiple biological scales, this symposium aims to support progress towards integration and cross-disciplinary collaborations to address these outstanding questions.

Roundtable discussion: parallels among perturbations

Emily Le-Sage, Jamie Voyles, Corinne Richards-Zawacki, Cheryl Briggs

By comparing different disturbances within the same system or similar systems, we can begin to understand which responses lead to resilience or a loss of function. Comparisons of thresholds, such as the duration or magnitude of disturbance that causes a system shift to a different functional state or become reorganized, will be instrumental in efforts to build a predictive resilience framework. Symposium speakers study a wide variety of perturbations, which vary in duration from short to long-term (i.e., pulse or press disturbances), and we invite our participants to think critically about how to quantify variation in their study system's responses to different disturbance types, magnitudes, and durations.

Roundtable discussion: connecting scales

Emily Le-Sage, Cheryl Briggs, Jamie Voyles, Corinne Richards-Zawacki

Theory suggests that cross-scale mechanisms are at work during a system response to perturbation, yet these connected processes are a challenge to study. As biological systems composed of different organizational levels are interconnected across scales, forecasting how changes at one organizational level affect the other levels requires synergistic research efforts. Thinking across multiple scales permits exploration of the general patterns of resilience and is fundamental in moving this research agenda forward. Thus, we plan to discuss current and new methods to study cross-scale mechanisms at our symposium activities, and spark synthesis and collaboration among invited researchers working at different scales in the study of resilience.

Roundtable discussion: common currencies

Emily Le-Sage, Corinne Richards-Zawacki, Cheryl Briggs, Jamie Voyles

Efforts in resilience biology are currently hindered by the lack of a common currency for measuring resilience. By comparing various ecological systems with similar perturbations, we can begin to understand whether specific system attributes or variables enable predictions of resilience. Finding common rules and system behaviors can transform the way we understand biological resilience and accelerate new discoveries of the rules of life on Earth. We plan to discuss outstanding questions of common currencies during our symposium forums such as: 1) What mechanisms generate variation in response to stressors of the same type, magnitude, and duration? 2) What are the relevant metrics that enable us to identify thresholds and estimate changes in system functionality? 3) How can we best train the next generation of transdisciplinary scientists to integrate across systems to better understand resilience?

Corresponding host-pathogen-microbiome seasonal rhythms across a latitudinal gradient

Emily Le-Sage, Brandon LaBumbard, Michel Ohmer, Laura Reinert, Karie Altman, Nina McDonnell, Ian Latella, Veronica Saenz, Mark Wilber, Jamie Voyles, Douglas Woodhams, Corinne Richards-Zawacki, Louise Rollins-Smith

As climate change is reducing the reliability of seasonal cues, studies of seasonal disease dynamics are critical for predicting future shifts in host-pathogen interactions. We studied 3 years of concordant rhythms of host peptide defenses, microbiome, body temperature, and the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) in leopard frog populations across five regions spanning 13° of latitude using generalized additive models. We examined cross-correlations among model estimates to determine if there is a temporal lag between host factors and Bd prevalence. We observed analogous seasonal patterns in Bd prevalence and stored antimicrobial peptide (AMPs) quantities all but one of our study regions. Northern populations (*R. pipiens*) had larger amplitude cycles in AMP quantities, which were more tightly associated with Bd prevalence (cross-correlation of 0.94 at 17 d). In

contrast, southern (*R. sphenoccephala*) populations displayed lower amplitude cycles in AMP quantities which lagged behind Bd prevalence with weaker cross-correlations (< 0.61 at 30 d). In piecewise structured equation models, Bd infection was predicted by the previous two-week average air temperature in all but one location. AMP defenses were never predictive of infection status, likely due to the temporal lag observed at the population level. Altogether, concordant seasonal rhythms in stored AMPs are expressed across latitudes in leopard frogs, though timing and strength of cross-correlations with Bd prevalence seasonality varied.

Break it 'til you make it: Mechanisms of flight muscle histolysis in the variable field cricket

Jacqueline Lebenzon, Tomas Diaz, Caroline Williams

Insect flight is one of the most energetically costly processes in the animal kingdom; both flying and maintaining muscles required for flight are expensive. Thus, when these flight costs outweigh the benefits of dispersal, some insects, such as the California variable field cricket, prioritize other expensive physiological processes such as reproduction. During this flight to reproduction transition, flight capable field crickets break down ("histolyse") their flight muscle. Further, this flight muscle histolysis is selective; crickets degrade their dorsolongitudinal (DLM) muscle first while maintaining their neighbouring dorsoventral (DVM) muscle. Although we know that histolysis is crucial for life history transitions in crickets, we have a limited understanding of the mechanisms underlying the massive cellular re-organization required for this histolysis. Here, our objectives were to understand the structural and functional changes associated with muscle histolysis, explore how crickets integrate upstream signals into downstream selective remodeling of DLM and maintenance of DVM, and use RNA interference to establish causal links between candidate regulatory pathways and selective flight muscle histolysis. While most animals have evolved ways to grow or maintain muscle, these insects have evolved the capacity to adaptively and selectively degrade flight muscle. Thus, understanding the mechanisms of insect flight muscle histolysis can yield insights into how muscle plasticity can evolve when it is required for energetic trade-offs during important life history transitions.

Non-invasive monitoring of surface temperatures and thermoregulatory behavior during competition

Josh Ledford, Raymond Danner, Juan Zuluaga

Recent laboratory studies have shown that stressful situations can cause instantaneous changes in surface temperature and are thought to influence thermoregulatory behaviors. We hypothesized that naturally occurring social interactions among wild songbirds would lead to a change in 1) surface temperatures and 2) thermoregulatory behaviors. We used thermal imaging to measure changes in birds before, during, and after competitive interactions and other disturbances among sixteen bird species at bird feeders. We predict that competitive interactions and other disturbances will lead to a decrease in surface temperature, which would support the 1st hypothesis. Further, we predict that the behavioral interactions will lead to an increase in behaviors associated with heat stress, such as panting and changes in stance, which would support the 2nd hypothesis. We captured interactions in over 200 non-invasive thermal videos of birds from June 6th to August 22nd, 2023, where birds engaged in various conflicts while feeding from three different bird feeders. We captured images at a range of intermediate to high ambient temperatures (26–35.7°C), when birds are known to exhibit a range of heat dissipation from surfaces and engage in cooling behaviors. Therefore, these measurements represent a range of typical environmental conditions experienced by wild songbirds during summer. The results of this study will help us further understand how naturally occurring social interactions influence thermoregulation in birds.

Sexual Dimorphism in Size-Adjusted Keel Lengths of Some Birds and Not Others

Adrian Lee, Elizabeth Cramer, Olivia Rataezyk, Patrick Wade-Wolfe, Erasmia Yager, Natalie Wright

Flight muscles are an integral aspect of avian morphology. Previous research found differences in keel lengths between male and female birds in some species of birds. To quantify the prevalence of these differences, we measured 458 specimens across 21 species in museum collections. A majority of species show no significant difference in keel size (relative to body size) between males and females. In three species, males had larger keels than females: Northern cardinal (*Cardinalis cardinalis*), Bullock's oriole (*Icterus bullockii*) and green-throated carib hummingbird (*Eulampis holosericeus*). Keel lengths of these species differed by 9.94%, 10.48%, and 8.47%, respectively. Of species without significant differences in corrected keel size, keel size differed by 3.01% on average between males and females.

Change for a dollar? Sand dollar larvae maintain food-induced plasticity throughout development

Ariana Lee, Isabel Villafuerte, Sarah Kasem, Ethan Nguyen, Douglas Pace

Echinoid larvae possess food-induced phenotypic plasticity, changing arm length and physiological growth efficiency based on food availability. While most studies examine plasticity at the onset of larval feeding, it is unknown if plasticity responses can be initiated throughout larval development. We assessed the morphological and physiological plasticity responses of larvae of the Pacific sand dollar, *Dendraster excentricus*, when switched from low to high (L2H) and high to low (H2L) food conditions at different developmental times. L2H larvae quickly acquired a high-fed phenotype by shortening their larval arms, suggesting that maintaining long arms is energetically expensive. Despite the differing times to metamorphic competency, high-fed larvae and L2H larvae had similar cumulative energetic costs of development, providing strong evidence for the adaptive value of phenotypic plasticity. All larval feeding treatments possessed similar protein biomass at metamorphic competency, suggesting that larvae need to have a minimum protein biomass to successfully transition to the juvenile stage. H2L experiments also demonstrated that larvae maintain morphological plasticity to changing food conditions. Our results establish that even in the backdrop of absolute requirements to achieve metamorphic competency, robust plasticity responses remain available to larvae throughout development in response to changing food environments, even after the establishment of the juvenile rudiment. This data further expands our understanding of the adaptive value and potential of phenotypic plasticity during echinoid planktotrophic development.

Splitting Hairs: The biomechanics of baleen in Gray Whales (*Eschrichtius robustus*)

Ferris Lee, Megan Vandenberg, Karly Cohen, Cassandra Donatelli, Shirel Kahane-Rapport

Rorqual whales, a group of Mysticetes (Cetacea), use an oral filter structure called baleen to filter-feed. Baleen is a keratinous material that hangs from the upper gums in a whale's mouth. Most rorquals lunge filter feed, engulfing enormous masses of both prey and water; the prey is then separated from the water using the baleen plates. Gray whales (*Eschrichtius robustus*), however, use a form of suction filter feeding by fluidizing mud and sand and scraping their heads and baleen along the substrate. While all baleen must be capable of withstanding the force of gallons of prey and water entering the mouth, gray whale baleen must also withstand repeated collisions with rough substrate. To better understand the durability of gray whale baleen, we measured the morphometrics and material properties of baleen plates from 18 gray whales varying in age and sex. Max load correlated with size ($r^2 = 0.42$, $p < 0.05$) and age of the animal ($r^2 = 0.44$, $p < 0.05$), although load consistently decreased towards the fringe of the baleen across all groups. Max load was found to be higher in males than in females. Scaling of baleen stiffness varied at each section: the middle and ends of baleen scale hyperallometrically and portions nearer to the gum scale hypoallometrically with standard length. Morphometric variation between age groups in baleen is primarily driven by rack spacing and major plate lingual thickness.

Mechanosensory role of elytra in beetle flight

Hyee Ryan Lee, Robert Dudley

Elytra, the modified and hardened forewings of Coleoptera, represent a key innovation in insect evolution, and can serve varied roles in physical protection, thermoregulation, water balance, transport of symbionts, sexual selection, and stridulation. However, in spite of their evolutionary origin as wings, the current role of elytra in flight has long been thought to be minimal. Here, we demonstrate a novel mechanosensory function for elytra in beetle flight by quantifying biomechanical changes during take-off following sequential elytral ablation in a False Blister Beetle (*Sessinia livida*, Oedemeridae). Elytral ablation to any extent resulted in loss of flight control for body orientation, an outcome also observed in dipterans with modified halteres. However, and unlike haltere manipulation in Diptera, elytral ablation yielded no changes in either wing-beat frequency of the hindwings or in oscillation frequency of the elytra. The elytra thus actively participate as sensory organs regulating flight control and likely function gyroscopically, albeit less effectively than dipteran halteres. A gyroscopic role for elytra may underlie evolutionary patterns of forewing shortening in beetles, and the substantial comparative diversity of this structure suggests a correspondingly wide range of sensory and biomechanical functions among the Coleoptera.

The Human Shoulder's Dual Challenge: Maintaining Torque and Speed During a High Demand Task

Kayla Lee, Erin Lee, Michael Rainbow

Evolution of the human shoulder complex likely resulted from selective pressures favoring the performance of diverse tasks requiring high mobility, speed, and torque, such as climbing, tool use, and throwing. In more constrained joints, such as the knee, torque decreases with increasing speed. Since the shoulder's functional demands require both high-speed and torque as seen in throwing, we questioned whether it can maintain torque over a large range of angular velocities. We introduce a novel approach that integrates a controllable cable machine (1080 Quantum) with markerless motion capture to compute angular velocity and torque using inverse dynamics. Participants ($n = 20$) executed a crossbody, isokinetic task at varying speeds and under a torso-constrained and unconstrained condition. We expected the shoulder to maintain high torque over a larger range of speeds in the unconstrained task. Linear regression on torque versus angular velocity revealed that most participants maintained high shoulder torque over a large range of angular velocities, while 7 subjects decreased torque as angular velocity increased. Although the torso-constrained condition was anticipated to cause greater reductions in torque as velocity increased, no definitive trend emerged. Differences in torque maintenance across speeds, conditions, and individuals may stem from postural changes, morphology, muscle recruitment, and elastic storage. This investigation highlights the variability in the human shoulder and may uncover form-function relationships that explain our exceptional throwing capabilities. Top of Form

Aerodynamics and Transition Dynamics of the Hind Wings of *Schistocerca americana* Grasshopper

Kyung Jun Paul Lee

True Colors: Investigating wing colorations through the eyes of Hummingbirds

Nora Lee, Yasmeen Erritouni, Kevin Epperly, Rosalee Elting, Alejandro Rico-Guevara

Hummingbirds are known to use plumage coloration as signals in the contexts of courtship display, territorial defense as well as camouflage. Past colorimetric studies on hummingbirds have focused on visually colorful (to humans) plumage patches such as gorgets, crowns, and chests, which ignore the potential functions of "dull" patches that could be conspicuous in the eyes of hummingbirds as a result of their tetrachromacy (ultraviolet + visible wavelength). One such example is the ventral side of hummingbird wings, on which our study focuses. During territorial defense, we observed that 1) some hummingbirds pause briefly during hovering flight with their underwings

held in visible position towards another hummingbird and 2) when perching on feeders they hold up their wing(s) with ventral sides facing approaching hummingbirds. We hypothesize that underwing colorations and patterns serve signaling functions in the context of territorial defense and are associated with dominance amongst individuals. To capture plumage coloration, we use a spectrophotometer to measure and a full-spectrum camera to photograph wing specimens from Burke Museum. Data from spectrophotometry and camera are put into R and ImageJ respectively to quantify and analyze plumage coloration and patterns through a hummingbird-specific visual system. After this study, future research on the behavior of live hummingbirds will be warranted as behavioral complements to this museum morphology study.

Free-ranging squirrels stabilize branch landing using nonprehensile, palmar foot grasps

Sebastian Lee, Stanley Wang, Duyi Kuang, Justin Yim, Eric Wang, Nathaniel Hunt, Hannah Stuart, Robert Full

An agile squirrel's survival can depend on dynamic, high-impact, stable landing on branches. Yet, squirrels don't grip branches using a prehensile grasp with thumbs; instead, they land stably using palms always ready for parkouring. Through high-speed video and force-torque measurements, we quantified the kinetics of direct landings for four free-ranging fox squirrels (*Sciurus niger*) during gap crossing maneuvers across three different gap distances. Previously, we found that as gap distance increased, velocity angle, event timing speed, and peak force-torque increased. Regardless of gap distance, squirrels managed effectively 85% of landing kinetic energy using their forelimbs. We tested control hypotheses for direct landings by modeling the front legs as a spring-mass system. We hypothesized two strategies to stabilize off-target landings. Control of front leg length as reflected by leg force could correct for under- or over-shooting by altering inertia to adjust swing up speed. We discovered that peak leg force did increase with landing error. Squirrels applied greater leg forces when overshooting and lower forces when under-shooting, supporting the leg force control hypothesis. Additionally, squirrels could correct for under- or over-shooting by generating pull up or breaking torques. We found that peak torque decreased with landing error, supporting the torque control hypothesis. Future research will investigate the embodied control of palmar grasps to give insight into high-impact, dynamic grasping and manipulation in devices.

A Morphometric and Behavioral Analysis of Everglades Mosquitofish Hybridization in South Florida

Rose Leeger, Jeffery Hoch

The Mangrove Mosquitofish is found in many brackish and freshwater ecosystems surrounding southeast Florida and Cuba. Historical range distribution in Florida has found these fish solely in the Florida Keys and parts of Miami. This research provides an update to the northernmost range that Mangrove Mosquitofish have been observed. As the name implies, Mangrove Mosquitofish reside in areas of critical habitat: Mangrove Forests. These forests are constantly battling habitat loss and reduction due to increased urbanization in native areas. Mangrove Mosquitofish are poeciliid fish species with a modified anal fin called a "gonopodium" that allows for internal fertilization. This research found novel hybrids of the Mangrove and Eastern Mosquitofish as well as a new discovery into the mangrove mosquitofish range expansion. Geometric morphometric analysis of hybrid gonopodia (Eastern Mosquitofish x Mangrove Mosquitofish) reveals an intermediate shape and shows the potential for genetic introgression between species. Hybridization may further threaten Mangrove Mosquitofish populations as they are more vulnerable than the Eastern Mosquitofish due to their use of threatened habitat and range limitation. The morphometric analysis between the two species and hybrids does confirm there is significant difference ($p < 0.05$) between the two species, and among sites. Boldness behavior trials of both male and female mangrove mosquitofish show slight differences in risk tolerance and exploration between the two sexes. The observations have important implications for the future of Mangrove Mosquitofish in the face of climate change and other anthropogenic habitat alterations.

Effects of predator-induced stress response on territorial aggression in convict cichlids

Joseph Leese, Aryssa Ellerbee, Nicole Moussa, Melissa Peller, Cora Zilinski

Territorial aggression is a critical behavior for many animals as a means to secure access to limited resources like food and mates. Convict cichlids, *Amatitlania nigroscia*, are a neotropical fish species that demonstrate territorial aggression as breeding pairs in the wild and as individuals in lab settings. In this study, we explored whether activation of the stress response, induced by exposure to a potential predator, would influence territorial aggression in this system. We hypothesized that predator-exposure would increase aggression and predicted that time to initiate contests would decrease and overall levels of aggression would increase. We acclimated individual male and female cichlids to a 'home' tank and then exposed them to a potential predator, *Parachromis dovii*, or a control treatment. Individuals were then returned to their home tank and presented with a territorial intruder. Surprisingly, predator exposure did not significantly affect aggressive behavior, but there were sex differences in the aggressive profiles toward intruders. Future work will measure the levels of glucocorticoids before and after predator exposure and contest to determine if individual aggression levels correspond to stress hormone levels.

Evolution of the syrinx of Apodiformes including the vocal-learning Trochilidae (Aves: Strisores)

Lucas Legendre, Carlos Rodriguez-Saltos, Chad Eliason, Julia Clarke

The vocal organ of birds, the syrinx, represents a key innovation in the evolutionary history of vertebrate communication. Three major avian clades—passerines, parrots, and hummingbirds— independently acquired both specialized syringeal structures and vocal production learning, between which a functional relationship has been proposed but remains poorly understood. In hummingbirds, the syrinx has never been studied comparatively alongside non-learning relatives in parent clade Strisores. Here we describe the syrinx anatomy in three swift species using enhanced-contrast computed tomography, which reveals structures previously unreported in the clade. We also test for correlations between syringeal and acoustic traits in a sample of hummingbirds and swifts using phylogenetic comparative methods. The swift syrinx presents lateral labia located on the first pair of bronchial half-rings, which are also present in hummingbirds and may be ancestral to Strisores. The further enlarged lateral labia of hummingbirds are found to be significantly correlated to the reduction in length of their trachea and m. tracheolateralis. Complex intrinsic muscles and loss of the sternotrachealis muscle co-occur with these shifts and with vocal learning. We recover a significant negative correlation between tracheal elongation and maximum vocalization frequency, suggesting that tracheal shortening in hummingbirds facilitated the acquisition of high-frequency vocalizing. Sampling additional Strisores clades, particularly those with undocumented syrinx morphology, will be essential to understanding the acquisition of vocal learning in hummingbirds.

The Potential for Increased Intake of Tributyltin Through the Ingestion of Microplastics

Layne Leggett, Robert Podolsky

The consumption of microplastics is of growing concern because they can leach toxins and interfere with normal feeding. Additionally, they can act as a vehicle for the ingestion of environmental contaminants that adsorb to their surface. Because pollution regularly exposes aquatic organisms to both microplastics and contaminants, it is essential to understand how they impact organisms independently and synergistically. We tested these possible effects using sea urchin (*Arbacia punctulata*) larvae, which ingest microplastics of similar sizes to the algae they normally consume. Larvae were exposed to one of several treatments: microplastic beads at low concentration, dissolved tributyltin (TBT), beads and TBT together, and two seawater controls, including one without food in case the TBT was harming the food supply. Based on studies with other contaminants, we predicted that the larvae exposed to the TBT contaminated microplastics would have greater effects on measures like growth and development than the sum of effects of exposure to TBT

or microplastics alone. We also carried out assays of glutathione oxidation by larvae under the different treatment conditions as a molecular indicator of stress. Our results will determine whether microplastics can have indirect negative effects on organisms at a lower concentration than where direct effects are apparent.

The Effects of Microplastic Ingestion on Zebrafish Physiology

Samantha Leigh, William Nguyen

Microplastics (MPs) are an emerging pollutant of serious concern. Despite their pervasiveness, we are in the early stages of understanding the full effects of MP pollution on marine organisms. Because MPs are the same size range as microzooplankton and phytoplankton, they are vulnerable for consumption by marine primary consumers. Some potential effects of MP ingestion include blocked and damaged digestive tracts, the leaching of plastic toxicants, and the introduction of foreign microorganisms; all of which could potentially lead to growth deficits, reproductive issues, and decreased lifespan. This is of particular concern for commercially important species since they represent a critical dietary and economic resource. In California, over 120,000 people are employed by the seafood industry, which generates over \$200 million per year to the state's economy. Furthermore, over 3 billion people worldwide rely on seafood as their primary protein source. This is why it is critical to understand how chronic MP exposure impacts fish physiology. We will be investigating the impacts of microplastic ingestion on the growth, fecundity, digestive efficiency, and gut microbiome community structure in the model organism *Danio rerio* (zebrafish) in order to understand what adverse effects microplastics could be having on vertebrates.

Endoparasites, but not microplastics, influence metabolic rate of pacific mole crabs

Rafael Leon, Oliver Coyle, Jonathon Stillman

Millions of tons of plastics are produced yearly, making microplastic pollution an increasing environmental threat. Microplastic ingestion has been linked to changes in metabolism and immune function. The pacific mole crab (*Emerita analoga*) is a filter feeder, and therefore prone to microplastic ingestion. On California beaches, 35% of mole crabs contain microplastics in their guts. Our prior studies found that thermal tolerance is positively correlated with microplastic ingestion in female pacific mole crabs. Here, we studied the effects of microplastics and other physiological stressors, endoparasites (*Acanthocephala profilicollis*), and temperature, on mole crab metabolic rate. Ingested microplastic enumeration was achieved via the collection, digestion, filtration and imaging of digestive tract samples. Metabolic rate was not influenced by measurement temperature (14 and 20°C) or microplastic ingestion. However, metabolic rate was negatively correlated with parasite load. Generalized linear mixed modeling (GLMM) showed that parasite load and to a lesser extent egg mass were significant predictors of oxygen consumption rate (metabolic rate). However, microplastic load, month of capture, and measurement temperature were not significant predictors of metabolic rate. Regardless of our findings, the number of microplastic particles in marine ecosystems will continue to increase due to industrial production and the degradation of existing plastics. This increase in microplastic pollution may amplify adverse effects of other stressors, heightening the need for further research on microplastic pollution.

Deforestation and elevation jointly determine morphospace occupation in anole communities

Gavia Lertzman-Lepofsky, Luke Frishkoff, D Mahler

In theory, the consequences of land-use change on community structure depend both on the composition of the original community and on how human impacts modify niche opportunities relative to the natural habitat. Empirically, however, this interaction is poorly understood, limiting our ability to understand the causes of Anthropocene biodiversity patterns. We harnessed an elevation gradient containing both forested and deforested habitats to test whether elevation-associated factors determine if land-use erases natural patterns of community structure and turnover among 50 local communities of *Anolis* lizards on Hispaniola. We quantified the distribution of individuals

projected into a community morphospace of functional traits and used linear models to test how habitat-type and elevation interact to shape alpha and beta diversity. In Hispaniolan anoles, deforestation enriches functional richness and evenness, particularly at high elevations where natural communities are depauperate due to thermal constraints, while simultaneously eroding turnover and truncating the exploration of peripheral regions of trait space. These results show that the way land-use affects communities depends on what was driving community structure originally: as deforestation reduces habitat complexity and increases daytime temperatures at high elevations, the environmental filters that typically shape these communities are relaxed, allowing high-elevation anthropogenic communities to functionally resemble low elevation communities. Our results highlight the necessity of examining Anthropocene communities in the context of underlying environmental gradients to understand community reassembly after land-use change.

Maternal aggression, brain metabolics, and neurotransmission: Insights from a feisty female bird

Emily Levy, Elizabeth George, Kimberly Rosvall

Aggressive behavior is ubiquitous across many competitive contexts, such as defense of mates during the breeding season, defense of territories outside of the breeding season, and defense of offspring (i.e., maternal or paternal aggression). Ethologists, behavioral ecologists, and neurobiologists have long sought to understand the physiological mechanisms triggered by these aggressive behaviors. Here, we measure the neurogenomic mechanisms triggered by maternal aggression. We hypothesize that maternal aggression primes the brain for future aggression by adjusting chemical signaling (e.g., neurotransmitters) or metabolism. Alternatively, the energetic and hormonal demands of reproduction may constrain an animal's priming response. In this case, maternal aggression may change gene expression in ways that reflect trade-offs with self-maintenance. We tested these hypotheses in incubating female tree swallows (*Tachycineta bicolor*). We exposed 10 females to a 30-minute simulated territorial intrusion, which elicited maternal aggression. After this challenge, we measured neural gene expression via RNA-seq in these females as well as 10 unchallenged controls. We saw a subtle treatment effect: the two groups had marginally different expression in gene networks associated with metabolism. Further, within the aggression-induced group, aggressiveness was correlated with expression in gene networks associated with diverse neuronal functions, including neurotransmission, synaptic plasticity, and metabolism. Thus, high levels of maternal aggression are associated with patterns of neural gene expression that should facilitate further nest defense.

AI in Ecology: Refining Microclimate Predictions and Thermoregulatory Tracking

Ofir Levy

Exploring why homoscleromorph sponges have ciliated epithelia when other sponges do not

Sally Leys, Veronica Price, Anudi Nanayakkara, Caroline Rocher, Carole Borchellini, Andre Le-Bivic, Emmanuelle Renard

Cilia are found on the epithelia of almost all metazoans, so their absence from the epithelia of all but one class of Porifera is strange. Homoscleromorph sponges possess ciliated epithelia, but the function of these cilia and their evolutionary history within Porifera are unclear. We examined the cilia of the homoscleromorph sponge *Oscarella lobularis* by comparing the ciliary beat frequencies (CBFs) of its buds at growth stages 1 and 4 with those of other animals in order to suggest possible functions for the cilia. Scanning electron microscope (SEM) images of the epithelial surface were also taken, some stained with ruthenium red to detect mucus. More mature buds were found to have a higher CBF than buds early in development. Mature buds had a similar CBF to mucus-transporting cilia from other aquatic invertebrates. Additionally, mucus was found to be present on the cilia in SEM images. These results suggest that *O. lobularis* likely uses its cilia for mucus transport. It is still unclear if the ciliated epithelium is an ancestral trait of Porifera and why it is present in Homoscleromorpha while other sponges manage well without it.

The structure-function relationship of sea turtle shell bone across ontogeny

Ivana Lezcano-Serra, Jeanette Wyneken

The boney shells of turtles, overlaid by keratin scutes, provide protection for the muscles, nerves, and viscera. The shells shield like use during predatory attack has resulted in the perception that it is both stiff and strong. Though this composite structure seemingly serves a protective role, how shells actually respond to applied loads has only recently been investigated. Much of this work focuses on only a few turtle species, mainly freshwater turtles and tortoises, with marine turtles receiving relatively less attention though their natural history and shell form differ from the species studied. We investigated the mechanical properties of juvenile and adult green turtles (*Chelonia mydas*), loggerhead (*Caretta caretta*), Kemp's ridley (*Lepidochelys kempi*) and hawksbill (*Eretmochelys imbricata*) turtles shells. Carapace samples were mechanically tested to quantify stiffness (Young's modulus), yield strength and toughness under quasi-static compression. Our data suggests that marine turtle shells are much less stiff than other testudines. Flexibility likely reflects the pressure oscillations encountered while diving. Additionally, juvenile shells are highly compliant, deforming substantially under relatively low stresses. Variation also exists between species with *C. mydas* and *E. imbricata* having much stronger and tougher shells, respectively, than the other species tested. These ontogenetic and inter-species differences may result from gradual changes in the boney microstructure. Factors such as trabecular thickness and porosity vary among groups with the former increasing across life stages.

Robotic and physics modeling of ground self-righting

Chen Li

Terrestrial animals may be flipped over during locomotion, fighting, courtship, etc. To survive, they must right themselves from an overturned state. Here I review recent progress in understanding ground self-righting using cockroaches as model organisms, by integrating biology experiments, robotic modeling, and physics modeling. Animal experiments (Li et al 2019 JEB) found that three cockroach species use diverse strategies to self-right, with each more frequently using a strategy with more body rolling than pitching. We then elucidated the physical principles by focusing on the discoid cockroach's winged self-righting. Robotic and potential energy landscape modeling (Li et al 2017 Adv. Rob., Othayoth & Li 2021 eLife) revealed that, although wing opening does not generate sufficient kinetic energy to overcome the high pitch potential energy barrier to somersault, it reduces the barrier for rolling, facilitating small kinetic energy from leg flailing to probabilistically overcome it to self-right. Finally, dynamic modeling (Xuan & Li 2020 RAL, 2021 B&B) discovered that the animal's large randomness in wing and leg motions helps it by chance find good coordination, which better accumulates mechanical energy to overcome the barrier. Overall, ground self-righting is strenuous. Often, the energy an animal generates is not sufficient for overcoming barriers. Only those strategies that lower the barrier sufficiently and uses generated energy appropriately can succeed. Thus, given many behaviors an animal displays, successful behavior is stereotyped.

Olfaction controls detection of reproductive odors and parenting avoidance in African cichlids

Cheng-Yu Li, Jessica Bowers, Theresa Alexander, Scott Juntti

Pheromones play essential roles in communication and reproduction in many species. In fishes, prostaglandin F2 α (PGF2 α) acts as a female reproductive hormone and initiates sex pheromone signaling. The female African cichlid *Astatotilapia burtoni* produces PGF2 α derivatives, which signal its fertility to conspecific males. However, how males perceive these reproductive cues remains unclear. To understand the neural mechanisms underlying the perception of reproductive odors, we silenced two major populations of olfactory sensory neurons (OSNs), ciliated and microvillus OSNs, using CRISPR to knock out sensory transduction channels, *cnga2b* and *trpc2b*, respectively. We found that only the males null for *cnga2b* are insensitive to PGF2 α -derived pheromones and have defects in courtship behavior, indicating that ciliated OSNs sense reproductive cues. We further discovered a subset of ciliated OSNs that express a beta olfactory receptor, Or113a, are activated by reproductive

odors. This revelation highlights the role of this receptor in detecting female reproductive status. Surprisingly, we also observed that *cnga2b* mutant males collect eggs during spawning. This behavior was not observed in other mutant lines or wildtype males. Furthermore, none of the ~1000 haplochromine cichlids, which are closely related to *A. burtoni*, exhibit male mouthbrooding behavior. These results indicate that olfactory signaling through ciliated OSNs plays a key role in inhibiting egg retrieval in males during spawning, enforcing the female-only parental care strategy of these species.

Shape matters: Effects of head morphology on flow entrance into olfactory organs in sharks

Meng-Yun Li, Yun-Hsin Lin, Ya-Yu Chiang, Kai-Jung Chi

Olfaction is vital for shark navigation and predation, requiring odorant transport by flow from the environment into their noses and then onto the receptors. Without muscular pumping, sharks rely on swimming to create inflow. Head geometry and nose locations inevitably affect the environmental flow and subsequently the flow's entrance into and passage through the olfactory organ. To address this, we first compared the morphology of shark heads and nasal chambers of specimens collected from fish markets in Taiwan. Measurements from dissection and computed tomography (CT) suggested that the head shapes and nose locations might be associated with habitats and diets. To examine the effects of head morphology on flow behaviors, we used the blue shark (*Prionace glauca*) as model species for flow visualization. We released dyes from varying positions at an upstream plane and traced each streamline's path to map the detectable range by the shark. We then projected the dye's trajectories onto the 3D-reconstructed head and examined how head shapes affect the flow locally. Our results show that under stable flow, only dyes within specific range could enter the nares, and releasing positions affected where the dye entered and subsequently the internal passages and travel times. These observations are consistent with our previous simulations and imply that the spatial information carried in the odorant plume could be deciphered by the shark's olfactory system.

Kinematic mobility and modularity of integro-cornuate hyoids during mammalian feeding

Peishu Li, Nicholas Gidmark, Kaleb Sellers, Teresa Lever, Zhe-Xi Luo, Callum Ross

The basi- and thyrohyals of the mammalian hyoid cradle the larynx, while an ossified chain called anterior cornu connect the basihyal to the basicranium. First seen among Jurassic mammals, the saddle-shaped basi-thyrohyal complex and ossified anterior cornu are hypothesized to be critical for the evolution of mammalian swallowing behavior. Extant mammals exhibit variable ossification of anterior cornu, but how this variation impacts swallowing biomechanics is unknown. One hypothesis suggests completely ossified cornua (integro-cornuate) constrain basihyal excursions compared to incompletely ossified (discreto-cornuate) cornua. In contrast, integro-cornuate hyoids may allow individual elements to move differently and expand hyoid functional repertoire. We used XROMM to quantify 3D hyolingual swallowing kinematics in dogs, which have integro-cornuate hyoids. During swallow-transport cycles, absolute and relative magnitudes of dog basihyal excursion exceed those of discreto-cornuate mammals like opossums; interspecific difference in basihyal excursion is driven by larger tongue protraction post-swallow in dogs. During dog tongue base retraction (TBR), proximal cornu elements (stylo- and epihyals) remain stationary while cerato- and basihyal protract and elevate. We hypothesize that kinematic decoupling between proximal and distal cornu elements help integro-cornuate hyoids perform two divergent functions during swallowing: airway protection and bolus propulsion. While distal hyoid elements could divert the larynx from the bolus, proximal elements could anchor the styloglossus muscle during TBR. Kinematic modularity of integro-cornuate hyoids may contribute to the diversity of mammalian TBR mechanisms.

Bacterial Population Inversion Near a Hydrodynamic Black Hole

Shengkai Li, Trung Phan, Robert Austin

We present a 2-dimensional microfluidic environment that utilizes concentric rings of funnel ratchets which generate effective fields which direct motile *E. Coli* bacteria towards an exit hole, entry into which sweeps the bacteria away via hydrodynamic flow. We show that the disappearance of bacteria and their signaling molecules near the hydrodynamic black hole triggers inter-bacterial chemotactic migration away from the black hole, leading to the emergence of a population inversion where the remaining bacteria collectively move away from the black hole in spite of the presence of nutrients within the flow stream. We reveal the fundamental significance of cell-cell communication in these phenomena by investigating different Brenner-Patlak-Keller-Segel models including those with and without a direct intercellular information flow, through analytical and numerical approaches.

Living with males leads to more physical injuries in female leaf-footed cactus bugs

Yichen Li, Christine Miller

Males in tens of thousands of species have evolved weapons to fight for access to mating opportunities. The use of weapons can make their combat especially vicious, which can lead to injuries for nearby individuals including females. However, very few studies have investigated the extent to which male-male competition leads to female injury. Here, we examined the effect of male-male competition on females using the leaf-footed cactus bug, *Narnia femorata* (Hemiptera: Coreidae). Males of this species use their elaborated hindlegs as weapons to fight for access to cactus territories where the females feed and lay eggs. Rival males typically attack individual males, but they have also been observed attacking mating pairs and females nearby. Thus, we predicted that females in the presence of multiple males would incur more injuries than females living without males. In this study, we assigned females into one of two social groups – a group with three males or three other females. We found that females living with multiple males were 22.1% more likely to suffer injuries, including broken wings and punctures through their cuticle. This result supports previous work in other insects showing that living with multiple males can harm females. We will next examine exactly how injuries are incurred in *N. femorata* and their long-term fitness consequences.

The effects of body length on undisturbed swimming patterns of Endler's guppies (*Poecilia wingei*)

Yihan Li, Kelly Diamond

Swimming is essential for the survival and reproduction of teleost fishes. Swimming patterns can be influenced by multiple factors, including health status, social status, size, age, environment, or the personality of an individual. In many sexually dimorphic species faster swimming ability has been associated with increased mating success. Endler's guppies (*Poecilia wingei*) are live-bearing fish that display sexual dimorphism in shape and color of adult fish. In other species of live-bearing fish from the same family (*Poeciliidae*), sperm-producing fish tend to display for larger egg-producing fish. In this study we quantified how body length of Endler's guppies affects undisturbed swimming patterns. We predicted that smaller fishes would have higher overall distance travelled but larger fish would move at higher peak velocities. Fish were filmed individually in an undisturbed, open environment and tracked fish position using machine learning tools (DeepLabCut). From the position data, we measured peak velocity, frequency of burst swimming, and overall distance traveled. We then tested how each metric varied with fish body length. Preliminary results indicate that larger Endler's guppies traveled further distances and have higher peak velocity. These data partially support our prediction and suggests that mating preference of larger fish might also be because it's an indication of faster swimming ability, and sexual and natural selection may favor larger and faster individuals.

Understanding three-dimensional flows in a fish school using wild menhaden and robots

James Liao, Subhra Shankha Koley, Edwin Rajeev

We investigated the hydrodynamics and schooling architecture of wild menhaden (*Brevoortia tyrannus*), an open-water clupeid. We subjected fish schools consisting of up to 30 individuals to flow speeds from 0.2 - 0.6 meters s⁻¹. School morphology transitions to more 2-D formations at higher flow speeds, suggesting that hydrodynamics plays a role in distributing individuals. We then fabricated a robotic, 4-fish school consisting of a flexible polymer having a refractive index similar to water to reveal the hydrodynamics within a school. We actuated each 3-D fish robot independently at a tailbeat frequency of 4-7 Hz over a range of Reynolds numbers (20,000- 50,000), altering the tailbeat phase differences between neighboring fish and characterized the hydrodynamic interactions using time-resolved stereo Particle Image Velocimetry (PIV). Two-dimensional schooling configurations revealed out-of-plane flow was substantial, approaching 25% of the freestream velocity. Our experimental approach investigates schooling hydrodynamics at relevant Reynolds numbers and incorporates the three-dimensional shape of the fishes to recreate realistic hydrodynamic interactions that are otherwise challenging to simulate with computational fluid dynamics. Measuring three-dimensional flows in fish schools, in addition to biological insights, promises to further our capacity to design optimal arrangements for autonomous underwater vehicles.

The Effects of Perchlorate Exposure on Zebrafish Behavior

Randi Libin-Straub, Michael Minicozzi

Perchlorate is an oxidizer and known endocrine-disrupting compound that essentially every individual of industrialized countries, including Americans, is exposed to through drinking water or agricultural products. Perchlorate has been shown to cause numerous developmental and behavioral effects in fishes and amphibians. We aimed to investigate when behavioral effects occur in zebrafish (*Danio rerio*) from perchlorate exposure. Zebrafish were exposed to environmentally relevant concentrations of perchlorate (10 ppm or 100 ppm) from fertilization through adulthood to investigate these behavioral outcomes. Sexually mature zebrafish were recorded in undisturbed water for 20 minutes and recordings were analyzed with NOLDUS software. We found that perchlorate-exposed zebrafish spent more time in the center of an arena and remained relatively still, while control (0 ppm) zebrafish spent much more time moving around the edge of the arena. The adult exposure experiments were repeated on larval zebrafish to obtain earlier time points for when perturbed behavior occurs. The perchlorate-exposed larval zebrafish showed differences from the control group in their velocity and time moving at both 5 days and 10 days post-fertilization. This altered behavior is noteworthy due to the conserved development of zebrafish and human nervous systems. This is particularly concerning considering the early onset of these abnormal behavioral patterns in fishes. Perchlorate can pass the blood-placenta barrier so it could impact early fetal human development.

Female reproductive costs: Trade-offs for females in an avian cooperative breeder

Andrea Liebl, AJ Steen, Katie Brust, Makayla Busskohl, Andrew Russell

In cooperatively breeding species, where non-breeding adults help breeding parents raise offspring, additional help may allow mothers to reallocate energy towards self-maintenance and their own survival. This may be particularly important because cooperative systems tend to be concentrated in harsh environments, which are expected to take a greater toll on offspring, breeders, and helpers alike. Although reproductive trade-offs have been considered for almost 100 years, the impacts of these trade-offs are most often considered in uniparental or biparental care systems. Here, using chestnut-crowned babbler (*Pomatostomus ruficeps*), a cooperatively breeding species endemic to the arid and semi-arid region of the Australian Outback, we focus on the physiological, behavioral, and survival trade-offs that females make with variable demands of reproduction generated with variable numbers of helpers. Previous work shows that breeding females in this species exhibit compensatory care during the nestling period by reducing her

own provisioning effort with additional helpers; however, during the incubation period, females incubate more when more helpers are present. However, how helpers in this system mitigate other maternal tradeoffs, such as stress physiology, reproductive effort decisions, maternal effects, and survival has not yet been shown. Using a cooperatively breeding system where breeding females have variable amounts of parental help in which we are able to follow individuals through time, allows us to better understand reproductive tradeoffs for breeding females.

Range expansion and behavioral variation in two *Gambusia* fishes in the Susquehanna River watershed

Isaac Ligocki

As a direct and indirect result of human activity, many species ranges are changing relative to their historical boundaries. Climate change has resulted in many species shifting to higher latitudes as new habitats become accessible. Additionally, many species have and continue to be intentionally or unintentionally introduced outside their historical range. In many species, individuals found at range boundaries display different behavioral and life history phenotypes (e.g. lower sociality, reduced neophobia, earlier maturation). We set out to investigate whether the eastern mosquitofish (*Gambusia holbrooki*) was expanding its range northward in the Susquehanna River watershed in the eastern United States as predicted by recent models. Our field surveys identified widespread *Gambusia* throughout the watershed far beyond previously described boundaries. Surprisingly, inspection of the gonopodia in male fish (and confirmation using molecular analysis) indicated fish further north in the watershed are introduced western mosquitofish (*Gambusia affinis*) which had not previously been documented in the Susquehanna River. We are currently conducting behavioral studies on fish of both species collected from multiple sites in the Susquehanna watershed to determine whether range expansion phenotypes exist in these populations, and how these species interact with one another where they are sympatric in the watershed.

Multigenerational patterns of maternal age effects on lifespan and reproduction in a rotifer

Alyssa Liguori, Sovannarith Korm, Alex Profetto, Emily Richters, Kristin Gribble

The age at which a female reproduces can affect offspring traits, including lifespan, reproduction, and health. Such maternal age effects are common across diverse taxa, but less is known about their dynamics across multiple generations, or whether grandmaternal and great-grandmaternal ages interact with maternal age to affect offspring fitness. We conducted multigenerational life table experiments on two strains of the rotifer *Brachionus manjavacas*. For each strain, young (3 day-old) and old (10-11 day-old) maternal age lines were maintained across three offspring generations. In the third generation, we initiated switched maternal age cohorts to achieve factorial combinations of maternal and grandmaternal ages. For each generation and line, we scored survival and reproduction daily throughout the lifespan of individuals, from which we calculated lifetime reproductive output (LRO), reproductive period, and rates of reproductive senescence. Maternal age affected offspring LRO in both strains, but in opposite directions. Reproductive period was longer for offspring of young mothers for both strains. For both strains, these effects were consistent across generations, with no evidence for the accumulation of either positive or negative effects across old and young lines. Switched maternal age cohorts revealed that effects were reversible within one generation, but only for certain response metrics and in a strain-dependent manner. These results will inform research on the mechanisms underlying maternal age effects and their importance in variable environments.

Importance of early experience for sea turtle magnetic field responses

Dana Lim, Jady Sethna, Isabelle Sechrest, Catherine Lohmann, Kenneth Lohmann

Many animals detect and use the magnetic field of Earth in their movements and migrations. Under laboratory conditions, hatchling loggerhead sea turtles

(*Caretta caretta*) from Florida respond to magnetic fields that exist along their Atlantic Ocean migratory route by swimming in directions that would, in each case, keep them within warm, safe currents. Previous experiments showed that these directional responses were disrupted if hatchlings experienced their first swim in a magnetic field other than that of their home beach, suggesting these responses are somehow reliant upon swimming first in their local magnetic field. One possibility is that oriented swimming is initiated by experiencing specific changes between the first magnetic field in which they swim and ones they subsequently encounter. To investigate this, we allowed turtles to first swim in a non-local field and then simulated the precise magnetic field change they would experience in moving from their natal beach to a location along their migratory route. Turtles that experienced this biologically relevant change in magnetic field, but did not experience their natal beach field during their first swim, failed to orient. This suggests that it may not be the change in field, but rather the magnetic field itself experienced early in life, that matters for initiating these crucial swimming responses.

Competitive environment selection in male *Hyla versicolor*

Rock Lim, Michael Benard

During intraspecific competition, organisms must assess whether conditions are favorable for success. Which individuals compete in larger or smaller groups? We investigated this question in an experiment on how perceived chorus size affects male *Hyla versicolor*'s (gray treefrogs) calling site choice. When mating, male frogs travel to and call from water. When selecting calling sites, frogs may use other males' vocalizations to glean information such as distance, number of competitors, and predation risk from loudness and number of calling frogs. Generally, larger choruses require more intense calling and competition, while isolated sites allow more distinct individual calls. Most papers focus on females' responses to calls, with few works on male phonotaxis. Thus, it is unclear which calling conditions males are likelier to move toward. We investigated this by giving male frogs a binary choice between recordings of groups of calling males or one male. Each recording was played at two volumes to simulate distance, quieter representing farther and louder nearer. Males were captured in wetlands from June through August 2023 and placed in a sound-insulated experimental chamber. Auditory stimuli were played from speakers on either end of the chamber, and males freely roamed while movement, orientation, and calling were videorecorded. We then evaluated preference for calls from groups vs. individuals, and for quiet vs. loud calls. Additionally, we tested whether choices were influenced by size.

Power output increases in single muscle fibers with more compliant and nonlinear tendons

David Lin, Bertrand Tanner

Locomotion involves coupling between muscles, tendons, bones, and joints, which intrinsically form a multi-scale mechanical feedback system. Namely, the forces and length changes that occur in a muscle fiber are influenced by muscle architecture, mechanical characteristics of the attached tendon, musculoskeletal geometry, and inertias of the limbs and body. To probe experimentally how muscle performance is affected by these elements, we developed a virtual mechanical load environment for single skeletal muscle fibers based on the concept of mechanical impedance and implemented by force-feedback. Within this environment, we can manipulate crossbridge mechanisms via solution-based methods, as well as muscle architecture, tendon, and inertial properties. For this study, we used this system to measure the mechanical power output of single muscle fibers from rat medial gastrocnemius muscles while being activated from rest to full activation (to mimic the generation of a movement from rest) and while coupled to simulated linear and nonlinear tendons. The linear tendons were varied two-fold in their compliance. We found that power output increased as tendon compliance increased, such that the increase in velocity outweighed any decreased in force due to force-velocity effects. Adding a nonlinear toe region, in which compliance was small but then increased with tendon length, caused the peak power to be generated earlier during the activation of the fiber, which would be advantageous for generating movements more quickly.

Robophysical modeling of how orb-weavers crouch legs to modulate prey vibration sensing

Eugene Lin, Yishun Zhou, Luke Moon, Andrew Gordus, Chen Li

Orb-weaving spiders use vibration sensors in their leg joints to sense prey on their webs. They can modulate this sensing by adjusting the vibration dynamics of the prey-web-spider system and thus the vibrations of leg joints induced by a given prey vibration input. This is done by longer-timescale web-building behaviors that modulate the properties of the web and by short-timescale postural changes during sensing. While the former strategies have been studied more, the latter has received little attention. In particular, with actively behaving spider and prey, it is difficult to measure the vibration dynamics of the entire spider-web-prey system required for understanding how spider behavior modulates vibration propagation through the system and systematically compare effects of different leg postures. To make progress, we created a robophysical model consisting of a prey robot, a physical web, and a spider robot, with multiple simultaneous measurements. Accelerometers measured spider leg joint vibrations, robot actuation was recorded, load cells measured web tension, and high-speed cameras measured web vibrations. We found that a more crouched posture increased the transverse vibrations induced by the prey robot experienced by the spider robot, whereas a less crouched posture increased the longitudinal and lateral vibrations sensed. We are investigating if dynamic leg crouching benefits sensing and systematically varying prey robot vibration and location, spider robot leg motions, and web pre-tension to discover principles.

Mechanisms of acid-base regulation of larval sheepshead minnows across a salinity gradient

Lu Lin, Andrew Esbaugh

Estuarine systems are highly dynamic with daily and seasonal fluctuations in salinity, pH, and oxygen that can impose significant physiological stress on larvae during recruitment. Sheepshead minnow (*Cyprinodon variegatus*) is a model estuarine teleost that is tolerant to changes in pH and salinity in early life stages; however, the mechanism of acid-base regulation and its interaction with different saline environments remains unclear. In seawater, acid excretion is thought to occur primarily through sodium-proton exchangers (NHE) owing to a favorable sodium gradient. This gradient becomes less favorable in freshwater where V-type H⁺ ATPase (VHA) is hypothesized to become more important for acid-base balance, while NHE continues to participate in osmoregulatory ion transport. Here we will empirically test this paradigm by acclimating embryos to different salinity conditions at 0 ppt, 25 ppt, and 60 ppt for 7 days (i.e., fertilization through hatch) and exploring the relative importance of various acid excretion pathways using a combination of real-time PCR, immunofluorescence microscopy and scanning ion microelectrode measurements of H⁺ flux. Studies will specifically focus on the contributions of NHE (2a, 2b, 2c, 3), carbonic anhydrase 17 (CA17), and VHA. Larval survivorship was not affected by salinity treatments and little evidence of phenotypic plasticity was observed; however, NHE3 was significantly upregulated at 0 ppt, while NHE2c was downregulated. H⁺ efflux studies are on-going and will be discussed.

How emus stand up: hindlimb kinematics, kinetics and muscle dynamics

Yuting Lin, Jeffery Rankin, Mehran Moazen, John Hutchinson

Animals not only need to walk and run but also lie prone to rest, and then stand up, involving near-maximal excursions of limb joints. This vital behaviour is little studied outside of humans but is a likely biomechanical constraint on limb design because it should require large length changes and force production from muscles. Here we integrated experimental and musculoskeletal simulation methods to analyse three-dimensional joint motions, ground reaction forces, as well as muscle fibre forces, activations and length changes during the sit-to-stand transition in a large (42 kg) terrestrial, bipedal and cursorial bird, the emu (*Dromaius novaehollandiae*). Our preliminary results suggest that the emu's hindlimb anti-gravity muscle fibres operate near their functional limits (~50% of shorten/lengthening following Hill-type model assumptions; Zajac, 1989) during the sit-to-stand transition. Sit-to-stand also requires high muscle activations (>50%) and force generation of major extensor muscles early in the

transition. We highlight trade-offs between muscle fibre force capacity and length changes in the sit-to-stand transition in emus and explore how they overcome the steep evolutionary constraints on sagittal motion imposed by locomotion. Our future research will investigate size-related variations in birds and the strategies species employ to stand up. The fundamental biomechanical mechanisms underlying sit-to-stand transitions in animals remain an important, mostly overlooked topic of investigation, offering potential insights into organismal morphofunctional specialisation and evolution, robotic applications, and animal welfare.

Evolution of the marsupium in Didelphimorphia: Insights from development

Shanti Lindberg, Karen Sears

The Infraclass Marsupialia gets its name from the Latin word “marsupium”, or “pouch.” The current assumption is that the pouch is an ancestral condition for members of this clade and that it has been variably retained or lost across lineages. However, many groups present extreme dissimilarities with this trait, which calls into question the current dogma. Developmental data could inform the evolution of this key characteristic of marsupials, but well-characterized developmental series are only available for a handful of species across the entire clade.

For my thesis, I am investigating the cellular and molecular basis of pouch formation, and lack thereof, across the Order Didelphimorphia (New World opossums, ~70 species). This information will be utilized, in tandem with an evolutionary analysis of adult anatomy, to inform the evolutionary history of the pouch within this clade. Didelphimorphia represents an ideal study group because its members exhibit significant variation in pouch anatomy, and it contains two species in which developmental staging has been well characterized (*Monodelphis domestica* [pouch absent] and *Didelphis virginiana* [pouch well-developed]).

While my project is in its earliest stages, my preliminary phylogenetic reconstruction of adult pouch anatomy suggests that the pouch has been lost within ten genera within this order but is retained in some form within eight genera.

Quantifying skull shape convergence between Eupleridae and other feliform carnivorans

Tate Linden, John Flynn, Chris Law

As the only mammalian carnivores endemic to Madagascar, Malagasy carnivorans (Eupleridae) diversified into a variety of niches, evolving body shapes, locomotion, and diets as disparate as the gracile, burrow-dwelling, and insectivorous falanouc and the robust, semi-arboreal, and lemur-hunting fossa. Euplerid body plans either retained ancestral similarities or evolved to resemble species from other carnivoran lineages. For example, the two euplerid subfamilies, Euplerinae and Galidiinae, bear striking similarities to civets and mongooses, respectively, leading to their historic and erroneous placement within Viverridae and Herpestidae. Similarly, early morphological analyses grouped the cat-like fossa *Cryptoprocta* with Felidae. Molecular phylogenetic data, however, clearly shows that euplerids are monophyletic and most closely related to herpestid feliforms. Using 3D geometric morphometrics and phylogenetic comparative methods, we measure the level of cranial and mandibular shape convergence between Eupleridae and other feliform carnivorans (Hyaenidae, Felidae, Herpestidae and Viverridae). Given prior observations of the morphological similarity of euplerids to other feliform taxa, we predict that: 1) members of the subfamily Euplerinae, which are hypercarnivorous and generally more robust than members of Galidiinae, will resemble viverrids and felids in skull shape, and 2) species within the subfamily Galidiinae, which are more gracile and insectivorous, will share more cranial and mandibular characteristics with herpestids. This study will reveal new insights into the evolutionary morphology of a unique clade facing rapid habitat loss and fragmentation in Madagascar.

Biology by Numbers: Scaffolding Data Literacy Learning in a Biology of Marine Organisms Course

Sara Lindsay

Quantitative reasoning and data literacy are core competencies practicing biologists use daily, and developing these competencies is an expected learning outcome for undergraduate Biology and STEM majors. Yet many students struggle with creating and interpreting figures. Providing repeated opportunities for students to engage with authentic data is one way to help them build these skills. In SMS 201, a large enrollment introductory Biology of Marine Organisms class, students engage with content and data related to marine biology core concepts in different ways during class meetings including through turn & talk discussions, multiple choice “clicker” questions, written notecard reflections and several guided data exploration activities that they finish for homework. These low stakes activities are facilitated by undergraduate learning assistants. Although overall student success in the course improved with these modifications, answers to exam questions in 2021 and 2022 showed that students still struggled to interpret and describe data presented in different formats and to make predictions based on data. Thus, in spring 2023 I aimed to incorporate at least one data-related question in every class meeting and to specifically scaffold working with types of data presentations that students struggled with on exams (e.g., graphs with multiple lines, diagrams of gene expression patterns). Comparing student performance on matched exam questions from 2021 and 2023 suggests this more intentional scaffolding may have contributed to better student outcomes.

PULSE: Helping Departments to Transform Undergraduate Education

Sara Lindsay, Judy Awong-Taylor, Fernando Nieto-Fernandez

Higher Education is on a trajectory that will focus more on recognizing how well institutions educate students rather than an institution's name recognition, Carnegie classification, or history. This value-based shift has the ability to level the playing field for all institutions by recognizing how well faculty engage in pedagogical best-practices and lead educational innovations that support learner outcomes and student success. The Partnership for Undergraduate Life Sciences Education (PULSE) is a lever of change that is helping drive the transformation of higher education in life sciences. Through its tools (PULSE Rubrics and Faculty Attitudes and Readiness Survey) and programs (Ambassadors Workshops, Recognition Program and Regional Networks), PULSE provides academic departments with resources, skills, and processes to align their undergraduate programs with national education initiatives to develop inclusive, student-centered, evidence-based teaching and learning practices, while removing barriers to access, equity, and inclusion. The PULSE Ambassadors Program deploys teams of trained facilitators to guide a department in crafting a shared vision and action plan for change. Using PULSE tools, the Recognition Program provides a process for departmental review and self-assessment and includes site visits to departments, detailed feedback reports, and follow-up support. Data from PULSE programs highlight common goals and areas for growth among departments nationwide. Visit our poster to learn how PULSE's Recognition and Ambassadors programs can be a lever of change for your institution.

Sexual selection and speciation in socially polyandrous shorebirds

Sara Lipshutz

Mating phenotypes influence whether species interbreed and can promote or impede reproductive isolation. Historically, research on speciation and sexual selection has focused on competitive males and choosy females. We explored the influence of female competition on speciation in a hybrid zone between two socially polyandrous Jacana species, for which female competition determines reproductive success. We compared a suite of pre- and post-copulatory phenotypes, including territorial aggression, body size, weaponry, vocalizations, plumage coloration, and sperm morphology. We found that female competition can promote hybridization, but gene flow between species is limited. We also found patterns of both convergent and divergent character displacement, in which phenotypic differences in sympatry are distinct from

those in allopatry, suggesting species interactions may facilitate morphological evolution. Altogether, this suggests that forces of sexual selection may be opposed by natural selection, thereby maintaining porous species barriers.

Phylotranscriptomics and the evolution of aggression in cavity-nesting songbirds

Sara Lipshutz, Mark Hibbins, Kimberly Rosvall

Phenotypic convergence sheds light on the predictability of natural selection; however, this process is poorly understood for complex behavioral traits. Here, we focused on obligate secondary cavity-nesting, which has evolved many times in birds. We hypothesized that the shared pressure to compete for a limited breeding resource would drive convergent behavioral evolution via convergent gene regulatory changes in the brain. We tested this hypothesis by contrasting ten species across five avian families, including transcriptomic and behavioral data collected in the wild. Our results reveal behavioral convergence among obligate cavity-nesting birds, particularly among females, which display higher territorial aggression than their more flexibly-nesting relatives. Behavioral convergence was associated with some elements of convergence in brain gene expression, but most trait-associated patterns of gene activity were unique to each species pair. Together, these observations suggest that natural selection uses diverse transcriptomic pathways to modify complex behavioral traits like aggression.

Elevated ammonia cues hatching in red-eyed treefrogs: a mechanism for escape from drying eggs

Astrid Lisondro-Arosemena, Karen Warkentin, Maria Salazar-Nicholls

Egg dehydration can kill terrestrial frog embryos, and this threat is increasing with climate change and deforestation. In several lineages that independently evolved terrestrial eggs, and retained aquatic tadpoles, embryos accelerate hatching to escape from drying eggs. However, the cues that stimulate drying-induced early hatching are unknown. Ammonia is a toxic, water-soluble metabolic waste that accumulates within eggs as embryos develop and concentrates as eggs dehydrate. Thus, increasing ammonia concentration may be a direct threat to embryos in drying eggs. We hypothesized that it could serve as a cue, stimulating embryos to hatch and escape. The embryos of red-eyed treefrogs, *Agalychnis callidryas*, hatch early to escape from many threats, including dehydration. To test if they also use high ammonia as a cue to hatch, we exposed stage-matched pairs of hatching-competent, well-hydrated sibling embryos to ammonia and control solutions in shallow water baths and recorded their behavior. Control embryos remained unhatched while ammonia-exposed embryos showed a rapid, strong hatching response; 95% hatched, on average in under 15 min. This demonstrates that elevated ammonia can serve as a hatching cue for *A. callidryas* embryos. This finding is a key step in understanding the mechanisms that enable terrestrial frog embryos to escape from egg drying, opening new possibilities for integrative and comparative studies on this growing threat.

Detecting Limiting Fitness Components in a Sensitive Early Life Stage as a Function of Reproductive

Jack Little, Emily Carrington

A key challenge to understanding climate change impacts is predicting the ability of sensitive early life stages to move on in their life cycles. Ectotherms must grow, develop, and survive in dynamic thermal environments that vary on multiple timescales. Little is known about which life processes are limiting to the probability of advancing in the life cycle in nature. We integrate data from field environmental monitoring and laboratory thermotolerance assays for embryos of the intertidal gastropod *Haminoea vesicula* to predict which fitness component is limiting across different timescales of thermal variation. We show that survival has a lower thermal optimum and higher thermal breadth than growth or development. As a result, fitness components trade off depending on seasonal shifts in the temperature. Embryos deposited in spring experience cooler temperatures that are less likely to exceed their critical limits, but because they grow slowly and have long developmental periods, tend to encounter the ~biweekly return of lethal temperatures caused

by tidal forcing. In contrast, embryos deposited in summer more frequently experience temperatures that exceed the thermal optimum for survival, but compensate with high growth rates and short developmental times that allow them to avoid exposure to periodic extreme temperatures. Overall, we show the fitness effect of trading off growth for survival depends on how long development time is relative to periods of habitat thermal variation.

Interactive effects of nighttime warming and prey availability on lizard energetics

Allison Litmer, Morgan Pelley, Steven Beaupre

Variation in physiological function and life history are driven by both temperature and food availability. Such organismal processes influence persistence and population dynamics. Many ectotherms thermoregulate daily but face challenges at night due to homogenization of the thermal landscape, which is often not considered in climate change studies. Temperature warming can increase energetic demands, with subsequent consequences amplified or mitigated based on prey consumption. We examined the interactions between warming nighttime temperatures and prey availability in affecting food consumption, digestive passage time, energy budgets, and fecal and urate production in a model organism, prairie lizards (*Sceloporus consobrinus*). We implemented a full-factorial experimental design of three temperature treatments (current conditions, +2°C nighttime increase, and +4°C nighttime increase) and three prey availability treatments. The effects of nighttime warming and prey availability were interdependent and varied based on the magnitude of change. Under a 2°C increase, lizards were efficient at consuming food and maintained or increased energy budgets, compared to current conditions. In contrast, under a 4°C increase, energy budgets were lower than current conditions under high prey, but comparable under low prey. Thermal performance can change due to nighttime warming, and can be antagonistic or additive, depending on prey availability. Future studies should focus on daily temperature cycles and the interaction with co-occurring abiotic and biotic changes to understand factors influencing organism energetics, physiology, and life history.

Thermal physiology of the Atlantic horseshoe crab, *Limulus polyphemus*

Kerryanne Litzenberg, Daniel Sasson, Fabio Casu, Jody Beers

Temperature has long been known to impact biological systems and physiological performance across the animal kingdom. Rising water temperatures are especially of concern to ectothermic marine invertebrates who cannot regulate body temperature. While the effects of temperature have been widely studied across various phyla, little is known about how it might impact metabolism, blood chemistry (e.g., amoebocyte density, and hemocyanin concentration), and metabolomic profiles in invertebrates. NMR-based metabolomics is extremely sensitive to external stressors allowing us to determine shifts in metabolic profiles caused by temperature. Due to the importance of their hemolymph in the biomedical industry, the Atlantic horseshoe crab (HSC), *Limulus polyphemus*, represents a unique model for investigating the effects of temperature. In this study, HSCs were collected during spawning season from April-June, 2023 and assigned to three temperature treatments (18, 23, and 28°C) where they acclimated for one week. A standard metabolic rate was obtained for each crab and approximately one-third of their total blood volume was collected. Temperature was found to have a significant impact on metabolic rate ($p = 0.003$); however, it did not affect hemocyanin concentration ($p = 0.311$), or amoebocyte density ($p = 0.622$). Preliminary results from untargeted NMR-based metabolomics analysis reveal that temperature might influence hemolymph metabolite profiles. These data will provide insight into the effects of rising temperatures on physiological stress in marine invertebrates.

Neuromechanical control centralizes as terrain complexity and speed increase in running cockroaches

Ellen Liu, Leo Wood, Izaak Neveln, Simon Sponberg

For biological and robotic locomotors centralization is an important parameter for neuromechanical control. We consider centralization as the coupling

between neuromechanical modules (e.g. limbs) that mediate responses to perturbations. Decentralized control may be advantageous for complex terrains because it allows for separate local responses to each heterogeneous perturbation. High speeds may also limit the time for processing a highly centralized neural response. Alternatively, centralization could increase to maintain dynamic stability at higher inertial forces induced by greater speeds or terrain complexity. We used an empirical, model-free measure of centralization based on information decomposition determined the degree to which a cockroach (*Blaberus discoidalis*) is centralized although it does not distinguish which features of performance are benefiting from centralization. The control signal was taken as the muscle action potentials from the femoral extensor muscle of the local leg. The local state was the position of the local leg's tarsi while the global state was the average position of each tarsi. We first confirmed earlier results that the control signal was found to share more information with the global state than the local state, indicating a centralized system. This difference grew with increasing speed and terrain complexity, showing that cockroaches can modify their control architecture through neural feedback or environmental interactions. This supports our hypothesis of increased centralization, which could arise from either increased neural or mechanical coupling.

Opening a Window to a Warm Earth: Age and Growth of Catostomid Fishes from the Eocene Okanagan High

Juan Liu

The Early Eocene Climatic Optimum (EECO, 50-52 Ma) is among the most extreme instances of global warming in Earth's history. Analyses of freshwater fish growth during the EECO provide a window into how these environmental indicators adapt to changing temperature regimes and extreme climate. I investigated organismal response to EECO by assembling and interrogating a new age and growth dataset of two catostomid species, *Amyzon aggregatum* and *Wilsonium brevipinne*, from lacustrine and fluvial deposits of the Eocene Okanagan Highlands. I measured opercular and scale annuli width and performed von Bertalanffy growth function analysis on digitized growth lines from photographs. I found that 1) the asymptotic size of lacustrine *A. aggregatum* is higher than that of fluvial *W. brevipinne* for both opercles and scales; 2) the Brody growth coefficient of the lacustrine species is relatively higher in scales but lower in opercles; 3) the small-sized fluvial species exhibited earlier sexual maturation. Additionally, dark and light annuli bands are similar in width, indicating relatively warm but prolonged winters. Interspecific differences in growth characteristics are consistent with micro-climatic differences between lacustrine and fluvial habitats and across broadening altitudinal gradients caused by the uplift of the Okanagan paleo-highlands. Both species responded to warm winters by growing fast nearly year-round. Fossil fish growth responses during past temperature peaks provide an informative model for how aquatic organisms respond to global warming.

Comparative cephalofoil morphology and diet of Florida bonnethead sharks (*Sphyrna tiburo*)

Kathy Liu, Jasmin Graham, Jayne Gardiner, Sora Kim, HyeJoo Ro, Tonya Wiley, Catherine Macdonald

The first described omnivorous shark species is the bonnethead shark (*Sphyrna tiburo*), a small hammerhead species with a rounded spade-shaped head. Studies have shown seagrass to make up a large component of bonnethead diet, though it may vary across life stage, region, and sex. Bonnetheads are also the first documented shark species to show sexual dimorphism in head morphology. Males have a significantly more pointed snout which was originally believed to develop at the onset of sexual maturity, however, upon further research, both male and female cephalofoils get rounder as they mature. Bonnetheads sharks can be found in shallow tropical and subtropical waters along both coasts of the Americas but the characteristics of only a few populations are well-defined. This project is the first in-depth assessment of Biscayne Bay, FL (25° N 80° W) bonnethead characteristics and uses bonnetheads from the greater Tampa Bay, FL area (27° N 82° W) as a comparison. Body length measurements were noted, photos of the cephalofoil were taken for analysis in ImageJ, and muscle biopsies collected were used for stable isotope analysis. There are overall physical similarities but distinct separation of diets between locations although not between sex or maturity. This study

provides more comprehensive information about bonnethead shark traits and diet across populations and is an initial step in longer-term research on bonnetheads in Biscayne Bay.

Diet drives varied rates of body shape evolution in reef fish

Shih-Na Liu, Peter Wainwright, Michael Burns

Disentangling the determinants of phenotypic diversity and their respective contributions is key to understanding the mechanisms that shape patterns of diversity across the tree of life. Among coral reef fishes, diet influences lineage diversification, as it underpins numerous crucial biotic interactions. We build on these insights by exploring the impact of diet on the evolution of body shape. Using phylogenetic methods, we compared average body shape, morphological disparity, and rates of evolution across 1,350 species of reef fishes grouped into 7 diet categories. We found that generalized carnivores, mobile invertivores, and planktivores occupy the region of morphospace characterized by long and slender forms, while groups that feed by biting, including herbivores, sessile invertivores, and durophagous invertivores tend to have laterally compressed, deep bodies. Rates of body shape evolution and disparity also varied with diet. Sessile invertivores outpaced all other groups in state-dependent rates of evolution but had the lowest disparity because they are phylogenetically young, while planktivores exhibited the highest disparity despite not being exceptional in age or rates. These findings highlight the complex interplay between frequent transitions between diets, morphological adaptation to those diets, and time in shaping patterns of body shape diversity in reef fishes.

A novel haplotype reference panel for the critically endangered coral, *Acropora palmata*

Nicolas Locatelli, Richard Karp, Keri O'Neil, Andrew Baker, Ross Cuning, Iliana Baums

For millions of years, *Acropora palmata* was one of the dominant reef-building organisms in the shallow reefs of the Caribbean. However, following a precipitous decline due to disease and anthropogenic impacts beginning in the 1980s, the species was listed as critically endangered. As corals continue to decline globally, it becomes increasingly important to deploy genomic tools to characterize the genetic diversity of natural populations, as well as to manage nursery stock and create restoration plans to generate resilient reefs. Here, we sequenced samples from 100 Florida *A. palmata* colonies using a combination of PacBio HiFi and Illumina sequencing and performed population demographic analyses using PSMC. We found an effective population size of only 1712 individuals with a slight bottleneck occurring ~25Kya. Additionally, we detected no population structure across the length of the Florida Reef Tract, in line with previous results. Utilizing the same data, we generated a haplotype reference panel using a combination of read-backed and population phasing with the goal of imputing low coverage sequencing data, and microarray SNP data. With this reference panel, we impute SNPs and small indels for 1,201 Florida *A. palmata* samples previously genotyped using the Axiom™ Coral-Algae Genotyping Array to generate the most comprehensive genetic resource for any coral species, to-date. This dataset will allow for large-scale GWAS studies of phenotypic traits while also reducing future experiment costs in this threatened foundation species of Caribbean reefs.

Heavy metal contamination in bird feathers from natural protected areas in the Amazon

Yeraldi Loera

Although one of the most biodiverse regions in the world, the degree and effects of heavy metal contamination from the mining and refinery of oil and gold in the Amazon remain unstudied. Here, we present a survey of heavy metal contamination in the feathers of wedge-billed woodcreepers (*Glyphornychus spirurus*), a resident neotropical bird. Despite ecological protection, samples collected near oil and gold mining activities generally confirmed the predicted elevation of heavy metals associated with those activities. Surprisingly, samples from reference sites also displayed elevated levels of certain heavy metals, most not associated with oil and gold mining activities. These results highlight the urgent need for further studies on the

ecological and biological impacts of heavy metal exposure on rainforest birds, how these impacts are occurring across space and time, and to what extent birds may represent indicator species for heavy metal exposure in other wildlife or human communities.

Rapid change in a rainforest anole: results from the first six years of a translocation experiment

Michael Logan, Karla Alujevic, Leah Bakewell, Albert Chung, John David Curlis, Samantha Fontaine, Daniel Nicholson, Renata Pirani, Adam Rosso, Claire Williams, Kelly Wuthrich, W. Owen McMillan, Christian Cox

The environments experienced by animals are changing quickly as human-induced disturbances such as climate change and habitat destruction take their toll. In the understudied tropical forests of the world, an open question is whether small, relatively non-vagile ectotherms such as lizards can withstand these changes with behavioral compensation, plasticity, or genetic adaptation. Here, I will discuss some of the major results from the first six years of a field-translocation experiment we have been conducting in the lowland tropics, where we have moved thousands of slender anole lizards (*Anolis apletophallus*) from a single mainland population to hot islands in the Panama Canal and measured population dynamics and phenotypic change over several generations. I compare this to “normal” change that occurred in parallel in the mainland source population. In general, the slender anole is highly flexible, experiencing rapid shifts in population growth, behavior, morphology, physiology, and gut microbial composition on the islands, as well as a surprising amount of year-to-year change on the mainland. Some of these changes appear to be adaptive, suggesting hidden resilience in this vulnerable species and raising the possibility that tropical ectotherms more generally may have some capacity resist rapid environmental change.

The Role of Olfaction and Magnetic Navigation in the Mass Nesting Behavior of Sea Turtles

Kenneth Lohmann, Roger Brothers, Vanessa Bezy, Catherine Lohmann

During mass-nesting in olive ridley sea turtles, thousands of adult females emerge from the ocean nearly simultaneously to lay their eggs along small, specific stretches of coastline. We posed two questions about this complex, collective behavior. First, how do turtles find the right nesting beach and, second, how do they synchronize emergence from the ocean? To investigate navigation, adult female turtles at Ostional, Costa Rica, were captured as they crawled onshore and briefly tethered to an electronic tracking system in a circular pool of water, where they were exposed to a magnetic field that exists 500 km northwest of their nesting beach. Turtles responded by swimming southeast, a direction that would facilitate return to the nesting beach had they actually been displaced geographically. To investigate whether mass-nesting is triggered by a chemical signal such as a pheromone or environmental cue, we disrupted olfaction in turtles just prior to a predicted nesting event. Gravid females were captured offshore and their nasal passages were flushed with either sea water (controls) or with zinc sulphate, which disrupts olfaction. During mass nesting, turtles treated with zinc sulphate nested significantly later than those treated with sea water. Results are consistent with the interpretation that turtles use magnetic navigation to reach the vicinity of the nesting beach and rely on olfaction to coordinate the timing of nesting.

The Notochord of Atlantic Salmon Continually Changes its Functional Morphology

John Long, Harald Kryvi

Among bony fishes, the Atlantic salmon, *Salmo salar*, is, to our knowledge, the only species for whom we know how the notochord's morphology changes over a complete life cycle and over the complete length of the axial skeleton. We review those continual changes and build an integrated understanding of the spatiotemporal reorganization and its possible functional consequences. In the embryo, the notochord is the first functional organ, signaling the formation of the body axes, the neural tube, and somites. Just after hatching, the notochord's functions change, as it builds extracellular sheaths that resist body bending, generate hydrostatic pressure in conjunction with the swelling

vacuolated chordocytes, and generate the first ossifications of the vertebrae, the chordacentra. With ossification in the sheaths comes clear changes in the notochord's structure along its length, with its rostral portion building an unsegmented bony cap and its urostyle portion building crenulated, irregular chordacentra. By the adult stage, the rostral cap and associated notochord have been eliminated by chordoclastic cells while the urostyle notochord remains and has been structurally reinforced with the addition of irregular transverse septae, internal extensions of the extracellular sheaths that may resist twisting.

Real-time sequencing can facilitate studies of infection biology in wild amphibians and reptiles

Ana Longo, David Rodriguez

Host persistence after initial outbreaks is a common outcome of wildlife diseases. However, the immune mechanisms allowing hosts to overcome infection and disease are less well-known, mainly due to the lack of genomic resources for non-model species. Here, we discuss emerging real-time sequencing applications (e.g., nanopore sequencing) to study immunity in amphibians and reptiles, which are two vertebrate classes that are threatened by the spread of invasive multi-host pathogens. From traditional genotyping of immune loci to direct RNA sequencing and methylation profiling, real-time sequencing technologies promise a new set of tools that could transform studies of individual responses in the field and in the lab without the need of specialized instrumentation. Faster and more streamlined data acquisition can reveal the precise timing of when within-host responses deteriorate after the pathogen invades and starts to multiply. Real-time sequencing technologies can also identify host regulatory genes used by pathogens to potentially silence pattern-recognition receptors or damage-associated molecular patterns. Even though large genome content in amphibians still remains bioinformatically challenging, emerging sequencing technologies are beginning to provide more accessible methods to interrogate non-model genomes. We anticipate that common workflows for genotyping and species identification will be easily implemented in portable labs in remote areas, thus, reducing technical gaps in early detection and rapid response to emerging infectious diseases.

The female perspective on disease risk

Patricia Lopes

When faced with deteriorating conditions (such as shortage of resources, temperature extremes, or infection), females can prioritize their own survival over investment in reproduction. Alternatively, it is also possible for females to adjust the phenotype of their offspring in ways that may change how offspring cope with, and thrive under, those stressful environments. Here, I raise the possibility that the perception of disease risk also triggers trade-offs between survival and reproduction in females. Mounting evidence suggests that animals can respond to disease risk. For example, the presence of a diseased groupmate can trigger changes in the behavior and physiology of animals not experiencing that disease. I will use recently published studies to illustrate how reproducing females respond both behaviorally and physiologically to high-disease-risk environments and how those responses can affect both their reproduction and the phenotype of their offspring. More research on this topic is necessary to fully comprehend the cascading effects that maternal responses to disease risk may have for offspring and the potential feedbacks of those on the mother. Given the expected rise in infectious disease events, this knowledge will be critical to predict how populations will respond to these upcoming challenges. My goal is that this overview and discussion will help the community arrive at a conceptual framework for understanding when parental responses to disease risk are costly or beneficial for parents and offspring.

Investigating ciliary-mediated hydrodynamics of sand dollar larvae via millifluidics

Bryant Lopez, Haley Obenshain, Wesley Chen, Ariana Lee, Douglas Pace, Siavash Ahrar

Micro/millifluidics are a valuable approach for investigating the hydrodynamics of organisms. We used millifluidics (channel width 4, height 1.5, length 50 mm) to study the relationship between morphology, feeding environment, and hydrodynamic performance in larvae of the Pacific sand dollar, *Dendraster excentricus*, which has a pluteus larval stage characterized by arms that support a ciliated band used for locomotion and feeding. Larvae display food-induced morphological plasticity so that when food is limited, larvae grow longer arms and ciliated bands to increase food capture ability. Larvae grow shorter arms and direct energy towards faster development when food is abundant. Our hypothesis is that changes in larval arm length, as typified by morphological plasticity, result in critical differences in the hydrodynamic performance of the ciliary band. Using high- and low-fed larvae, we first confirmed that we can visualize the flow patterns generated by the ciliary behaviors for both phenotypes using fluorescent beads and video microscopy. Next, we observed the hydrodynamics of low-fed larvae at different developmental stages. Hydrodynamic recordings for each larva with and without flow were obtained. We measured particle velocity and vorticity. Additional ongoing analyses are examining the relationship between morphological parameters (e.g., post-oral arm length) and hydrodynamics. Collectively, results demonstrate the use of millifluidics to investigate ciliary-mediated hydrodynamics of marine larvae and further understand the adaptive value of morphological plasticity in feeding larval forms.

Understanding Amphibian Life Cycle Ecology and Evolution through Agent-Based Modeling.

Jesus Lopez, Ashley Teufel, Robert Page

Many amphibian species experience a complex life cycle in which larvae develop in a deteriorating environment. Larvae must balance consuming resources and metamorphosing quickly enough to escape their shrinking pond. This process has been difficult to model given the complexity of the environmental, physiological, and genetic factors influencing metamorphosis. Utilizing agent-based modeling, we have created a model leveraging data on *Ambystoma maculatum* to capture the key features of the ecology and evolution of many amphibian life cycles. In our model, agents have four developmental stages: egg, larva, juvenile, and adult. The agents are characterized by parameters corresponding to the real-life attributes of amphibian organisms, including chromosomes with genes that can contribute additively to metamorphic risk or be functionally neutral. The agents' environment consists of patches with parameters for mortality risk, habitat permeability, and food availability. The model is highly configurable, allowing for the modeling of different amphibian species and heterogeneous environments across various parameters. Additionally, the model can accommodate many use cases, offering configurations and result interpretations through the interface or through the parameter and output files for more specific needs. We have utilized the model to study gene flow and natural selection under several spatial scenarios with varying numbers of ponds with unique hydroperiods. The model has the potential to serve as a powerful tool for conservation efforts, population genetics research, and education.

Cranial landmark variation in mammals gives diverse alignment for temporomandibular biomechanics

Sonia Lopez, Emily McParland, Peishu Li, Nicholas Gidmark, Courtney Orsbon

The mammalian jaw joint, the temporomandibular joint (TMJ), connects the lower jaw to the cranium and has pivotal functions in chewing and speech. Understanding normal and pathologic TMJ function requires a functionally- and anatomically-relevant coordinate system. A common clinical tool for mammalian coordinate system orientation is the Frankfurt plane, fit to the external auditory meatuses and the ventral margins of the orbits. However, it is unclear how the orientation of Frankfurt plane affects the kinematic outputs analyzed for comparative TMJ biomechanics, especially among taxa

commonly employed as TMD model system. Moreover, alternative planes may be more biomechanically relevant to TMD. We compared three separate coordinate systems: the Frankfurt plane, the orbital-occipital plane, and the upper molar tooth cusps in six common model organisms (mouse, rat, rabbit, macaque, sheep and pig). We found variable orientation in the Frankfurt plane across different model systems, largely driven by the differences in the external auditory meatuses to the jaw joint and orbitals, especially in pigs against other model species. However, orbital-occipital and tooth-cusp alignments are relatively similar across species. Our results provide new insights into the sensitivity of different reference planes to interspecific variation in craniomandibular anatomy for TMD research. Understanding skeletal variation impacts the usage of plane orientations better informs animal model selection for TMD research.

Timing is Everything: Effects of Temperature on Embryonic Development and Spawning in Fiddler Crabs

Paola López-Duarte, Ruth Wright, Caitlin BrabbleRose

Decapod crustaceans inhabit a variety of habitats throughout the world. From the most stable to the most dynamic environments, these ectotherms exhibit physiological, behavioral, and morphological mechanisms of thermoregulation. Fiddler crabs (Family Ocypodidae) inhabit intertidal areas across the world. During their reproductive season in the Southeastern United States (May–October), they experience daily temperature fluctuations of ~20°C (20°C–40°C) at the marsh surface and < 5 °C in burrows that are 20–30 cm deep. Female crabs incubate developing embryos inside these burrows for ~14 days (½ lunar cycle) before synchronously releasing larvae during the high tidal phase of the spring tides (new and full moon) to achieve transport away from adult habitats and towards coastal developing areas. We sought to measure the effects of temperature on incubation times and the timing of larval release with respect to the tidal and lunar cycles by exposing ovigerous fiddler crabs to different temperature treatments (15°C to 40°C). Embryos from *Leptuca pugilator* from South Carolina and *Minuca longisignalis* from Louisiana developed faster with increasing temperatures, but did not survive at 15°C and 40°C. Surprisingly, crabs kept in the laboratory for 1–3 weeks still synchronized spawning around the time of high tide and spring tides. Our results suggest that the mechanisms controlling embryonic development in fiddler crabs are flexible to temperature conditions while remaining synchronized to the tidal and lunar cycles.

Taking the Long Way Around: Ecology from Morphology in Chondrichthyan Cranial Lateral Lines Canals

Marco Lopez-G, Vishruth Venkataraman, Michael Coates

The lateral line canals of the Bonnethead Shark (*Sphyrna tiburo*) differ wildly from the expected morphology relative to its close and distant relatives, even accounting for differences in head shape. Instead of being markers of phylogenetic constraint, the morphological characteristics of the lateral line system may reflect, and thus be a proxy for, fish ecology. The mechanosensory lateral line organ is conserved in all fish and is essential for behaviors such as schooling, predation, predator avoidance, orientation, and navigation. The diverse forms of the system have been investigated phylogenetically, but little research has been done to determine how the pattern and structure of the line correlates with the behavior and life history of the animals. With an ecomorphological disparity comparable to mammals, morphological convergence in distant lineages, and a well-conserved lateral line pattern throughout, sharks are used as the model clade for quantifiably analyzing and comparing the lateral line organ. Using a representative sample of sharks, non-invasive and non-destructive digital data of the system is acquired from tomographic reconstructions of contrast-stained heads for testing the connection between ecology and lateral line canal morphology. In addition to length, depth, caliber, and surface area analyses of the system, histological sections will be taken to better understand the canals between taxa and in different regions of the head.

A Tale of Structure and Function: Histology of the Tail Membrane of *Carollia perspicillata*

Alberto Lopez-Resendiz, Adam Kuuspalu, Brooke Quinn, Melina Hale, Sharon Swartz

Bats are known for their ability to fly, being the only mammals to do so. This is possible due to their flexible wing membranes, which serve as actuators to adjust lift and as sensors to detect changes in airflow and/or wing strain, which could contribute to navigation control. We investigate the histology and innervation of the uropatagium, the membrane between the legs, that may contribute to flight stability and reduce airflow separation, in *Carollia perspicillata*, Seba's short-tailed fruit bat. We compare its structure to other areas of the wing membrane and other membranous locomotor structures in vertebrates. We performed immunohistochemical analyses on uropatagial tissue samples using Alexa Fluor 488 Phalloidin, AAT, and DAPI staining to visualize muscle, nerve, and cell structure. Uropatagium tissue samples exhibited muscle fibers with distinct layouts based on anatomical position. Within the hypodermis, caudally located muscle fibers ran proximodistally, while cranially located fibers had oblique arrangements. Similar to the rest of the wing membrane, there was no indication of muscle spindles in the uropatagium. Nerve fibers were also observed next to the muscle and in the skin. The abundant innervation in the skin decreased closer to the trailing edge, indicating differences in sensory inputs between regions. These findings may help elucidate the physiological bases for the uropatagium's function in flight and how membrane-based locomotor structures evolved in distinct vertebrate groups.

The eco-evolutionary dynamics of elemental homeostasis and nutrient cycling

Andrés López-Sepulcre, Jeferson Amaral, Nimisha Gautam, Amina Mohamed, Saismit Naik

Stoichiometric homeostasis, the capacity to uphold chemical stability amid environmental fluctuations, is crucial to living systems. Because maintaining a constant inner environment requires altering the external environment through the selective uptake and recycling of nutrients, the study of stoichiometric homeostasis becomes central to understanding the ecological effects of species. Organisms vary in the degree to which they are homeostatic. Strict homeostasis occurs when the organism maintains a strictly constant composition regardless of the environment. Other organisms show flexible homeostasis, whereby the chemical environment changes their composition to some degree. Stoichiometric homeostasis is central to ecological stoichiometry theory, which tries to understand how the elemental composition of organisms affects and is affected by their environment. To date, however, the dominant assumption is that organismal stoichiometry, and therefore homeostasis, is a fixed property of a species. However, some recent evidence shows that homeostasis can vary within species, suggesting it can evolve on rapidly. This has important consequences because intraspecific variation in homeostasis implies intraspecific variation in the ecological impacts of organisms, a necessary component of eco-evolutionary feedbacks. In this article, we will review recent evidence for intraspecific variation in elemental homeostasis, lay out the hypotheses for its evolution, and discuss its ecosystem consequences. We argue that understanding microevolution of elemental homeostasis is central to our understanding of eco-evolutionary feedbacks.

Synchronization of Daily Rhythms in Human Odor Production and Mosquito Olfactory Preference

Lan Lou, Karthikeyan Chandrasegaran, Shajoesza Diggs, Emilie Applebach, Sneha Sapkota, Adaline Bisese, Richard Rust, Dana Hamad, Zhijian Tu, Chloe Lahondere, Clement Vinauger

In response to daily rhythms in environmental conditions and resource availability, insects such as mosquitoes display biological rhythms that regulate their foraging behaviors to optimize their chances of survival and minimize energy expenditures. However, while the importance of olfaction in mediating mosquito-host interactions has been established, the chemical and molecular rhythms modulating these interactions are yet to be fully characterized. In this context, we quantified the daily variations in human odor and identified odorant volatiles that are differentially produced throughout the day. We then

created artificial odor mixtures mimicking human scents at multiple times of the day based on these differentially emitted chemicals. Next, behavioral assays allowed us to quantify the interaction between human odor rhythms and the time-dependent olfactory preference of *Aedes aegypti* females. Our results indicate that mosquitoes taken in the last hours of the photophase are most attracted to the odor of humans characteristic of the late evening. The female antennal transcriptome analysis identified sensory genes whose expression showed daily variations and represented likely candidates to explain the observed behavioral rhythms. Altogether, these results contribute to improving our understanding of the underlying mechanisms governing mosquito-host interactions, and the molecular aspects we identified could open new research avenues for developing control strategies against mosquito-borne diseases.

Cellular contexts and regulatory logic underlying shared features of development and regeneration

Katy Loubet-Seneor, Mansi Srivastava

Development and regeneration both result in a complete adult organism. Despite intrinsically different starting points (the single-celled zygote during development versus many differentiated cells in a regenerating adult animal), they share many key processes such as establishing major body axes and differentiating cellular lineages. Given these similarities, it is perhaps unsurprising that transcriptome-wide studies in multiple species have revealed broad overlap between genes deployed in development and regeneration. However, the mechanisms allowing genes to accomplish similar tasks in both processes remain unclear. For example, are developmental genes reactivated in the same cellular contexts? Do distinct regulatory landscapes drive the activation of similar genetic pathways in development and regeneration? We have leveraged the regenerative capacity and accessible embryos of the acoel worm *Hofstenia miamia* to investigate these questions within a single organism. Combining *in situ* hybridization, scRNAseq and bulk ATACseq datasets, we compared molecular trajectories, chromatin landscapes, and cellular contexts of pathways shared between development and regeneration. We find both broad similarities in pathway progression, and development and regeneration-specific features. For example, early genetic factors in neural specification and patterning networks exhibit broader chromatin accessibility and cellular contexts in development versus regeneration, despite concordance of downstream gene relationships. This reflects a general trend that later features are more similar between the two processes. Together, these data provide a rigorous comparison of regenerative and developmental pathways.

Differential foraging response to multiple microplastic types by bluegill *Lepomis macrochirus*

Michael Louison, Molly Cannon, Isabel Montano, Preston Pennington, Anthony Dell

Microplastics (MPs) are an emerging contaminant and potential threat to aquatic and terrestrial organisms. Uptake of MPs by animals can happen passively or actively as a result of the direct consumption of MPs. Therefore, the ability to successfully recognize and avoid MP consumption is beneficial to animals (including fish), but this ability may be impacted by the type of MP present as well as competition related to density. In this study, we examined the willingness of bluegill *Lepomis macrochirus* to forage on microplastics (high density polyethylene bag films or polypropylene rope fibers) and whether the response to them would change over time. Bluegill were stocked at densities of 3 or 6 individuals into 37 L aquaria and offered flake food (controls) or MPs over a period of 4-6 days. Results showed that foraging on MPs declined over time while foraging on food increased, indicating learned avoidance of MPs. Total foraging however was impacted by plastic type – Bluegill were more likely to forage on films than on fibers. Following group testing each fish was assessed for swimming performance in a modified swim tunnel apparatus, however no significant differences were found in swimming performance between feeding groups. Our results add to the growing body of knowledge of how fish may consume MPs, particularly in relation to the risks posed by different MP types.

Multilevel examination of radiation stress and signatures of selection in Chernobyl wolves

Cara Love, Brian Arnold, Bridgett vonHoldt, Shane Campbell-Staton

Examining the intricate relationship between contaminant exposure, physiological responses, and evolutionary changes in wild populations is critical for understanding the impacts of anthropogenic environmental change. We explore the consequences of chronic multigenerational radiation exposure on gray wolves (*Canis lupus*) in the Chernobyl Exclusion Zone (CEZ). By analyzing regulatory and genetic variations, we uncover molecular signatures of stress and adaptive evolution. Whole blood transcriptome data reveal significantly altered leukocyte populations among Chernobyl wolves and regulatory divergence at both single gene and co-regulatory levels in CEZ wolves. These results highlight radiation-induced immune modulation, cellular apoptosis, and antitumor immune response as potential physiological consequences of radiation stress and targets of selection within the CEZ population. To examine genomic signatures of selection, we examined whole genome sequencing data for lineage-specific signatures of selection within the CEZ wolf population. Several genomic regions exhibit lineage-specific divergence, overlapping genes linked to cancer physiology. These genes encompass crucial functions such as anti-tumor immunity, cellular invasion, and migration. Through a composite of multiple signals approach, we pinpoint specific variants under selection, and functional modeling reveal potentially relevant protein alterations in immunity regulation and cancer physiology.

Illuminating odorant receptor diversity and sex-biased expression in the common eastern firefly

Sarah Lower, Samuel Pring, Hanh Tran, Mathew Price, Robert Mitchell

Olfaction is essential for many aspects of insect biology, from foraging to mating. In insects, olfaction is mediated by odorant receptor (OR) proteins in the dendritic membranes of olfactory receptor neurons inside of olfactory sensilla, located primarily on the antennae. However, ORs remain uncharacterized in the vast majority of insect groups. Here, we use genome and RNA sequencing to identify 101 ORs in the genome of *Photinus pyralis*, the common eastern firefly. Phylogenetic analysis shows that specific OR clades have diversified in *P. pyralis* versus other beetle species. Differential expression analysis reveals that a subset of ORs are upregulated in male antennae as compared to female antennae and back legs of both sexes, suggesting that they may be involved in mating. One OR is more highly expressed in female antennae than male, suggesting that it may have female-specific functions, such as detecting suitable oviposition sites. These findings increase known diversity of insect ORs in an understudied beetle family (Lampyridae) and suggest that bioluminescent fireflies may rely on multimodal mating signals more than expected.

Swimming biomechanics as a driver of temperate inland lake fish abundances

Kelsey Lucas, Kevin Wehrly, Karen Alofs

Fish body and fin morphology is linked to swimming ability and so may constrain the types of habitats a species can occupy. Here, we explore the relationships between swimming morphology and habitat occupancy in inland lakes. We collected linear measures of body and fin morphology from museum specimens of 30 of the most common Michigan inland lake species and show that strongest sources of morphological variation are traits related to streamlining, stabilization, pectoral and caudal fin use, and diet. Alongside, we developed species distribution models to identify how these species' abundances vary along environmental gradients using data from standardized lake surveys conducted between 2003-2019 by the Michigan Department of Natural Resources. We find that environmental factors like temperature are the most important predictors of fish abundances, but factors related to swimming biomechanics, like flow levels and presence of refuges, correlate to a lesser extent with fish species abundances. We suggest that after regional filters set which species are found in a water body, swimming ability may influence relative abundance or microhabitat use. Along with future swimming studies using live fishes, this work ultimately will aid in identifying selection pressures that may have influenced fish diversity and clarifying swimming specializations that may constrain habitat use and ecological role.

Diet and Chemical Defenses of the Sonoran Desert Toads

Marina Luccioni, Jules Wyman, Lauren O'Connell

The Sonoran Desert Toad (*Incilius alvarius*) is the only animal known to secrete the psychedelic compound 5-MeO-DMT as a chemical defense, but the source of 5-MeO-DMT in *I. alvarius* remains unknown. Some amphibians endogenously produce chemical defenses while others acquire them from specialized diets. In this study we analyzed toxin gland secretions and diet profiles from wild *I. alvarius* and sympatric anurans from native and urban habitats around Tucson, Arizona to explore possible links between diet and 5-MeO-DMT production. All *I. alvarius* secreted high concentrations of 5-MeO-DMT, whereas other sympatric toads did not. The diet of *I. alvarius* was similar to that of sympatric anurans, indicating that *I. alvarius* does not exhibit relative dietary specialization. Slight dietary differences between toads in native and urbanized habitats were observed. Taken together, these lines of evidence suggest that diet is not directly linked to 5-MeO-DMT production, and support the alternative hypotheses that Sonoran Desert toads synthesize 5-MeO-DMT endogenously or via a microbial symbiont.

Black and White crappie age-growth characteristics exhibit divergent responses to a thermal gradient

Elva Lucero, Michael Newbrey, Ashley Desensi, Jennifer Newbrey

We have a poor understanding of how fish age and growth respond to a warming climate. Therefore, age and growth characteristics of two closely related fish species were examined with the goal of identifying adaptations for warm or cool climates from an evolutionary perspective. We assessed 50 populations of White Crappie (*Pomoxis annularis*), which evolved in a warm climate, and 28 populations of Black Crappie (*Pomoxis nigromaculatus*), which evolved in a cooling climate. Published age and growth characteristics were compared to three types of mean annual temperature gradient data; minimum (MAT_{Min}°C), 24-hour (MAT_{24hr}°C), and maximum (MAT_{Max}°C). We examined the relationships among MAT_{Min}/MAT_{24hr}/MAT_{Max}°C and longevity, maximum total length (MTL), and total lengths at ages 3, 4, and 6 years (TL_{age3,4,6}) of chronological age. Using least squares regression, we found significant positive relationships among MAT_{Min}/MAT_{24hr}/MAT_{Max}°C and TL_{age3,4,6}, but significant negative relationships between MAT_{Min}/MAT_{24hr}/MAT_{Max}°C and longevity for Black Crappie. White Crappie only showed two significant relationships between MAT_{Min}°C and TL_{age3} and MTL. Black Crappie in warm climates had a shorter lifespan but grew (faster) to longer lengths at ages 3, 4, and 6 years old, while White Crappie only weakly responded to the cold temperatures of a thermal gradient. White Crappie were not strongly influenced by a thermal gradient, suggesting that they are pre-adapted for a warming climate unlike their sister species the Black Crappie.

Shape analysis reveals distinction between South African and Chinese specimens of *Lystrosaurus*

Jacqueline Lungmus, Zoe Kulik, Yu-Tai Shi, Christian Sidor, Kenneth Angielczyk

Representing both the quintessential disaster fauna and survivor taxa, the genus *Lystrosaurus* has long been recognized for its abundance, geologic longevity, and cosmopolitan distribution. *Lystrosaurus* is increasingly being used to test macroevolutionary hypotheses pertaining to the End Permian Mass Extinction and the resiliency of terrestrial vertebrate communities. However, this focus has largely emphasized specimens from the southern hemisphere, with northern hemisphere and equatorial samples not being the focus until more recently. As in the Karoo Basin of South Africa, *Lystrosaurus* is one of the most abundant terrestrial taxa in the Bodda strata of North-West China, providing a unique opportunity for comparing two taxonomic groups from through time and space. Here, we present updated results comparing a large sample of South African *Lystrosaurus* skulls to a sample of Chinese *Lystrosaurus*.

Using 2D geometric morphometric analyses, we compared skull morphology in dorsal, lateral, and anterior views to quantify shape difference between these two paleo-communities. We found that the Chinese specimens possess a truly distinct morphology from that of South African *Lystrosaurus* specimens in all three views, with only minor overlap in morphospace. Besides

representing larger overall body-sizes, the skulls of Chinese specimens are distinct for being more elongated, with less lateral flaring and a proportionally longer snout. This data adds critical insight into the regional differences between this cosmopolitan group.

Ecomorphology and morphometrics of head, body, and fin shapes in parrotfishes (Scarini: Labridae)

Linnea Lungstrom, Q.T. Elizabeth Van-Ha, Mark Westneat

Investigating the ecomorphology of fishes provides insight into how the interaction between morphology and ecology relates to their adaptive diversification. In the parrotfishes (Scarini: Labridae), feeding mechanisms and cranial morphology have been associated with trophic variation. However, lateral morphometrics of the head, body, and fins and their association with feeding modes and ecomorphology remains to be explored. Here, we quantified head and body morphological variation among 71 parrotfish species using traditional and geometric morphometrics and investigated associations related to feeding modes in the group. Results revealed a strong association between feeding mode and the morphological characteristics of head size and shape, pectoral fin aspect ratio (AR), and body shape. Browsers had lower pectoral fin ARs, smaller, flatter heads, and small beaks, while excavators had higher pectoral fin ARs, larger, peaked heads, and large beaks. Scrapers also had higher pectoral fin ARs and larger, peaked heads, but the apex of the forehead was located anteriorly in comparison to excavators. Additionally, browsers were significantly less disparate than scrapers and excavators and accounted for a small proportion of the total morphological disparity. Larger foreheads and beaks as well as higher pectoral fin ARs may facilitate the more mechanically strenuous feeding modes of scraping and excavating. In scrapers, the anterior apex of the head may allow for more contact with coral, potentially increasing scraping success.

Organization of medial octavolateralis nuclei activity during different flow stimuli in zebrafish

Elias Lunsford, Claire Wyart

During navigation, animals rely on sensory systems to relay information about changes in their environment to motor command circuits in the brainstem that select optimal motor actions. The organization of sensory inputs coupled with motor commands is essential to our understanding of decision-making processes underlying navigation. We have leveraged the versatility of responses of fish to water flow using the larval zebrafish (*Danio rerio*) in which we combine microfluidics and functional calcium imaging to decipher where and how stimuli is integrated in the hindbrain to inform action selection. The flow sensitive lateral line (LL) system is innervated by afferent neurons that project into the brainstem to the medial octavolateralis nuclei (MON). Current methods have yet to discern how MON neurons are recruited as a function of stimulus parameters such as flow direction, intensity, symmetry, and steadiness. MON neurons must precisely coordinate the selection of stereotyped motor actions based on said flow parameters. Therefore, input-output connectivity of the MON is key to solve how hindbrain neurons compute the flow and select motor actions. Our findings suggest an organization of the sensorimotor network that enables each stimulus perceived on the body to recruit a MON subset, which integrates sensory information, that likely projects to command neurons to recruit subsets of command neurons responsible for during ipsilateral turning and forward swimming.

Adhesive performance under different strain rates in *Strongylocentrotus purpuratus*

Keegan Lutek, Alyssa Stark

Many benthic invertebrates, like the purple sea urchin, reversibly attach using an adhesive system at the distal end of extensible tube feet to facilitate adhesion and mobility in the variable, wave-swept intertidal zone. When experiencing high forces, tube foot failure may occur at two places: the site of adhesion or the tube foot stem. In some species, faster rates of applied force increases tube foot stem strength due to the viscoelastic properties of the stem (i.e., the material stiffens when pulled faster). This suggests that whole

animal adhesive performance may also be strain rate dependent. However, no work has investigated whether the tube foot performance or whole animal performance (a combination of the tube foot stem and adhesive properties) is strain rate dependent. Therefore, we performed a preliminary experiment testing tube foot disc adhesive performance and whole animal adhesive performance independently but found no strain rate dependence at either level. This unexpected result, that purple urchin whole animal performance is not strain rate dependent, prompted a second experiment measuring tube foot disc adhesion, whole animal performance, and tube foot stem properties under different strain rates to gather a wholistic view of the purple sea urchin adhesive system. These results have important implications for the ecology of purple sea urchins and how this highly plastic adhesive system may adapt in specific hydrodynamic conditions.

Operation brainstorm: developing molecular biomarkers to detect the pathology of cerebral malaria in

Kenedi Lynch, Christine Lattin, Tosha Kelly

Cerebral malaria, the most severe and fatal form of malaria, is grossly understudied in avian models. Due to a lack of effective non-lethal methods, diagnosing cerebral malaria currently requires finding malaria parasites in brain tissue post-mortem. The ever-present threat of malaria to avian biodiversity and the risk of spillover from wild to captive birds makes it essential to diagnose cerebral malaria in living birds as a first step before treatment. We conducted the first investigation of avian cerebral malaria pathology using qPCR to identify a potentially non-lethal biomarker of the disease in wild birds. Using cerebral tissue from wild-caught house sparrows (*Passer domesticus*) experimentally inoculated with *Plasmodium relictum*, we correlated cerebral inflammatory cytokine expression (a hallmark of cerebral malaria) with cerebral parasite load. We predicted that the ratio of pro-inflammatory cytokine expression to anti-inflammatory cytokine expression would be higher in birds with cerebral malaria than in birds with simple malaria, and that this would positively correlate with cerebral parasite load in brain tissue. Future work will correlate cerebral cytokine expression with cytokine expression in whole blood to assess whether qPCR could potentially be used as a non-lethal diagnostic tool to detect cerebral malaria infections in wild birds.

Web applications as a tool for highly collaborative, multi-institutional research initiatives

Leigha Lynch, Eric Lynch, Timothy Campbell, Terrence Ritzman, Heather Smith

Many researchers are involved in highly collaborative, multi-PI, multi-university studies using big data. These studies typically include many trainees who must learn uniform terminology, collection methods, and data entry/analysis to ensure data fidelity. We encountered these challenges in our collaborative study quantifying human anatomical variation. Our study requires simultaneous data collection by 4 PIs and 12 students on both Midwestern University campuses (AZ and IL) from cadaveric specimens acquired via body donation programs utilized in multiple gross anatomy courses at the university. To address these challenges, we developed a web application that can be accessed by multiple simultaneous users from any location. Our secure web application allows for: 1) easy data recording via multiple electronic devices; 2) instant access to new data; 3) consistent terminology/definitions through drop-down form inputs providing text or images of each trait; and 4) automated visualization of basic statistics. The back-end database allows for long-term data storage of anonymized anatomical and user data, which can be exported for further analysis. We can, therefore, flexibly use the data to address multiple research questions and record student involvement to ensure appropriate acknowledgement years after data collection. Our web application allowed us to develop a highly integrated network of researchers and data, while easily controlling data accuracy, longevity, and usability and we feel it is an ideal tool for any collaborative research project.

Establishing tardigrades as an emerging model organism for systems neuroscience

Ana Lyons, Boyeong An, Lilia Dow-Yuzawa, Richik Pal, Saul Kato

Foundational discoveries in neuroscience have often stemmed from studying optically transparent non-mammalian organisms like *C. elegans*, *Drosophila*, and an increasing variety of non-model organisms. Tardigrades—with transparent bodies, a few thousand neurons, and a rich behavioral repertoire—are a promising model for systems-level neuroscience. We present a framework for mapping the neuroanatomy of the tardigrade (*Hypsibius exemplaris*) and capturing whole-brain and whole-body dynamical neural activity in live, behaving specimens. We introduce imaging techniques for live tardigrades across life stages using holographic tomography and live-cellular dyes. We also develop neuroanatomy mapping methods of fixed tardigrades via confocal microscopy, exploring Hybridization Chain Reaction (HCR) in situ methods as well as expansion microscopy with heavy metal staining. We share guidelines for whole-brain and body imaging sample preparation of live tardigrades. We establish a custom bioinformatics pipeline, alongside an adapted Tardi-Vec transgene protocol, to probe the expression of neuron-specific promoters and marker proteins like eGFP and GCaMP throughout the nervous system. We present preliminary results and challenges of delivering genetic cargo into the tardigrade germline, using PiggyBac and CRISPR-Cas9 and a variety of delivery methods. Future objectives include creating a stable *H. exemplaris* strain expressing pan-neuronal GCaMP to investigate behaviors like goal-driven locomotion, phototaxis, thermosensation, and dormancy. We invite the community to collaborate in developing tardigrades as a new model organism for neuroscience.

Every body, every mind: A framework for building community among disabled & neurodiverse scientists

Ana Lyons, Mashel Fatema Saifuddin, Kevin Daigle, Laura Persson

We are a group of UCSF postdocs, graduate students, researchers, and educators working to establish a peer-led student and postdoc interest group with community events, with the goal to counteract ableism in academia. We hope that our model of a peer-to-peer postdoc and student-led interest group can serve as a model for increasing support and retention of diverse scientists among academia and science, at large. In particular, the approach of this peer-led special interest group aims to increase community-building and advocacy for scientists with diverse experiences related to mental health, neurodivergence, disability (short or long term), chronic illness, chronic pain, and the collaboration of allies. This group's framework for engagement includes inviting guest speakers and panelists to discuss and workshop challenges that neurodiverse and disabled scientists face, establishing a peer-mentorship program for people who identify with neurodiversity and/or disability, having a regular reading group on anti-ableism in academia, and maintaining a group website—to serve as a framework for establishing disability affinity groups at other institutions. In future years, the group hopes to help collaboratively organize social mixers at relevant academic conferences and campus events, such as first-year orientations. Intended positive outcomes of forming disability-centered, peer-led groups include increased visibility and awareness at academic institutions, improving support networks, reducing stigma, cultivating a more diverse and equitable scientific community, mentorship opportunities, empowerment and advocacy, and educating allies.

Fish larvae trade-offs: Pelagic larval swimming may constrain body proportion in reef fishes.

John Lyons, Kathryn Kavanagh

The vast majority of reef fishes, including damselfishes, have a pelagic larval stage that ends when any surviving larvae swim to a reef to settle. An extremely rare alternative life style is 'larval brooding', where both parents protect larvae on the reef for months until they disperse nearby. The monophyletic clade of larval brooders includes two genera *Acanthochromis* and *Altrichthys*. In examination of the skeletons of these damselfishes, we found that all species of damselfish that brood larvae on the reef have a uniquely increased precaudal vertebral count, while all typical damselfishes have an invariable vertebral count with a greater proportion of caudal vertebrae. To explore the

significance of the vertebral differences, we measured body proportions of larval brooders vs relatives with the typical pelagic larval stage. We found increased body cavity area and reduced muscle area in larval brooders. In a comparison of known larval swimming ability among reef fishes, the larval brooders performed most poorly. We propose that when larval brooding evolved, relaxed selection on larval swimming performance allowed a shift in body proportions to favor a larger body cavity and altered axial patterning. An enlarged body cavity gives a fitness advantage to larval brooders as females could hold more or larger eggs, and provides additional storage for resources expended during the biparental care that characterizes these species.

Science of Attraction: Investigating Sexual Dimorphism in the Vocal Organ of Fruit Bats

Miya M-Khoo, Peishu Li, April Neander, Bruce Patterson, Zhe-Xi Luo

Sexual selection is often hypothesized to drive novel phenotypes in the vocal apparatus of vertebrates that use specialized vocal signals for mate attraction and intrasexual competition. Yet, interspecific variation in vocal anatomy can also stem from differences in phylogeny and/or body size. We use pteropodid fruit bats as a model to examine the effect of sexual dimorphism, body size, and phylogeny on the evolution of hyoid and larynx. Within Pteropodidae, the male hammer-headed fruit bat (*Hypsignathus monstrosus*) is known to have one of the largest larynxes relative to body size among mammals. Previous hypotheses attribute laryngeal hypertrophy in *Hypsignathus* to sexual selection as it enables males to produce loud calls during lek mating behavior. However, this hypothesis is untested given laryngeal morphology of female *Hypsignathus* is poorly known. Moreover, it is unclear if male-specific laryngeal hypertrophy is unique to *Hypsignathus* or characterizes other related epomorphine bats. Our μ CT visualization suggests sexual dimorphism in laryngeal size and degree of calcification increases with body size among epomorphines. After correcting for body size, the thyroid of male *Hypsignathus* is much taller but narrower compared to other pteropodids. We hypothesize that laryngeal hypertrophy in male *Hypsignathus* is mainly driven by positive allometry along the superoinferior axis, while circumferential expansion is partially constrained by the smaller hyoid, and by narrow thorax opening which the larynx passes through during descent.

Characterizing limb-girdle muscular dystrophy type 2G from Titin cap mutation in mouse models

Aria Ma, Sarah Holbrook, Jennifer Stauffer, Greg Cox

Titin cap (TCAP) encodes for telethonin, a protein necessary in sarcomere assembly in muscle myofibrils. Deleterious mutations in TCAP result in total deficiency of telethonin in muscle, causing the adult-onset autosomal recessive myopathy, limb-girdle muscular dystrophy type 2G (LGMD2G). This disease is characterized by progressive muscle weakness and wasting in the upper and lower limbs, and mild deterioration of the respiration system. The impact of the mutation on different muscle fibers in affected muscle groups is currently unknown. This study examined hindlimb tissue from 8-month, 12-month, and 24-month old mice to characterize neuromuscular degeneration progression and investigate phenotypes of different muscle fibers. Through examining four key muscles (tibialis anterior, peroneus longus, soleus, and medial gastrocnemius), a variety of fibers are represented (e.g. slow and fast-twitch) to understand how the mutation affects muscle impairment. Progression of disease is recorded through analysis of the muscle fiber area, instances of central nucleation, and occurrences of an unknown aggregate formation. The results show that progression of disease affects slow and fast-twitch muscles differently with instances of central nuclei and aggregates differing at unique time points. Higher instances of central nucleation may be repair mechanisms at work as muscle deteriorates. Further investigation into aggregate material properties and specific response mechanisms of slow and fast-twitch fibers is needed to understand how progression of LGMD2G in mouse models parallels human symptoms.

Analysis of resting status reveals distinct elevational variation in energy dynamics of lizards

Liang Ma, Zhongwen Jiang, Shi-ang Tao, Cheng Wenda, Chu-yu Cheng, Dan-yang Wu, Wei-guo Du

Animals spend a considerable proportion of their life span at rest. However, resting status has often been overlooked when investigating how species respond to environmental conditions. This may induce a large bias in understanding the local adaptation of species across environmental gradients and their vulnerability to potential environmental change. Here, we conducted an empirical study on montane agamid lizards, combined with mechanistic modeling, to compare elevational variations in body temperature and energy dynamics (digestion and metabolism) between resting and active status. Our study on three populations of an agamid lizard along an elevational gradient revealed a trend of decreasing body temperature towards higher elevations, the main contributor of which was resting status of the lizards. Using population specific reaction norms, we predicted greater elevational variation in hourly and cumulative digestion for resting lizards than for active lizards. Climate-change impacts, estimated as the change in cumulative digestion, also show greater elevational variation when resting status is factored into the analysis. Further, our global analysis of 98 agamid species revealed that in about half of their combined distributional range, the contribution of resting status in determining the elevational variation in cumulative digestion and metabolism of lizards was greater than the contribution made by a lizard's active status. Our study highlights the importance of considering resting status when investigating how species respond to environmental conditions, especially for those distributed over tropical and subtropical mountain areas.

The tempo of the invasion of new adaptive zones in mammals

Fabio Machado

An adaptive zone is a restricted region of the phenotypic space occupied by species with relatively similar form-function relationships. While within-zone evolution is usually small in scale and does not involve major ecological changes, when opportunity arises, lineages might invade new adaptive zones, leading to extreme and rapid evolutionary changes. Implicit in this idea is the notion that within-zone evolution is slower and less extreme than between-zone transitions. However, the actual timing (or tempo) of adaptive zone invasion has rarely been modeled, let alone compared to the tempo of within-zone transitions. Here, I investigated the necessary time to produce evolutionary changes observed in the skulls of 10 mammalian groups that invaded new adaptive zones. The results show that all transitions can happen fairly rapidly, even under strong restrictive conditions. Additionally, almost all between-zone transitions did not take longer than within-zone changes. Exceptions to this are among some fossil groups, like saber-tooth tigers and glyptodonts, which involve radical changes in form-function relationships. These results reinforce the extreme potential of change in complex phenotypes and reinforce the role of contingencies as the defining feature in macroevolutionary change.

Aerobic and anaerobic metabolism after injury in regenerative and non-regenerative annelids

Joseph Mack, Alexandra Bely

Animals display extensive variation in regenerative ability. To understand how and why regeneration might be gained or lost among lineages, it is important to study the physiological mechanisms that fuel injury repair and regrowth. Toward this end, we are investigating aerobic and anaerobic metabolism following injury in both regenerative and non-regenerative species of water nymph worms (Annelida: Naidinae). We predicted that aerobic metabolism would increase in response to injury, which has been reported in other invertebrates. However, using microrespirometry, we found that neither regenerative nor non-regenerative species show an aerobic response to amputation, suggesting that regeneration does not incur a cost through increased aerobic metabolism in annelids. Meanwhile, recent research on vertebrates has found increased anaerobic metabolism to be associated with regeneration. To determine if water nymph worms also show an anaerobic response to injury, we are using qPCR to investigate the expression of a variant of the coq-2

gene associated with anaerobic metabolism in protostomes. With this data, we will test the hypothesis that anaerobic pathways, rather than aerobic pathways, are elevated during regeneration in annelids. Overall, this research will deepen our understanding of the physiological mechanisms that fuel regeneration in annelids.

Effect of a strong magnetic pulse on the map sense of loggerhead sea turtles

Alayna Mackiewicz, Kayla Goforth, Abigail Glazener, Catherine Lohmann, Kenneth Lohmann

Diverse animals, including loggerhead sea turtles (*Caretta caretta*), can detect Earth's magnetic field and use it as a cue to navigate during long-distance migrations. Turtles can use magnetic field information to determine their geographic location, an ability known as a magnetic map sense. Despite extensive evidence for magnetic sensitivity, how turtles actually detect and process magnetic information remains enigmatic. One hypothesis is that crystals of the mineral magnetite underlie the magnetic sense. To test the magnetite hypothesis, a strong, brief magnetic pulse can be applied, which realigns the magnetic dipole moment of magnetite and potentially disrupts magnetoreception for a time afterward. To determine whether turtles use magnetite-based magnetoreceptors, juvenile loggerhead turtles ($n=16$) were conditioned to associate a magnetic field with a food reward using an established assay. After two months of conditioning, a strong magnetic pulse was applied to determine if their ability to recognize the rewarded magnetic field was disrupted. Findings from this study will provide insight into the mechanism of magnetic sensing and map sense of marine animals.

Morphological diversity of the oral lures of Stargazers (Percomorphacea: Uranoscopidae)

Leo MacLeod, Kate Bemis, Stacy Farina

The family Uranoscopidae, commonly known as Stargazers, are found worldwide in tropical to temperate oceans. Stargazers are ambush predators that bury into the sediment until only the eyes and dorsal region of the mouth are visible. While buried, members of three genera (*Uranoscopus*, *Kathetostoma*, and *Genyagnus*) use water flow to flick a lure out of the mouth to entice prey closer. In this study, we examined the extreme morphological diversity in the lures of these genera. The lure is attached to a membrane on the inside of the lower jaw and is diverse in size, shape, and colour. For example, in some species, the lure is black with a simple thread shape, while in others it is an orange filamentous pompom. We describe the variation both within and between species and discuss use and challenges of the lure as a phylogenetic character.

Plastome phylogenomics of Agave subgenus Manfreda

Bryan MacNeill, Fae Bramblepelt, Michael McKain, Aaron Rodriguez, Eduardo Ruiz-Sanchez, Juan Pablo Ortiz-Brunel

Agave is a well-known plant genus that comprises several economically and culturally important species involved in alcohol, fiber, and sugar production. Agaves serve as ecological keystones in many arid or semiarid ecosystems and comprise over 250 species. Subgenus *Manfreda* consists of 52 herbaceous species that exhibit highly variable floral architecture compared to other Agave lineages, attracting specialist pollinators like hummingbirds and hawkmoths and generalist pollinators like bees. How these floral phenotypes diversified has yet to be precisely understood, as there is little molecular evidence for the phylogenetic relationships within *Manfreda*. Here we present a robust plastome phylogeny for *Manfreda*, showing hybridization and chloroplast capture are likely prominent in the group. Short branches of the phylogeny backbone suggest little divergence of chloroplasts during the group's diversification. We recovered non-monophyletic origins of floral phenotypes, suggesting convergent evolution of pollination syndromes. We also discovered a ~1000 base pair inversion in the plastomes of non-*Manfreda* Agave species used in our analyses. These results will contribute to the knowledge of Agave systematics and this system as an emerging model for studying floral evolution. Future research will incorporate nuclear genes from

an Asparagaceae custom-probe set to identify hybridization events and try to obtain better phylogenetic resolution for this economically and ecologically important genus.

A Shellfish Journey: Characterizing an Inhibitory Receptor in the Crustacean Molt Cycle

Ashlynn Madril, Jorge Perez-Moreno, Mihika Kozma, Neha Gandhi, Luisanna Hernandez-Jepesen, Tomer Ventura, Donald Mykles

Molting is an essential process that crustaceans undergo to grow, develop, and regenerate lost appendages, which involves the shedding of the old exoskeleton and the hardening of a new exterior shell. It requires coordination between multiple complex pathways, one of which involves the molt-inhibiting hormone (MIH), essential for regulating the molting process itself. MIH, a member of the crustacean hyperglycemic hormone (CHH) superfamily, maintains the crustacean molting gland, the Y-Organ, in a basal state, preventing crustaceans from continuously initiating the molt cycle. The MIH receptor has not been fully identified or characterized. It is expected it is a type of Class A G-protein coupled receptor (GPCR) since previous research has found that MIH binding elicits a canonical GPCR signaling cascade through adenylyl cyclase activation. The existence of GPCRs known to bind CHH-superfamily peptides in insects (i.e., ITP) also supports this hypothesis. This study examines seven possible CHH-family GPCRs that have been previously identified and aims to determine the most likely MIH receptor candidate. Using *Gecarcinus lateralis*, the blackback land crab, as a model organism, bioinformatic analyses and polymerase chain reactions (PCRs) were used to identify the expression of these putative MIH receptors across tissues and molt stages. Identifying the MIH receptor is a significant step towards understanding the intricate molting process, with implications across fields and industries. Supported by NSF grant IOS-1922701.

3D Slicer and SlicerMorph: A new frontier for 3D digital morphology

Murat Maga, Sara Rolfe, Chi Zhang, Arthur Porto, Adam Summers, Steve Pieper, Andras Lasso

3D digital morphology has emerged over the last decade as a new field. Efforts in digitization and the emergence of aggregate specimen archives are transforming morphology-based research. Yet interacting with 3D specimen data is fraught with difficulty due to the myriad of formats and lack of standardized workflows. Some researchers turn to powerful, but expensive proprietary software, which in short term mitigates some of the operational challenges, but creates long-term ones such as cost of maintenance, difficulty in data sharing, and collaboration. Perhaps more importantly, such tools interfere with efforts of integrating digital morphology techniques as part of the curriculum or outreach activities.

We will present how using 3D Slicer and its SlicerMorph extension facilitate collaboration and data-sharing, improving the accessibility and application of digital morphology in teaching and research contexts. Slicer is an open-source platform poised to revolutionize the anatomical sciences, much as other open-source platforms (e.g., R) have revolutionized the fields of statistics and scientific computing. Within the Slicer ecosystem, the SlicerMorph extension streamlines digital morphology by enabling data import, visualization, measurement, annotation, and geometric morphometric analysis on 3D data, including volumetric (CT, MRI) and surface scans. The extensible nature of 3D Slicer allows users to develop new functionality that is then available to all users. Expanding the open-source digital morphology ecosystem must be a community-led effort, including training and identifying challenges/barriers.

Tooth root shape/orientation and its relationship to bite force in the jaws of two therian mammals

Isaac Magallanes, Peishu Li, Zhe-Xi Luo

Teeth and jaws are powerful indicators for interpreting mammal life history, ecology, and evolution because their great diversity in shape reliably reflects mammalian feeding function and dietary ecology. To date, extensive studies exist on the transformation of tooth crowns and jaws across major phylogenetic

transitions and ecological diversifications. However, fewer studies address the other half of dental morphology: the tooth roots, which are integral to dental anatomy and essential for the masticatory function of all toothed mammals. Tooth roots and their surrounding soft tissues (periodontal ligaments) experience complex loading regimes during mastication. The periodontal ligaments help transmit sensory afferents to the central nervous system and assist in the sensorimotor coordination required for precise occlusion. While human tooth root morphology has been examined through dental clinical practice and research, the diversity of tooth root morphologies in other major mammalian groups remains poorly documented. We present new data on intraspecific variation in the tooth roots of the marsupial, *Monodelphis domestica* (n=12) and the placental, *Tupaia glis* (n=8). Using morphometric measurements like root surface area and root orientation, we explore their relation to the distribution of estimated maximum bite forces and bite force orientation along the post-canine rows and the jaws. Our preliminary results show that the permanent molars in both these taxa are more consistently aligned with the orientation of the bite force during mastication.

Saltwater to freshwater transitions in stingrays result in diversification, but not convergence

Autumn Magnuson, Mason Dean, James Weaver, Joao Pedro Fontenelle, Nathan Lovejoy, Matthew Kolmann

Why are some groups of organisms more diverse than others? Classic hypotheses for explaining this consider distinctions in time, place, available resources, and competition as the catalysts for differential diversification. Despite lakes and rivers accounting for less than 1% of Earth's total water, freshwater and saltwater environments have similar levels of diversity, so studying transitions between these environments can provide insight into differential diversification. Stingrays have made the transition from saltwater to freshwater multiple times across different continents, making them an apt model system for understanding how habitat transitions can shape diversification. We collected data on habitat preference and diet from the literature, as well as measured functional traits from radiographs of stingrays. We evaluated the frequency of saltwater-freshwater transitions in stingrays, compared ecological and phenotypic diversification among freshwater and saltwater lineages, and assessed the degree of convergence among freshwater species. Like other marine-derived fishes, stingrays have overwhelmingly only transitioned from saltwater to freshwater and not the reverse. After independent transitions to freshwater, river rays did not demonstrate increasing morphological or lineage diversification. Although freshwater and saltwater stingrays overlapped morphologically, the former were more diverse and expanded the margins of morphospace. Piscivores and molluscivores occupied novel regions of morphospace. Freshwater stingrays did not converge morphologically, which may be because there has not been enough time for this to occur among more recent freshwater lineages.

Environmental DNA metabarcoding of biodiversity in regions of the Antarctic continental shelf

Andrew Mahon, Madeline Armstrong, Kenneth Halanych, Christopher Jerde

Assessing the biodiversity of any system is difficult, but due to the logistics, it is especially difficult in the polar continental shelf systems of the Southern Ocean. While traditional sampling in these remote regions can be difficult, using molecular tools is gaining momentum for biodiversity studies worldwide. Environmental DNA (eDNA) metabarcoding is a reliable, cost-effective, noninvasive method for sample collection and analyses. This approach has been limited in Antarctica partly by access, the absence of robust databases for analyses, and the slow development of laboratory and data analysis pipelines. Here, we present eDNA metabarcoding results from multiple sites around the continental shelf of Antarctica, including the rarely sampled East Antarctic shelf region. Along with our study results, we include sample collection information, laboratory methodology, and our detailed data pipeline. We assess the scale of biodiversity using eDNA metabarcoding data, including an assessment of alpha and beta biodiversity. There is considerable uncertainty in the gamma diversity in the Antarctic, but we provide estimates of composite eDNA water samples needed to estimate species richness in a given region. Additionally,

we find that nearshore and offshore samples have unique species communities detectable by eDNA metabarcoding. Our results demonstrate the utility of eDNA applications in Antarctica and support its future use in the region.

Heat waves and prior infection improve survival of *Manduca sexta* to *Bacillus thuringiensis* bacteria

Katherine Malinski, Christopher Willett, Joel Kingsolver

Climate change may alter ecological interactions between hosts and their pathogens. Exposure to heat waves can impact immune responses, but host immunity can also change with prior pathogen exposures. History of prior infection events can confer a survival advantage to later pathogen encounters through immune memory, known as immune priming in insects. Here we examine the impact of a heat wave during an initial bacterial pathogen exposure on the strength of immune priming protection in the tobacco hornworm, *Manduca sexta*. We tested naturally-occurring field and domesticated lab populations of *M. sexta* to explore the impact of recent evolutionary history and selective pressures for immune and heat responses. We found that the magnitude and directionality of the immune priming effect was both population and temperature-dependent. For both populations, prior pathogen exposure reduced survival to a second infection under control temperature conditions, indicative of a stress effect. The heat wave treatment reversed the directionality of this effect in the field population, conferring a survival advantage to a later infection, but increased the magnitude of the stress effect in the lab population, further reducing survival to the secondary infection. This study implicates both environmental context and evolutionary history as important mediators of host-pathogen ecological outcomes in response to heat waves caused by climate change.

Reducing meta-analysis bias in ancient DNA studies

Swapan Mallick, Matthew Mah, Adam Micco, Nick Patterson, David Reich

Many comparative genomic studies using next-generation sequence data typically add a number of newly sequenced individuals to repositories of previously studied organisms.

This can induce batch effects, where different protocols create artifactual genetic relationships, compounded by institution-specific computational pipelines where subjective choices of aligners, parameters, recalibrations and filtering are made.

The process of making raw variant calls is relatively simple – many tools exist. However, filtering calls to a trustworthy set is still an unreliable art. The typical approach of applying hard thresholds to quality metrics, and to do so 'consistently' across heterogeneous sets of raw data assumes a homogenous error distribution across samples and datasets – which is optimistic.

Tools for defining and assessing filter choice in a robust way exist for high coverage diploid calls, for example, in variant score recalibration methods from the Genome Analysis Toolkit (GATK). However, these are not usable for low-coverage samples, such as those where experimental design mandates low-pass sequencing, or in particular, for comparative ancient DNA studies where samples may be uniquely degraded, and where each sample has its own error profile.

We propose a novel adaptive framework for assessing the error associated with sequencing which operates on a per-sample basis, or at even finer resolution if multiple sequencing protocols have been used for a single sample. This now replaces our own in-house strategies for variant calling.

Dissecting the contributions of neural activation and strain trajectories to muscle force production

Carissa Mallonee, Monica Daley, Kiisa Nishikawa, Caitlin Bemis

We previously developed a novel ex vivo 'avatar' technique that enables dissection of the contributions of neural activation and muscle intrinsic properties in the control of animal locomotion. The approach combines in vivo measures of muscle strain, activation, and force during steady and perturbed locomotion with ex vivo work-loop experiments, in which in vivo strain trajectories and activation timing serve as inputs for mouse EDL muscles, with force and

work measured as outputs. We used in vivo data from guinea fowl (GF) walking on a treadmill with obstacles. Half of the GF underwent a self-reinnervation protocol in which the peripheral nerve branch to the lateral gastrocnemius (LG) muscle was transected and repaired. Motor output and steady gait kinematics recover by 6 weeks, but there is a chronic loss of the autogenic stretch reflex. Length trajectories, activation timing, force, and work output differ between intact and re-innervated LG muscles during perturbed locomotion. To dissect the contributions of strain and activation to force and work output, we used scaled data from the GF experiments to perform work loop experiments on mouse EDL muscles by mixing and matching intact vs. reinnervated strain and activation patterns. We hypothesized that the different strain patterns with the same activation would differ more in peak force and work per cycle than the same strain patterns with different activation. This result indicates that muscle intrinsic properties and activation interact to produce the differences in force production observed in vivo.

Extreme long-axis rotation: the functional consequences of fibular reduction in theropod dinosaurs

Armita Manafzadeh, Stephen Gatesy, Bhart-Anjan Bhullar

Reduction of the fibula – one of two bones comprising the tetrapod crus – has been thoroughly documented in theropod dinosaurs. In at least three distinct theropod lineages, the once robust and tubular fibula became increasingly gracile and splint-like, ultimately losing its distal articulation with the ankle. We propose the previously unexplored hypothesis that fibular reduction enabled extreme long-axis rotation (LAR) at the theropod knee joint. Although centuries of paleontologists and zoologists assumed that birds and other dinosaurs have hinge-like knees capable of only simple flexion-extension, 3-D studies of avian kinematics have demonstrated that the tibiotarsus LARs nearly 70 degrees on the femur during locomotion. This additional degree of freedom is essential to avian locomotor control, allowing maneuvering by a limb with an otherwise sagittally restricted hip and ankle. Understanding how extreme knee LAR arose is thus essential to more fully understanding the evolution of theropod locomotor kinematics, agility, and performance. Here we present the results of a marker-based X-ray Reconstruction of Moving Morphology analysis of the avian knee joint with attention to the role of the reduced fibula. After describing the functional complex that facilitates extreme knee LAR in birds, we trace its assembly throughout theropod evolution and discuss the implications of this novel mobility for the behavior of dinosaurs able to exploit it.

The Mitochondrial Constellation: Phylogenetic Relationships of Sea Stars based on mitogenomes

Harrison Mancke, Lauren Baena, Andrew Mahon, Kenneth Halanych

Members of Asteroidea (Echinodermata), starfish or sea stars, are ecologically important, diverse, and span from the intertidal to the abyssal zone (~6000m). Understanding the relationships among sea stars has received surprisingly little attention despite their diverse and interesting life histories. To address this shortcoming, this study aims to explore evolutionary relationships within Asteroidea by taking advantage of recent collections in the Antarctic. Here we sequence the mitochondrial genome of ten sea star species. They were included in maximum likelihood and Bayesian inference analyses of forty-one mitochondrial genomes, whose representation spans Valvatida, Spinulosida, Velatida, Forcipulatida, Brisingida, Notomyotida, and Paxillosida. Twenty of the mitochondrial genomes belong to Valvatida, an order that has traditionally been considered monophyletic, a finding not supported by molecular data. Preliminary results suggest the gene order for all sampled asteroids is identical with thirteen protein-coding genes, two rRNA genes, twenty-two tRNA genes and one origin of replication region. The A+T content of polar species was 64.58%; temperate was 61.68%; tropical was 62.47%; deep-water was 66.91%. We expect our data to recover Valvatida, as well as Paxillosida and Forcipulatida, as paraphyletic. This study will provide a robust analysis of the complicated relationships of Asteroidea, allowing a more informed understanding of sea star phylogeny and evolution, and how it could potentially be influenced by habitat ecotype.

Macro photogrammetry on live animals: Hummingbird bills as a case study

Lucas Mansfield, Joshua Medina, Kevin Epperly, Nora Lee, David Cuban, Rosalee Elting, Ana Melisa Fernandes, Felipe Garzón-Agudelo, Summer Delehanty, Kathryn Stanchak, Sharlene Santana, Duncan Irschick, Alejandro Rico-Guevara

3D measurements made with digital tools are increasingly useful for studying morphology, but methods that capture sub-millimetric detail are rarely portable, inexpensive, or usable on live animals. These issues are especially troublesome when studying bird bills, which are often small, complex, and delicate to handle. Using photogrammetry, we scanned Burke museum specimens of several hummingbird species modifying aspects such as light, number of cameras, and number of photographs, to determine the ideal conditions for generating bill models. We then applied the methodology developed in the museum to live hummingbirds in the US and Colombia. This system is capable of rendering high-resolution 3D models, field-amenable, allows color analyses, adaptable to subjects of a variety of sizes, and easy to update. In hummingbirds, fine-scale details like sharpness, curvature, and minute variations in bill-tip shape can have large behavioral implications and can tell us more about how bills are used for feeding, fighting, and preening. However, bill tips are delicate, and fine details are difficult to preserve and easily lost in museum specimens due to wear and tear. A complete picture of bill morphology requires individuals that cover a wider range of life history than those commonly available in a museum collection. The 3D imaging of these traits in the field represents a powerful new way to learn about bird bills and other fine-scale features in live animals.

Approaches for Minimally Invasive Long-Term Ecological Monitoring

Christine Mantegna, Steven Roberts, Camille Gaynus

Long-term ecological monitoring plays a pivotal role in advancing our understanding of ecological processes, supporting evidence-based conservation decisions, and promoting the long-term health and resilience of intertidal ecosystems. The San Juan Island archipelago is located in the Salish Sea and includes about 170 islands with only a handful of them inhabited by humans. Yellow Island is an uninhabited nature and marine preserve in the archipelago, and has been managed by The Nature Conservancy since 1980. While terrestrial monitoring on Yellow Island is robust, there has been limited attention to the intertidal. We processed water samples taken from Yellow Island using two DNA sequencing platforms to compare accuracy of taxonomic characterization to our physical survey methods. DNA sequencing provides effective support for our physical surveys and offers novel insight into taxa present. The Salish Sea is not immune to the human drivers of climate change and as such requires monitoring measures in the least invasive way possible. Using physical and molecular techniques we created an intertidal species inventory, baseline community composition and biodiversity analyses to complement the terrestrial dataset.

Phenological Species Monitoring at OWC NERR: Integrating Programmatic Enhancements

Jason Manzon, Emily Kuzmick

Understanding how sensitive and indicator species will react to future climate change scenarios can inform management decisions and contribute support for these species. Old Woman Creek National Estuarine Research Reserve (OWC NERR) is home to many sensitive species including bald eagles (*Haliaeetus leucocephalus*), cavity nesting avian species such as the eastern bluebird (*Sialia sialis*), lungless salamanders, and a variety of migratory avian species. While certain monitoring initiatives like the System-Wide Monitoring Program (SWMP) gather weather and water quality data from NERR sites, how native populations change over time is less understood. The Phenological Species Monitoring Program at OWC began in 2016 and bases data collection efforts in citizen science. Volunteer data collection yields sizable data seasonally, though these data can be highly variable and therefore questionable for analysis. Integration of programmatic enhancements can streamline data collection and analysis to yield more effective outputs. Addition of normalized

ambient data, revision of data collection methods, and development of a methodology for repeated, meaningful analysis will add to program reliability and volunteer activity. This long-term research project combines field research with data analytics to observe trends in species presence and abundance to answer phenological questions. We conclude that continued work is needed to optimize data collection and use, and we give recommendations regarding annual analyses that will best answer respective research questions.

Stress inhibition of fish growth: cortisol effects on insulin-like growth factor-1 (Igfl) pathways

Hayley Mapes, Janae Shew, Henry Marden, Meredith Journey, Brian Beckman, Sean Lema

Fish experiencing stressful conditions increase production of the glucocorticoid hormone cortisol, which alters energy intake, turnover, and allocation with the aim of recovering physiological homeostasis. When cortisol remains elevated, however, those stress-induced changes in energy allocation impair fish growth. While it is known that cortisol inhibits anabolic growth processes in part via changes in growth hormone (GH)/insulin-like growth factor-1 (Igfl) signaling, it is not understood in fish which components of GH/Igfl pathways are regulated by cortisol during the stress response. Here, we explored how cortisol influences the GH/Igfl system in blue rockfish (*Sebastes mystinus*) by treating fish with exogenous cortisol and examining the effects on Igfl pathways. Cortisol-treated fish showed an increase in plasma cortisol 24-hrs post-injection. Cortisol-treated fish experienced a decline in plasma Igfl concentrations despite no change in levels of igfl gene transcripts in the liver. Cortisol-treated fish also showed higher liver gene expression for Igf binding protein (Igfbp) 1a and 1b, two transport proteins that inhibit Igfl stimulation of somatic growth. Cortisol-treated fish also expressed reduced mRNA levels of igfbp3a, which promotes growth by facilitating Igfl transport. Cortisol-treated fish expressed reduced mRNA levels within muscle tissue of myoblast determination protein 2 (myoD2), a key gene promoting muscle growth. These findings indicate a role for cortisol-mediate modulation of Igfbp expression and myogenic factors as a mechanism underlying growth inhibition in rockfish experiencing stress.

Integrating GM and XROMM illuminates the role of the quadrate as a keystone of cranial kinesis

Miranda Margulis-Ohnuma, Armita Manafzadeh, Elizabeth Brainerd, Bhart-Anjan Bhullar

The quadrate has been referred to as a “keystone” of the avian skull, dynamically linking the braincase, jugal bar, and pterygoid-palatine complex to enable cranial kinesis, an avian innovation that allows the upper beak to move independently of the rest of the skull. The quadrate of modern birds is dramatically altered compared to that of early theropod dinosaurs, appearing substantially more compact and lacking a prominent pterygoid flange. However, the morphological transformation of this critical bone during panavian evolution has not been thoroughly studied. We integrated data from (1) a 3-D geometric morphometric (GM) analysis of fossil and extant reptilian quadrates and (2) a marker-based X-ray Reconstruction of Moving Morphology (XROMM) analysis of avian cranial kinesis during feeding. Our morphometric analysis revealed variation in the quadrate predating the evolution of Ornithurae, but we found that ornithuran quadrates occupy a distinct region of morphospace, having substantially reoriented articular surfaces from the ancestral condition to yield the modern configuration of articulations for the jugal bar and pterygoid. Viewing joint motions in our XROMM animations in consideration of these results, we conclude that the distinctly avian articular surface configuration is essential for the motion of the quadrate that drives the avian kinetic apparatus.

Mitochondrial function exhibits sex-specific aging among painted turtle (*Chrysemys picta*) populations

Jamie Marks, Alex Sills, Elizabeth Addis, Luke Hoekstra, Fredric Janzen, Beth Reinke, Anne Bronikowski

How and why males and females differ in their life-histories, including rates of aging, remains a persistent question in life-history evolution. Specifically, most

studies on sex-specific aging have focused on species with genotypic sex determination, in which sex-specific genome architecture and content may exist. In this study, we tested for sex-specific differences in a mechanism of aging in painted turtles - a species that has environmental sex determination and is therefore an excellent model for understanding sex-specific life-histories without the confound of differing genomes. We measured mitochondrial function as a proxy for age-related physiological deterioration in males and females from three populations to quantify both within and among population variation in the sex-by-age interaction. We measured basal, ATP-coupled, and maximal cellular oxygen consumption in white blood cells and tested the prediction that cellular respiration would decline with age faster in the faster-aging sex. We further tested whether the age-by-sex interaction was repeatable across three populations where our long-term data allowed us to characterize sex-specific demographic aging. We found significant differences between males and females in their age-related patterns of mitochondrial function, and variation among populations in these trends. These results will be discussed within the context of aging as this study is part of the larger goal of understanding sex-specific aging across the animal kingdom.

The life-history of maternal effects

Jamie Marks, Simon Lailvaux

Context-dependent allocation of resources drives trade-offs among fitness-related traits and other components of the integrated organismal phenotype. In addition, the amount and type of acquired resources can also affect the phenotypes of other organisms through indirect genetic effects, for example the maternal provisioning of offspring. Despite a large literature on maternal effects, the extent to which mothers might affect the phenotypes of their offspring, as well as the various mechanisms by which they do so, remain understudied, particularly with regard to many functional traits that affect key aspects of survival and reproduction. Our goals in this talk are to provide a better understanding of what maternal effects are; to review the various approaches to measuring and understanding maternal effects; and to provide a cohesive overview of the mechanisms facilitating such effects, including how maternal effects themselves might change over a mother's lifetime. We also critically consider the challenges involved in distinguishing between the adaptive/non-adaptive value of such effects, particularly for functional, whole-organism traits. Finally, we discuss the logistic and practical limits of quantifying these effects in many animal systems.

The evolution of chromosomal and regulatory architecture in deuterostomes

Ferdinand Marlétaz, Elise Parey, Laura Piovani

A survey of ethanol concentrations within floral nectars at a Mediterranean climate botanical garden

Aleksey Maro, Ammon Corl, Rauri Bowie, Jimmy McGuire, Robert Dudley

Floral nectar is a major part of many animal diets; small-bodied pollinators such as hummingbirds consume over 80% of their body mass in nectar daily. Saccharide-rich nectar becomes inoculated with a variety of microbes as nectaries are exposed to atmospheric particulates and are visited by various animals. We hypothesize that such microbes can fermentatively produce low concentrations of ethanol, and accordingly used an enzymatic assay to estimate ethanol concentrations of 221 samples of nectar from 42 species of flowering plants at the University of California Botanical Garden at Berkeley. Ethanol concentrations among all study species averaged 0.012% (mass/mass) with a maximum species average of 0.040%. Nectar samples collected on a hot day showed significantly higher ethanol concentrations (average of 0.035%; $n=18$) than samples collected from the same individual plants a week later when air temperatures were 15°C lower (0.013%; $n=11$; t -test, $p=0.001$). Dicot species (average of 0.015%; $n=26$) showed significantly higher concentrations than monocots (0.008%; $n=16$; t -test, $p=0.009$). Future studies are necessary to determine to what extent such ethanol concentrations are physiologically or ecologically relevant for pollinators, including intra- and

interspecific variations in ethanol metabolic rates. Despite the geographically constrained nature of this study, the results here imply that exposure to low levels of ethanol amongst nectarivores is likely to be widespread.

Effects of variable cold temperatures on metabolic rates and organ masses of house sparrows

Chelsi Marolf, David Swanson

Climate change continues to threaten species as global average temperatures rise and extreme weather events become more frequent. Small birds living year-round in temperate regions may be particularly susceptible; northern latitudes are experiencing greater rates of warming, and extreme cold weather events in a warmer climate might create physiology-environment mismatches. To address the question of whether variable cold temperatures impose an additional energetic burden on birds, we exposed house sparrows to three 6-week temperature treatments: warm (25°C), stable cold (3°C), and variable cold (mean of 3°C, range: -10° to 16°C). We measured basal and summit (maximum thermogenic) metabolic rates before and after the acclimation period. Following acclimation, we also measured masses of nutritive and exercise (pectoralis and supracoracoideus) organs and total body mass, to understand if body composition remodeling to cold is exacerbated by variable cold temperatures. We found no significant difference between the fluctuating and stable cold temperature groups for basal or summit metabolic rates, while the warm treatment group had significantly lower basal metabolic rates following treatment. We detected little evidence for muscle responses to cold, but digestive organs increased in both cold and variable cold groups, along with BMR. These results suggest that variable cold temperatures did not impose an additional energetic burden, but that cold temperatures, in general, generate phenotypic changes in small birds.

Female house cricket acoustic environment and mate choice decisions

William Marquart, Zack Majd, Kerianne Wilson

Female mate choice in house crickets is a complex process, affected by a variety of environmental factors including risk of predation, seasonal changes and perceived population density. For example, female field crickets raised under silent conditions display less discrimination in mate choice and no preference for particular song traits compared to females exposed to song during rearing. Mate choice decisions can affect female's reproductive allocation and lifetime fitness since, among other things, males of varying quality may transfer different concentrations of specific semen components during mating. Domesticated house crickets have been reared at exceptionally high population densities and exposure to different conspecific acoustic environments may reflect their unique recent evolutionary history. We analyzed the effect of adult acoustic environment on female house crickets mating behavior by housing females with or without exposure to male calling prior to an encounter with a male. We hypothesized that females reared in a silent environment would be more willing to mate, which would indicate that female crickets compensate for reduced mate availability by decreasing selectivity towards potential mates. In line with our hypothesis, preliminary results showed that females housed in silent conditions as adults mated males less frequently but spent more time in proximity to males during a mating trial.

The mechanisms of correlated evolution: Aposematism in *Phyllobates* poison-dart frogs

Roberto Márquez

A wide array of organisms use conspicuous signals to warn predators of secondary defenses in order to avoid predation, a strategy known as aposematism. In poison-dart frogs aposematism has evolved quickly and dynamically, with multiple independent origins of conspicuously colored and chemically defended lineages. Although the ecological and behavioral processes guiding the evolution of aposematism in poison frogs are relatively well known, the underlying cellular, genetic, and molecular mechanisms remain largely unknown. In this talk I will explore the possible molecular and evolutionary genetic and developmental mechanisms behind the correlated

evolution of aposematic coloration and toxicity in *Phyllobates* poison-dart frogs from population genetic, biogeographic and developmental perspectives, focusing on how these processes lead to convergent evolution of integrated multi-trait phenotypes.

Evaluating Sexual Dimorphism in Brain Morphology of *Lontra canadensis*

Kristin Marsh, Dominik Valdez, Leigha Lynch

By exploring sexual dimorphism in animals with relatively large brains, researchers can get a better understanding of what factors influence brain morphology. This is because many species exhibit variation in behavior, diet, and environment between sexes, all of which are selective pressures that may contribute to shape and size variation in brain morphology. Yet for many species, little is understood about how these factors influence brain development and evolution. To fill this gap, we are investigating endocast morphology of the North American river otter, *Lontra canadensis*, a species that exhibits ecological, dietary, and behavioral variation between sexes. Specifically, we investigated *Lontra canadensis* sexual dimorphism in endocast volume and shape. We found a significant difference between male and female *Lontra canadensis* endocast volume ($p=0.0027$), with males exhibiting relatively larger endocast volume to body size than females ($p=0.031$). There is also significant sexual dimorphism in endocast shape ($p=0.0085$, $F=4.935$) despite no allometric signal ($p=0.864$, $R^2=0.060$). We found that 49.41% of the variation, determined by principal component analysis, was due to sex with males having larger anterior sigmoid and pro-anterior ectosylvian gyri. We also found that males displayed wider lateral and preoreal gyri. These results suggest that the differences in size and shape of the endocasts are not because male *Lontra canadensis* simply have larger bodies. We can predict that males have larger and more complex endocasts because of their higher quality diets and increased environmental variation, which may act as the primary selective pressures on brain evolution, compared to females.

Movement Patterns of Upper Texas Coast Loggerhead Sea Turtles

Christopher Marshall, Madelyn Rupp

Identifying movement patterns, core and home ranges, and foraging habitats are necessary to the conservation and management of endangered species, such as sea turtles. Loggerhead sea turtles (*Caretta caretta*) are part of a rich Texas marine megafaunal community. However, little is known regarding movement patterns and foraging habits of adult loggerheads in the western Gulf of Mexico. Even fewer data are available for male loggerhead turtles. A recent (2022-2023) loggerhead stranding event provided an unusual opportunity to satellite tag male and female rehabilitated loggerheads. The resulting telemetry data was used to identify core and home ranges, habitat use, and foraging grounds. Movement data were fit with a state-space models (SSMs) to account for the location and observation error. Core and home ranges were calculated using the SSMs to calculate Kernel density estimates based on the 50% and 95% isopleths. Loggerheads released from Galveston, TX either moved (1) northeast, offshore of Louisiana, (2) southwest, offshore of Matagorda Bay, TX, or (3) up Galveston Bay, as far as Trinity Bay. All loggerheads repeatedly moved between their eastern- and western-most points back to Galveston and spent time in Galveston Bay. Loggerheads that spent time offshore also inhabited areas deemed to be critical Kemp's ridley sea turtle foraging habitat. Benthic habitats offshore of the upper Texas coast are important foraging grounds for both loggerheads and Kemp's ridley sea turtles.

Iron contamination mediates chytrid infection in Eastern newts: evidence from a field mesocosm study

Vanessa Marshall, Wesley Neely, Samantha Siomko, Shannon Buttimer, Jack Boyette, Carlos Becker, Ryan Earley

Disease ecology considers how hosts, environments, and pathogens interact to drive disease dynamics, but neglects organismal physiological responses to stressors. Environmental pollutants are physiological stressors associated with increased glucocorticoid (GC) hormone levels, and chronic exposure

can lead to reduced body condition, immunosuppression, and increased susceptibility to disease. In this study, we tested the hypothesis that environmental metal contaminants would mediate amphibian-Batrachochytrium dendrobatidis (Bd) host-pathogen dynamics. Eastern Newts (*Notophthalmus viridescens*) at Ruffner Mountain in Birmingham, Alabama were surveyed to quantify Bd prevalence and loads, iron metal contamination in two water sources, and GC stress hormone levels in a 7-week mesocosm experiment. Ruffner was an iron-ore mining site until the 1950s and the man-made iron-ore cleaning ponds ("Red Lakes") have visible metal contamination. Dead newts with high Bd loads were documented in the "Red Lakes" in 2010 and 2021. Ruffner also has ponds fed by underground wells ("Wetlands") that were not used in iron mining process. Because exposure to iron mining contamination may increase susceptibility to Bd by inducing an immunosuppressive physiological stress response, we predicted that 1) newts inhabiting "Red Lake" water would have higher Bd loads and that 2) "Wetland" newts transferred to "Red Lake" water would develop higher Bd loads. Preliminary results reveal that newt body condition, sex, and water source (contaminated or not) drive Bd loads, while water source alone impacts Bd prevalence.

Thermoregulatory behaviors and effects of water characteristics on dive duration in *Anolis aquaticus*

Alexandra Martin, Diane Cordero, Alva Mihalik, Lindsey Swierk

Semi-aquatic ectotherms that use water to escape predation must balance anti-predator behavior against the thermoregulatory and metabolic constraints of reduced water temperatures and water turbulence. The water anole, *Anolis aquaticus*, evades predators by diving underwater for long durations, facilitated by rebreathing an air bubble that forms above its nares. To understand the fitness tradeoffs and compensation tactics associated with aquatic anti-predator behavior, we performed two experiments. To assess how water characteristics, such as flow and dissolved oxygen (DO) affect dive duration, we submerged lizards in varying levels of water flow and DO. We found that with greater flow, lizards dove for significantly shorter durations, likely due to the energetic strain of remaining submerged, or due to loss of oxygen via disruption of the bubble. We also found a positive trend between DO and dive duration, which indicates oxygen from the surrounding water is likely diffusing into the bubble, as has been documented in some semi-aquatic invertebrates. We also assessed whether *A. aquaticus*, a thermoconformer, performs thermoregulatory behaviors after diving by cooling lizards and allowing them to behaviorally thermoregulate in a thermal gradient ranging from ~4°C to ~40°C. Preliminary results suggest that *A. aquaticus* does not exhibit thermoregulatory behaviors after cooling and instead may passively return to normal body temperature. Our research expands our understanding of how ectotherms balance predator risk and extreme physiological costs.

Course-based undergraduate research (CURE): the evolution of spectacular adaptations in fishes

Christopher Martin

CURE courses provide an opportunity to bridge innovative teaching with major research programs. Here I overview some recent undergraduate course research projects on scale-eating pupfish, aestivating lungfish, Cameroon crater lake cichlids, and four-eyed fish conducted in my IB177LF class: Ichthyology, an introduction to the scientific process through research on fishes. For example, students have investigated an adaptive radiation of Cyprinodon pupfishes to measure the relationship between feeding kinematics and performance during adaptation to a novel trophic niche, lepidophagy, in which a predator removes only the scales, mucus, and sometimes tissue from their prey using scraping and biting attacks. We used high-speed video to film scale-biting strikes on gelatin cubes by scale-eater, molluscivore, generalist, and hybrid pupfishes and subsequently measured the dimensions of each bite. We then trained the SLEAP machine-learning animal tracking model to measure kinematic landmarks and automatically scored landmarks from over two hundred strikes. Two distinct performance peaks strongly predicted gel-biting performance, corresponding to a significant nonlinear interaction between peak gape and peak jaw protrusion. A performance valley separating scale-eaters from other species may have contributed to their rapid evolution. In addition, student teams have investigated the antimicrobial properties

of the cocoon produced by aestivating lungfish, functional trait divergence in Barombi Mbo cichlids, hatchetfish flight mechanics, and the visual field of live four-eyed Anableps fish. By focusing on diverse study systems, students receive a broad perspective on how to brainstorm, investigate, and test their own independent hypotheses about adaptation in the field and lab.

Turtle tears? Characterizing the magneto-microbiome of the sea turtle lacrimal gland

Julianna Martin, Robert Fitak

The magnetic sense of sea turtles has been well documented, however the underlying mechanism for this sensory system remains enigmatic. The symbiotic magnetic sensing hypothesis recently proposed a possible origin for this sense, suggesting that an organism's magnetic sense may be conferred by relationships with a group of bacteria known as magnetotactic bacteria (MTB). These bacteria are characterized by their ability to align with magnetic fields due to magnetic particles stored inside organelles called magnetosomes. MTB have been documented in soils and sediments, but their presence in the microbiomes of organisms has yet to be systematically described. To study the possible link between magnetotactic bacteria and magnetoreception in sea turtles, we must first describe the community of MTB living with these organisms, hereafter called the magneto-microbiome. The lacrimal gland has been identified as a location of interest due to its proximity to nerves implicated in magnetoreception. We are using 16S metabarcoding of green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) sea turtle lacrimal gland secretions (ie. tears) to characterize the community structure of the magneto-microbiome associated with the eye. Preliminary data have indicated that MTB populate the lacrimal gland of both green and loggerhead sea turtles, however higher-resolution sequencing analysis is necessary to adequately describe the community structure. Results from this study may provide insight into possible mechanisms underlying magnetoreception.

Major histocompatibility complex immune gene evolution in four sea turtle species

Katherine Martin, Jamie Adkins, Vipheaviny Chea, Anna Forsman, Erin Seney, Lisa Komoroske, Kate Mansfield, Anna Savage

Major histocompatibility complex (MHC) immune genes comprise one of the most diverse vertebrate gene families. Several non-mutually exclusive evolutionary mechanisms, including pathogen-mediated balancing selection, favor high allele number and sequence diversity within individuals and populations. Similar and identical MHC alleles are shared by species millions of years diverged, in a phenomenon known as trans-species polymorphism (TSP). TSP may arise from: 1) maintenance of ancestral polymorphism in descendant species via balancing selection, and 2) convergent evolution of identical alleles in different species that confer similar pathogen resistance. Immune genes like MHC are ideal candidates for understanding selective processes shaping genetic variation across millions of years of diversification. Sea turtles are likewise ideal for studying immune system evolution given their long lifespans, conservation threats, and species-level susceptibility differences to the viral-associated tumor disease fibropapillomatosis. However, sea turtle immunogenetic variation is minimally explored. We sequenced MHC class I and class II genes from 88 loggerhead (*Caretta caretta*), 268 green (*Chelonia mydas*), 62 leatherback (*Dermochelys coriacea*), and 38 Kemp's ridley (*Lepidochelys kempii*) sea turtles. We reconstructed phylogenetic relationships among MHC alleles and recovered extensive variation, and evidence of TSP and positive selection shaping allele evolution. We assess whether identical alleles arose from common ancestry or convergent evolution. Our study is the first comparative MHC analysis across turtle families, providing broader insight into the evolutionary forces shaping immunity in reptiles.

Variation in Toll-like receptor expression in native and non-native house sparrow populations

Lynn Martin, Elizabeth Sheldon, Kailey McCain, Massamba Thiam, Jorgen Soraker, Henrik Jensen, Roi Dor, Kate Buchanan, Jim Briskie, Blanca Jimeno, Kim Mathot, Thinh Vu, Ho Thu Phuong, Cédric Zimmer, Aaron Schrey

House sparrows (*Passer domesticus*) are among the world's most broadly distributed vertebrates, and successful spread of individual sparrows into new areas does not appear to be random. For instance, many behavioral and physiological patterns have been detected between native and non-native populations, including particular differences in the regulation of immune responses. An element of the immune response that seems especially important to colonization is the regulation of the Toll-like receptors (TLRs). TLRs provide first lines of defense against various pathogens, and previous work in the very recent (<70 years old) house sparrow range expansion across Kenya revealed that TLR-2 and TLR-4 expression increased towards the western vanguard of that invasion. This pattern suggests that colonizers might mitigate the risk of succumbing to comparatively novel infections in novel places by conducting more pathogen surveillance. To determine whether TLR expression was generally relevant in house sparrow invasions, we compared expression of TLR-1, 2, 3, 4, 7, and 15 in liver, spleen and gut samples from native (i.e., Spain, Israel, Norway, Vietnam) and non-native (i.e., Australia, New Zealand, Alberta, and Senegal) birds. We expected higher TLR expression in all tissues in the latter than former populations. Our broad geographic sampling also allowed us to disentangle invasion history from climatic heterogeneity and evolutionary relatedness among populations, other forces apt to affect TLR expression. Moreover, by measuring TLR expression in blood before and after standardized, simulated infection (i.e., lipopolysaccharide exposure), we could discern whether non-native birds exhibited greater immune regulatory flexibility than native ones.

The 'Competitive exclusion – tolerance rule' explains species turnover along environmental gradients

Paul Robert Martin, Cameron Ghalambor

Closely-related, ecologically-similar species often segregate their distributions along environmental gradients of time, space, and resources, but previous research suggests diverse underlying causes. We reviewed reciprocal removal studies in nature that experimentally test the role of interactions among species in determining their turnover along environmental gradients. We found consistent evidence for asymmetric exclusion coupled with differences in environmental tolerance causing the segregation of species pairs, where a dominant species excludes a subordinate from benign regions of the gradient, but is unable to tolerate challenging regions to which the subordinate species is adapted. Subordinate species were consistently smaller and performed better in regions of the gradient typically occupied by the dominant species compared to their native distribution. These results extend previous ideas contrasting competitive ability with adaptation to abiotic stress to include a broader diversity of species interactions (intraguild predation, reproductive interference) and environmental gradients, including gradients of biotic challenge (e.g., food availability, predation). Collectively, these findings suggest that adaptation to environmental challenge compromises performance in antagonistic interactions with ecologically-similar species. The consistency of this pattern across diverse organisms (plants, invertebrates, vertebrates), environments (marine, freshwater, terrestrial), and locations (tropics, temperate, subarctic) suggests generalizable processes structuring the segregation of ecologically-similar species along disparate environmental gradients, a phenomenon that we propose should be named the Competitive exclusion – tolerance rule.

Is anyone even dioecious, anymore?: The preponderance of leaky males in Australian *Solanum*

Chris Martine, Melody Sain

Dioecy is a plant sexual system in which individuals are either "male" (sperm/pollen-producing) or "female" (egg/seed-producing), but never both. More than a dozen dioecious *Solanum* species have been described from northern

Australia, but what has been historically described as dioecy appears to require a number of caveats – among them a proclivity for "male" plants to "leak." In other words, male flowers occasionally produce functionally female organs that render these flowers cosexual. Recent observations from both in situ and ex situ plant populations suggest that true dioecy may not exist at all in this clade – and point further to sexual fluidity as the normative condition in this and many other plant groups.

Geometric kinematics: analyzing motions as shapes

Christopher Martinez

The ability to move is a defining feature of animals, yet our understanding of motion diversity and how motions evolve remains limited. I introduce geometric kinematics as a method for studying motions as trajectories of shape change. By leveraging analytical tools from landmark-based morphometrics, this approach allows for a wide range of applications for characterizing and statistically comparing kinematic variation. I show how these methods may be used for intraspecific comparisons of whole-motion variance during suction versus biting feeding motions in a freshwater fish. Next, I describe a framework for evaluating the integration of motions across disparate functional units. Finally, I present an example illustrating how these methods lend themselves to direct comparisons of morphological and kinematic diversity at macroevolutionary scales. These examples highlight the ability of geometric kinematics to address questions at the single species level as well as broad comparative analyses. Future expansion of this toolkit will allow for new ways of studying organismal motion diversity and evolution.

Pilot Electronic-Probe Diagnostic Nucleic-Acid Analysis (EDNA) For Early Breast Cancer Detection

Gerardo Martinez, Francisco Ochoa-Corona

Breast Cancer (BC) is a rampant problem in The United States and worldwide. According to the American Cancer Society, an estimated 43,700 deaths will be in the United States alone in 2023. Aiming to minimize BC death rates, we are creating a BC diagnostic platform based on a pathogen detection method known as E-Probe Diagnostic Nucleic Acid Analysis (EDNA). EDNA uses High Throughput Sequencing (HTS) metadata screened using the Microbial Finder (MiFi) bioinformatics platform to detect pathogens in an HTS raw sample. Specific cancer diagnostic assays are available, but most cannot test multiple cancer-related mutations at once. EDNA allows unique pathogen-specific sequences (or e-probes) design to improve the bioinformatic efficiency of searches of sequence data. EDNA technology has already been successfully applied to detect viral RNA/DNA, bacteria, and fungi associated with several diverse plant pathogens. We are now translating this technology for use in cancer for early detection of BC. We present a prototype for querying BRCA/II gene mutations in HTS metafiles in silico. BC mutation sequences were retrieved from various databases to test software against cancer-simulated populations of mutations created using Metasim. This seed data will serve as a platform to expand the project, adding other BC-related genes and further human trials using raw patient HTS data.

Testing the threshold trait model to predict plasticity of flight dimorphism in *Gryllus* crickets

Laurenco Martins, Jacqueline Lebenzon, Lisa Treidel, Colin Meiklejohn, Kristi Montooth, Caroline Williams

The threshold trait model explains how dichotomous traits (such as life cycle or trophic polymorphisms) are produced by a continuously distributed quantitative marker (known as the liability) combined with a threshold of expression. An individual's liability depends jointly on their genetic predisposition (frequently with a complex multigenic architecture), and on their environment through phenotypic plasticity. We aimed to test the ability of the threshold trait model to predict the plasticity of a threshold trait, wing dimorphism in insects.

The variable field cricket, *Gryllus lineaticeps*, consists of flight-capable long wing (LW) or flightless short wing (SW) morphs. In other *Gryllus* crickets, wing length has a polygenic basis and is environmentally sensitive. We reared split broods under LW-inducing (warm temperature, long-photoperiod) or

SW-inducing (cool, short photoperiod) conditions, and measured both morph frequency and an endocrine proxy - juvenile hormone esterase activity - that is hypothesized to underlie physiological liability. We first established that the critical period for morph determination is the last juvenile stage, and that esterase titers in that stage predict adult morph. We then tested the hypothesis that plasticity will shift the underlying physiological liability consistently across families, but that shift will only result in a corresponding shift in morph frequency for families whose liabilities lie close to the threshold of expression. These findings further our understanding of environmental impacts on dichotomous traits like insect wing dimorphism.

Go with the flow: Developing a scaffold to examine the energy flow within click beetles

Teagan Mathur, Liyuan Zhang, Josh Gibson, Ophelia Bolmin, Jake Socha, Marianne Alleyne, Sara Wilmsen, Kamel Fezzaa, Aimy Wissa

Click beetles (Elateridae) are one of many small organisms that use a system of springs and latches to achieve incredible accelerations. This strategy is referred to as latch-mediated, spring-actuated (LaMSA) movement and consists of latching, loading, and release phases. We hypothesized that during the loading phase, the large M4 muscle compresses portions of the beetle and loads the mesonotum, a saddle-shaped sclerite connecting the body segments on the dorsal side. Previously, the release-phase dynamics were identified as being governed by nonlinear damping and elastic forces. In this study, we examined three components of the energetics of the loading and release phases of the click beetle *Parallellostethus attenuatus* (length, ~18 mm), combining data from high-speed x-ray videos with micro-CT scans of the M4 muscle and mesonotum. First, we determined the M4 muscle's energy input using 3D morphological data of the mesonotum. Then, we calculated the mesonotum's stored potential energy using FEA. Finally, we compared the energy output from similar unconstrained jumps from this species to the energy input to estimate the energy dissipated. The results of this study support the hypothesis that the mesonotum is the primary spring structure. The methods developed here also create a framework relating geometry and kinematics to the resulting dynamics and can be extended to other biological and bio-inspired LaMSA systems.

Tradeoffs of hosting heat tolerant symbionts: coral growth modulated by light and temperature

Shayle Matsuda, Mariah Opalek, Raphael Ritson-Williams, Ross Cunning

The coral-algal symbiosis plays a crucial role in the health and survival of corals and coral reef ecosystems. Algal symbionts (Symbiodiniaceae) transport photosynthates to the host meeting the majority of energetic demands and support calcification. Some symbionts, such as *Durussdinium* spp., can impart greater heat tolerance to the host. However, there may be physiological trade-offs associated with harboring heat-tolerant symbionts during periods where water temperatures are within their normal range. *Montipora capitata* corals associate with both heat sensitive symbionts (*Cladocopium* C31) and tolerant symbionts (*Durussdinium* glynnii). Here, we investigate whether a growth trade-off exists when hosting heat-tolerant *D. glynnii*, and whether trade-offs are influenced by light availability. Corals dominated by C31 exhibited a 77% higher growth rate compared to those dominated by *D. glynnii*, but this disparity was observed only under higher light levels, and only during the period of cooler temperature (median 23.4°C); no growth differentiation was evident at any light intensity during warmer periods (median 25.5°C). Interestingly, *M. capitata* dominated by *D. glynnii* are more common in shallower regions of the reef. This may mean that despite a growth advantage that C31 has during the cooler months of the year, heat tolerance may play a more important role in survivorship and distribution under ocean warming, and should be considered when weighing conservation interventions selecting for specific traits.

Cross-ocean coral transplantation to restore resilience of Caribbean reefs

Mikhail Matz

Caribbean coral reefs are declining precipitously, while Indo-Pacific reefs largely manage to maintain their ecological function and diversity. The main reason for this difference is the profoundly different capacity for reef recovery post-disturbance. In the Indo-Pacific there are multiple coral species (“super-recruiters”) that recruit extremely efficiently to devastated reef areas and are able to promote full ecological recovery in less than a decade even after complete reef destruction. In contrast, the Caribbean does not have corals with comparable life history traits; instead, its major reef-builder species are experiencing multi-decadal region-wide recruitment failure. The state of Caribbean reefs is already so poor because of this that I believe it is time to consider the “11th hour” solution: introduction of the Indo-Pacific “super-recruiters” into the Caribbean to restore the reef recovery capacity. I discuss potential challenges and risks of such a radical intervention and outline several lines of research that I believe must be permitted and funded to prepare for its implementation in the nearest future, lest we lose the whole Caribbean reef ecosystem.

Flight and Climbing Performance Maps to Mito-Nuclear Interactions in *Drosophila*

Olivia Maule, David Rand

Much like any eukaryotic organism, *Drosophila melanogaster* has a two-part genome consisting of nuclear chromosomes and mtDNAs inside the mitochondria cytoplasm. These genomic components must communicate effectively to ensure proper metabolic measures of performance such as climbing speed, flight efficiency, and reproductive output. To understand interplays between the mitochondria and nucleus, genetically different *Drosophila* populations have been bred upon two distinct environments: a control diet, and an experimental diet, which contains Rotenone- an insecticide that modifies *Drosophila* functions by inhibiting electron transport in the mitochondria, ultimately limiting oxygen availability and therefore reducing overall performance. By cross-breeding two nuclear backgrounds with eight mitochondrial genotypes, the extent to which mito-nuclear interactions affect *Drosophila* performance have been analyzed using flight, climbing and protein assays. These quantitative results have identified specific mitonuclear genetic interactions that increase or mask sensitivity to rotenone. Notably, Rotenone has a clear genotype-specific impact on fly climbing but has a limited impact on flight performance. The extent to which mito-nuclear backgrounds can be considered efficient is then measured against variations in sex and environmental conditions, the latter determined by diet. Although there is evidence of Rotenone diets limiting mitochondrial efficiency, circumstances where Rotenone-fed *Drosophila* outperform control-diet *Drosophila* counterparts hint at the possibility of nuclear genotypes being better predictors of performance. Ultimately, this could assist in the creation of insecticides that better target inhibition of nuclear, rather than mitochondrial, function. This observation is integral in evaluating the extent to which mito-nuclear interactions influence adaptations to alternative environments or influence susceptibility to mitochondrial diseases.

The Influence of Temperature and Humidity on Metabolic Rate Across Diverse Insect Species

Alexander Mauro, Eric Riddell, Cameron Ghalambor

The earth's climate is rapidly changing and driving shifts in the distributions and abundance of organisms. A changing climate has been invoked to explain global declines in insect populations that have important roles in ecosystem function. However, these models have largely used temperature as the main climatic variable, even though other climatic variables are changing rapidly. Changing humidity levels and increases in drought pose a severe threat to insects because of their vulnerability to desiccation due to their small size and limited capacity for storing water. Insects lose water with increasing temperature through cuticle water loss and elevated respiration rates, but how temperature and humidity interact to drive water loss and shape the thermal performance curve is largely unknown. Here, we investigated this by

using flow-through respirometry to measure resting metabolic rate and water loss across a wide range of temperatures and multiple humidities in a diverse group of insect species in southern France. We found that temperature, humidity, and water loss can all influence the thermal performance curve. We further explored how the ecology of different insect groups can make them vulnerable to changes in temperature and humidity. Ultimately, our results emphasize that incorporating multiple climatic variables into performance curves will be important when investigating the mechanistic link between climate change and population decline in insects.

A hull reconstruction-reprojection method for pose estimation of free-flying fruit flies

Roni Maya, Noam Lerner, Omri Ben-Dov, Arion Pons, Tsevi Beatus

Understanding the mechanisms of insect flight requires high quality data of free-flight kinematics, e.g for comparative studies or genetic screens or for analyzing flight maneuvers. While recent improvements in high-speed videography allow us to acquire large amounts of free-flight data, a significant bottleneck is automatically extracting accurate body and wing kinematics. Here, we present an experimental system of four fast cameras and a hull reconstruction-reprojection algorithm for measuring the flight kinematics of fruit flies. Camera configuration was analyzed to minimize wing occlusion and the experimental system can automatically record hundreds of flight events per day. Our algorithm resolves a significant portion of the occlusions in this system using a reconstruction-reprojection scheme that integrates information from all camera views, allowing us to detect even partially occluded voxels. Wing and body kinematics, including wing deformation, are then extracted from the hulls of the wing boundaries and body. The method was validated with respect to a synthetic fly model and demonstrated on a dataset of thousands of wingbeats. Analyzing this dataset, we find, for example, that the fly's volitional turns are coordinated, i.e., body rotation is typically aligned with a distinct axis that combines body yaw and roll – an axis we term 'yoll'. This model-free method is automatic, accurate and open-source, and can be readily adjusted for different camera configurations or insect species.

The activation dependence of optimal length: effect of added compliance and force depression

Dean Mayfield, Natalie Holt

Tetanic force varies with muscle length according to differences in myofilament overlap and cross-bridge formation. Less understood is why the optimal length for force generation (LO) increases as activation becomes submaximal. Although the activation dependence of LO is consistent with the length dependence of Ca²⁺ sensitivity, this mechanism alone is insufficient. A direct comparison of the activation dependence of LO between fibre bundles and intact muscles implicated in-series compliance. Active fibre shortening induces force depression (FD) and increases in proportion to activation and force. Because FD would disproportionately affect high-force contractions, and FD normalised to muscle work increases at longer lengths, LO may decrease as force and shortening increase. This prediction was tested by determining LO and maximum tetanic force (P₀) for tetanic contractions with and without added compliance in single bullfrog semitendinosus muscles. Springs attached to muscles gave added fixed-end compliances between 13 and 51%. We found strong, negative correlations between added compliance and both LO and P₀ ($P < 0.001$). The reduction in LO and depression of force were exaggerated by stretch-induced muscle damage resulting from spring recoil. The reduction in LO owing to series compliance is unlikely to exceed 5-10%, while FD could be double this amount. As the activation-related shift of LO exceeds 30% for the bullfrog plantaris muscle, far-outweighing the contributions of compliance and Ca²⁺ sensitivity, further investigation is warranted.

Adrenergic signaling in *Ciona robusta* is required for maintenance of a cardiac stem cell population

Keren Maze, Bradley Davidson, Hannah Gruner

Innervation is important for heart function. Neural inputs appear to also impact heart growth but the exact mechanism is not yet well characterized. We aim to

address this question by studying the impact of neuronal signals on heart stem cell populations in the invertebrate chordate *Ciona robusta*. Our previous work indicates that stem cells form a single file line in the developing *Ciona* heart, referred to as the 'undifferentiated line.' We have also found that knockout of the neuropeptide tachykinin in extrinsic heart neurons severely disrupts heart growth. According to our current model, secretion of tachykinin from extrinsic neurons stimulates intrinsic cardiac neurons which then produce a signal that promotes proliferation of stem cells within the 'undifferentiated line.' We have not yet identified this intrinsic signal, but single-celled RNA sequencing data indicates that adrenergic signaling from the intrinsic neurons promotes 'undifferentiated line' proliferation. Here we show that inhibition of adrenergic signaling with doxazosin mesylate (a competitive antagonist of alpha₁-adrenergic receptors) results in a reduction of 'undifferentiated line' cells. However, the doxazosin treatment showed only an average of ~1 less 'undifferentiated line' cell, and our prior quantification of 'undifferentiated line' growth indicates 3 cells were added during the treatment period. Thus, our data suggests that adrenergic signaling is not the only factor impacting 'undifferentiated line' growth. These results serve as a starting point to discover and characterize how neuronal signaling coordinates heart growth.

Communicating science via public art in Philadelphia

Sarah McAnulty

Skype a Scientist is an informal science education nonprofit that is lowering barriers for people to connect with science. The need for science literacy has always been important, but in light of a changing climate, it is more critical now than ever. Many people are turning toward pseudoscience and conspiracy theory in the face of scary realities. Our organization promotes engaging directly with accurate scientific information at the source, by offering friendly, accessible connections with science.

Science communication exists within an ecosystem of messaging, and each approach to relaying critical science information will engage different sections of the broader population. Our recent methods in communicating science break through the siloed media structures by putting engaging science content directly in the paths of people's every day lives via murals, street art, and other unconventional approaches. This poster will cover three vignettes 1) Our Aquatic Neighbors: two large murals engaging Philadelphians on the biodiversity of the Delaware River ecosystem 2) The Squid Facts Project: a whimsical street art campaign sharing information on the biology of squid 3) Learn More About the Shore: a project that put information about Jersey Shore wildlife on bar coasters in Philadelphia.

This poster will share experiences in executing these projects, including finding funding, developing projects that connect with specific communities, and community engagement in design and implementation of the projects.

The Effects of Nonylphenol on Male Crayfish Aggression in Acute and Chronic Exposures

Jonathan McCabe, Daniel Bergman

Nonylphenol is used in a variety of industrial, domestic, and agricultural products. It is classified as an inactive ingredient that has little monitoring in the U.S. with a high possibility of exposure occurring via its disposal into the water supply. It is an endocrine disruptor by mimicking estrogen, influencing the behavior of organisms like crayfish. Crayfish utilize aggressive behaviors to find food, mates, and establish social hierarchies. We examine the effects nonylphenol has on aggression after acute and chronic exposures. We hypothesize a decrease in aggressive behaviors as the concentration of nonylphenol increases. Crayfish were obtained from the Grand River watershed. Crayfish were filtered by carapace length from 2.6 cm – 3 cm for carapace length. The crayfish were stored in individual 1L tanks and were exposed to nonylphenol on week 1 of the 4-week trial. Agonistic behaviors were recorded and scored. Analysis: A T-test and a Tukey multiple comparisons test were used to calculate statistical significance. For the behavioral assay there was a general decrease in aggressive behavior with an increase in both non-aggressive and submissive behaviors. In some individuals there was a decrease total time of aggressive behavior by over 50% from the pretreatment to week 4. The

results indicate a decrease in aggressive behavior with the increased doses of nonylphenol. As exposure duration increased there was a decrease in overall aggression.

Immune modulation in native and non-native house sparrows

Kailey McCain, Elizabeth Sheldon, Roi Dor, Kim Mathot, Blanca Jimeno, Henrik Jensen, Kate Buchanan, Jim Briskie, Aaron Schrey, Massamba Thiam, Jorgen Soraker, Thinh Vu, Ho Thu Phuong, Gabriela Mansilla, Lynn Martin

Immune modulation is crucial for invasive species' success and house sparrows (*Passer domesticus*) exemplify this. Colonization likely relies on inflammation control, with Toll-like receptors (TLRs) playing a key role in pathogen recognition. We found elevated TLR expression (TLR2 and TLR4) in invading sparrows; however excessive expression of TLRs may lead to an overly robust inflammatory response. We hypothesize that non-native house sparrows will exhibit an elevated baseline TLR expression, while concurrently relying on the co-expression of anti-inflammatory cytokines (IL-10 and PPAR δ) to attenuate downstream inflammatory pathways and non-native house sparrows will hasten the resolution phase of their inflammatory response. To examine the differences in immune modulation, we captured house sparrows and measured TLR2, TLR4, IFN γ , IL-10, and PPAR δ gene expression in the blood at three-time points (repeated measurements): pre-LPS injection, 8 hours post-injection, and 48 hours post-injection from native (Spain, Israel, Norway, Vietnam) and non-native (Australia, New Zealand, Alberta, and Senegal) sparrow populations. Our study revealed significant TLR expression differences between native and non-native populations, with distinct rate-of-change patterns between 8 and 48 hours. Notably, non-native sparrows showed a shallower TLR4-IL-10 relationship, indicating higher IL-10 levels relative to TLR4, except under extreme conditions.

CUREs at scale: offering a fly genetic research experience to high enrollment labs

Shelly McCain, Jimmy deMayo, Greg Ragland

Barriers from lack of time to knowledge of the process often limit undergraduate access to authentic research experiences for first-generation or students from underrepresented groups. We designed a Course-based Undergraduate Research Experience (CURE) for a biology majors introductory lab to provide an early and equitable research experience to hundreds of students per semester rolled into required coursework. Our CURE provides opportunities to design and test hypotheses bounded by the guidelines of genetic crosses among *Drosophila melanogaster* transgenic lines to test the effects of RNAi-mediated, tissue-specific knockout on a phenotype of interest. The major learning objectives revolve around the process of scientific inquiry, with content-based objectives in basic genetics, transgenics, transcriptional regulation, ecophysiology, and principles of statistical estimation. This CURE takes place in the second course in the freshman biology lab series. Our CURE was designed following the general principles of CUREs, where students use scientific practices for inquiry, perform collaborative work and learning, and use replicated trials to determine the unknown outcome of an experiment. In this presentation, we discuss solutions to logistical challenges involved in coordinating the experiments and teaching assistant training at scale, and opportunities to scaffolded curricula that build on these early experiences. We also share some encouraging, but sometimes perplexing results of student perception post-surveys about how students learn to do science and to identify as scientists.

Reconstructing the Origin of Reproductive Function for the Flower Development Gene LEAFY

Hannah McConnell, Jancee Lanclos, Nicholas Gjording, Genevieve Stockman, Julin Maloof, Andrew Plackett, Verónica Di-Stilio

The transition from vegetative growth to flowering in the diploid shoot (sporophyte) of angiosperms is controlled in part by LEAFY (LFY), a flower meristem identity gene. Less is known about whether LFY plays a role in the angiosperm

haploid (gametophyte) phase, and what that role might be. LFY orthologs also exist in non-flowering plants, and in mosses they control the first division of the zygote. Recent evidence indicates that LFY has a vegetative role in fern meristems, but it is unclear where its reproductive function arose. Here, we continue to bridge the gap in functional investigations into this key floral regulator between mosses and angiosperms to reconstruct the evolution of LFY's reproductive function across land plants. We ask whether LFY was co-opted into its specialized flowering function from a previously-identified generalized role in regulating meristems by characterizing its two paralogs (CrLFY1/2) in the model fern *Ceratopteris richardii* through constitutive overexpression.

Transgenic ferns experienced delayed gametophyte fertilization, suggesting a novel role in gametophyte reproduction, and produced overgrown lateral meristems and decreased leaf compounding in sporophytes, consistent with prior findings. Overall, our results suggest that LFY's reproductive role in angiosperm sporophytes may have been ancestrally co-opted from the gametophyte, and supports previous evidence that fern LFY orthologs are involved in cell division in both phases of the fern life cycle.

Evolution of phototransduction genes and implications for light sensing in the eyeless Anthozoa

Kyle McCulloch, Hansen Stacey, Meghan Payne, Emma Lilly

Opsin-based light sensing systems are extremely diverse, yet evidence suggests these are all related and evolved early in the common ancestor of animals. Understanding of visual system evolution is obscured by limited sampling, both in taxonomic breadth and in classes and contexts of opsins. Anthozoa, despite having no eyes, have three classes of opsins and a variety of light-mediated behaviors. Anthozoa are a major lineage in Cnidaria, sister to bilaterally symmetric animals, and are sister to jellyfish (Medusozoa) which have evolved eyes multiple times. Anthozoans therefore are a phylogenetically important comparison to better understand visual system evolution but have been often overlooked in this context. The starlet sea anemone, *Nematostella vectensis* is a model anthozoan amenable to functional genetics and has 29 opsins expressed throughout tissue types and developmental stages. This is optimal for studying evolution of light-sensing traits, but first we must have a baseline characterization of the gene candidates and behaviors for further functional work. Here we investigated evolutionary patterns of genes in *N. vectensis* classically associated with phototransduction. We also detail wavelength-specific photobehaviors and link them to opsins. After establishing wildtype genes and behaviors, ongoing work is determining the functional genetic basis for these traits. By characterizing the *N. vectensis* visual system at multiple biological levels, we can compare this to better known visual systems and better understand their evolution.

Biomechanics of rapid aerial righting in waxy, wingless planthopper nymphs

Christina McDonald, Saad Bhamla

Planthoppers, recognized for their rapid jumping abilities, consist of several taxa that produce intricate waxy adornments as nymphs. The wax, which may cover the body or extend out as filamentous tails, is speculated to serve ecological roles such as microclimate regulation, predator deterrence, and camouflage; however, its precise functions remain largely unconfirmed. This research aims to determine the biomechanical role of wax during jump-propelled aerial locomotion in wingless planthopper nymphs.

Using high-speed videography, we captured the movements of nymphs during take-off, while airborne, and during landing, both with and without their waxy adornments. Our results reveal striking contrasts in aerial performance: nymphs with wax removed experience uncontrolled tumbling, whereas those with wax intact initially rotate, counterrotate, then stabilize their aerial trajectory. This work suggests a previously unrecognized biomechanical function for insect wax structures and highlights the intricate interplay between insect morphology and biomechanics. The ability of these wingless insects to control their aerial trajectory and stabilization raises questions about the evolutionary pressures and precursors that may have preceded winged flight in insects.

Investigating the spectral responses of insect ocelli

Marisa McDonald, Daniel Chappell, Martin (Ric) Wehling

In insects, the majority of visual tasks are completed by the two main compound eyes. However, many groups also utilize a secondary visual system, the ocelli. The ocellar system is generally composed of two or three single lensed eye spots that reside on the head between the compound eyes, typically forming a triangle composed of two dorsal and one ventral receptor. While early studies thought that ocelli were basic light sensors, with no focusing power, spatial detection, or specializations, we now know this is not the case. Ocelli are hypothesized to be used for a variety of visual tasks, the best described being flight stabilization and sky compass orientation. Because of the sophisticated compound eyes of insect systems, the ocelli are seemingly redundant; however, they may be used for quick decisions and rapid processing in a matched filter system. It is likely that, much like the compound eyes, ocelli will be varied and adapted for specific functional requirements of the given insect's environment. In this study, we used electroretinogram responses to examine spectral sensitivity of ocelli across a variety of different insect species. Typically, ocelli are described to have two spectral receptors: a blue/green, and a UV. We aim to determine how sensitivity is shifting between species with different ecological niches.

Design of an insect scale robophysical model of the cockroach antenna with integrated sensing

Parker McDonnell, Lingsheng Meng, Kaushik Jayaram, Jean-Michel Mongeau

Insect antennae are distributed biological sensors that provide rich spatial and temporal multimodal information about the surrounding environment, enabling informed decision making even in poorly lit conditions. While high-fidelity tactile perception is common in insects, there is no engineering analogue for insect-scale robots due in part to their low weight and power budgets, placing strict limits on their capacity for autonomous navigation. The American cockroach (*Periplaneta americana*) antenna-with its approximately 140 strain sensing segments making up the passive flagellum-is one such example of a highly integrated and distributed mechanosensory system enabling tactile navigation with low overhead in weight and energy consumption. Inspired by this highly capable biological system, we have designed a near scale (40x4mm) modular eight element robophysical antenna prototype with integrated sliding capacitive sensing and readout electronics that are capable of measuring deflection (~1µm resolution) along the its length with high accuracy and speed (~1 KHz, 2 mrad). The antenna is constructed using the stack laminate approach, which has been successfully applied to actuators, sensors, and hinges in a variety of millimeter scale systems. This model will ideally enable biologists to validate mechanical principles governing tactile sensing and roboticists to achieve autonomous touch-based navigation at the insect scale.

Sustained, Steady, or Precipitous? Testing how sprint performance decreases with fatigue in a lizard

Eric McElroy, Christa Joby

Fatigue occurs when animals run for extended periods and exhibit a reduction in locomotor performance. This study examines changes in locomotor performance as a small lizard species is fatigued due to repeated sprinting locomotion. Texas horned lizards, *Phrynosoma cornutum*, were sprinted down a racetrack repeatedly in rapid succession to elicit a degradation in sprinting performance. The position of the mid-pelvis marked was digitized and used to quantify sprint speed and acceleration for each successive run. These data were used to examine how performance degrades, testing the three alternative possibilities of: 1) precipitous performance degradation after the first rapid sprint, 2) steady performance degradation over successive sprints or 3) sustained performance over the first several sprints followed by precipitous decrease in performance just before fatigue. Support for one of these alternatives may provide insight into which proximate mechanisms determine fatigue and also might determine how fatigue influences lizard behavior.

Arca zebra genome enables phylogenomic comparison of evolution and function in bivalve eyes

Kyle McElroy, Sivanandan Chudalayandi, Rick Masonbrink, Jorge Audino, Andrew Severin, Jeanne Serb

Eyes are important sensory structures that have evolved numerous times in mollusks. We know very little about how these morphologically diverse eye types have evolved, including the sets of genes recruited to support their development and function. Pteriomorpha, the subclass of bivalves that includes scallops, oysters, mussels, and ark clams, evolved eyes independently at least five times, making them an excellent setting to explore the molecular basis for convergence of novel traits. Until recently, genomic comparisons between eyed and eyeless lineages have been hampered by scallops being the only sequenced eyed species. We generated a chromosome-level genome assembly for an eyed ark clam, *Arca zebra*. Combined with our assembly for the eyed limid, *Ctenoides ales*, we used these data for phylogenomic comparisons across eyed and eyeless lineages of Pteriomorpha. We also performed RNA-seq on photosensitive and non-photosensitive tissues from light vs. dark treated *A. zebra*, along with the bay scallop, *Argopecten irradians*, and *C. ales* to identify candidate genes for light response and characterized expression across the diverse opsin repertoire. The core components of the molluscan visual cycle: r-opsin, retinochrome, and RALBP exhibit relatively high eye-expression in all three species, but we find that fewer opsins exhibit bias towards eyes in *A. zebra* vs. *A. irradians* and *C. ales*. Furthermore, other opsins like Go-opsin and xenopsin appear to have more distinct expression patterns across species.

Investigating the effects of increased temperatures on the grazing rates of the Caribbean king crab

Jaclyn McFadden, Jason Spadaro

Tropical algae grazers, such as the Caribbean king crab, are vital to coral reef health and longevity because of their ability to consume algal overgrowth covering coral and blocking out sunlight from their symbionts. Rising global temperatures have had significant impacts on grazers in a variety of environments; however, its effect on the grazing ability of these herbivores remains understudied despite their importance in coral reef restoration and recovery. In this study, we evaluated grazing ability in Caribbean king crabs through the juvenile to sub-adult stage by placing them into tanks with three distinct temperatures (28°C, 30°C, 32°C). After a 24 hour feeding trial, the algae was dried and weighed to determine how much algae was consumed. The one way ANOVA indicated there was no significant difference between grazing rates in each temperature, suggesting that these crustaceans may already be adapting to warming water temperatures. Regression analysis comparing carapace width, as well as cheliped length, against algae consumed showed that these had no significant impact on the amount of algae consumed. Caribbean king crabs, as well as other macroalgae grazers, will be useful in combating algal overgrowth on the reef as rising ocean temperatures continue to stress coral. Further exploration is needed to study the impacts of increased ocean temperatures on other important grazers, including the long spined sea urchin and herbivorous fishes and crustaceans.

A Role for the Neural Crest in the Development of the Zebrafish Anterior Lateral Line System

Noel McGrory, Vishruth Venkataraman, Theresa Christiansen, Victoria Prince

Two cell populations play key roles in constructing vertebrates' diverse cranial sensory systems: the neural crest cells (NCCs) form much of the skull and peripheral nervous system, while placode cells contribute to sense organs such as the eye, ear, and, in aquatic vertebrates, the lateral line (LL). The LL system detects water currents using sensory hair cells located within neuromasts, small organs innervated by the LL ganglia. Development of the posterior section of the zebrafish LL, which forms a linear array of neuromasts along the trunk, has been well studied. The more complex anterior LL, which has branches including supraorbital and infraorbital lines around the eye, is less well understood. To test the hypothesis that NCCs might influence development of the placode-derived anterior LL, we blocked zebrafish

NCC development using morpholino knockdown of *Ap2a* and *Foxd3* transcription factors and evaluated impact on anterior LL development using live microscopy and immunolabeling. We found significant disruptions to anterior LL development in manipulated embryos. Specifically, in the absence of NCCs, migration of the supraorbital line around the eye stalls, and there is near complete absence of the infraorbital line. Moreover, we observed similar phenotypes when NCCs were ablated using a NCC-specific Nitroreductase transgenic model. Our ongoing experiments aim to investigate interaction mechanisms between these cell populations as well as specific roles for NCC subpopulations.

Investigating the Role of Evaporative Water Loss in Chameleons

Madison McIntyre, Etti Cooper, Krystal Tolley, Christopher Anderson

As the global climate becomes rapidly more extreme and unpredictable, the successful maintenance of body temperature and hydration at physiological levels suitable for survival will become ever more critical for organisms. Squamates are particularly challenged by fluctuating aridity and temperature due to the nature of their integument and need to thermoregulate. Total evaporative water loss (TEWL) has served to measure adaptations that extant organisms use to combat environmental pressures on maintaining hydration necessary for survival. Cutaneous evaporative water loss (CEWL) may prove to be an important way of quantifying water loss in reptiles, however research on CEWL in reptiles is limited and neither have been studied in chameleons. We measured both TEWL and CEWL in five species of chameleons living along an environmental gradient. TEWL was measured using closed system respirometry with a dew point analyzer. CEWL was measured using the AquaFlux evaporimeter across body regions. For three species, CEWL was measured in the field and after a 2-month acclimation period in the lab. Our results provide some of the first data on chameleon evaporative water loss (EWL), including valuable insights into how chameleons compare to other squamates, how varying thermal environment shapes EWL in chameleons, and the plasticity of CEWL following acclimation to a lab environment. Finally, variation in EWL observed across the body suggests there may be regional variation in the integument impacting CEWL.

A thread on the convergent evolution of silk in crustaceans

Siena McKim, Thomas Turner

Despite the wide diversity across the animal kingdom, we often find similarities in distantly related lineages, suggesting convergent evolution is at play. This phenomenon is particularly pronounced within Arthropoda, the most diverse animal phylum. Arachnids, myriapods, insects, and crustaceans have often independently evolved silk, a versatile biomaterial that shapes arthropods' ecological interactions such as food capture and habitat construction. Despite extensive research on silk in insects and spiders, the origin and evolution of silk production in crustaceans remains relatively unexplored. Our comprehensive literature review of crustacean silk systems uncovered evidence suggesting independent silk evolution in crustaceans on at least five separate occasions. Among these, silk produced by amphipods and tanaids exhibit the characteristics of the canonical silks of distantly related silkworm moths and caddisflies, yet they are not genetically homologous. This distinction arises from differing amino and carboxyl terminus sequences in Tanaidacea silk proteins, indicating an independent origin. To verify amphipod silk independent origins, we are employing transcriptomic and histological methods on Corophiida and Ampeliscidae amphipods. Due to the evolutionary distance between Corophiida and Ampeliscidae and differences in silk gland morphology, we hypothesize that amphipod silk production has originated twice and been lost once. By investigating the evolution of silk production in crustaceans, we discover novel silks, silk genes, and silk-production systems, and ultimately elucidate a more complete evolutionary history of silk production in arthropods.

Gridfinder: A computerized method for efficient mass μ CT scanning of otoliths and dense objects

Maximilian McKnight, Adam Summers, Cassandra Donatelli, Arthur Porto

We produced an extension for 3D Slicer that automatically segments objects in a gridded holder. Our motivating application was high throughput data collection on pairs of otoliths. Micro computed tomography (μ CT) scanning is an increasingly useful tool as technology gets cheaper and more widespread. μ CT scanning is seeing increasing uses in biomechanics and morphology. One potential use is analyzing otoliths, which indicate many useful properties of a fish such as its shape and species. Research suggests a potential 100 fold increase in speed of obtaining useful results mass μ CT scanning otoliths, but a streamlined automatic method hasn't yet been developed to obtain those benefits.

Here we propose and implement a design for a holder to batch scan otoliths alongside our algorithm, Gridfinder, for auto segmentation and labeling of items. What's more, our methods generalize to any object dense enough to be thresholded out of the holder, and do not require any pre-registration. This will allow fields beyond otolith sciences to benefit from fast efficient batch μ CT scanning.

To allow Gridfinder to be used out of the box, enabling simple mass μ CT scanning by those without programming experience, we implement our methods as a module for the popular open source program 3D Slicer. We anticipate providing a platform for faster scanning methods will open doors to large scale data collection of many specimens previously unexamined.

Turgor Pressure Affects the Flexural Rigidity of Poricidal Anthers

Jenna McNally, Mitchell Alvord, Mark Jankauski

Buzz pollination is a behavior where bees latch onto an anther and vibrate it using their indirect flight muscles to extract pollen. The plants that require this specialized method of pollen removal have a unique tube-like anther morphology known as the poricidal anther. Though buzz-pollinated crops are valued at 130 billion dollars annually, little is known about the mechanical interactions between the bee and anther that facilitate pollen removal. Here, we identified how turgor pressure influences the anther's flexural rigidity, which is critical to pollen extraction and affected by drought and other environmental conditions. We assessed stiffness experimentally using flexure testing and estimated turgor pressure from mathematical modeling.

We found that anther stiffness decreased by over 50% within 30 minutes of being removed from the flower, which corresponds to an estimated pressure loss of 60%. Though modulus may also affect flexural rigidity, our mathematical model indicates flexural rigidity is much more sensitive to changes in pressure. Thus, environmental factors that affect turgor pressure may in turn affect anther flexural rigidity and bees' ability to successfully remove pollen from poricidal anthers. This is to be considered while investigating the ecology of buzz pollination.

Defining the mechanism by which Rap1 regulates Canoe and cell-junction cytoskeletal connections

Emily McParland, Noah Gurley, T Butcher, Corbin Jensen, Mark Peifer

Embryogenesis requires robust connections between cell-cell adhesive junctions and the actomyosin cytoskeleton to allow for dramatic movements of cells without disrupting tissues. Drosophila Canoe (Cno) and its mammalian homolog Afadin are conserved multidomain cell-junction-to-cytoskeleton linker proteins, with relatives across the animal kingdom. They share the same domain structure and parallel functions in maintaining epithelial integrity. They are activated by the small GTPase Rap1, which can bind each of their two RA domains. Deleting both RA domains dramatically reduces function. However, individual RA domain contributions are unknown. Using CRISPR/Cas9 engineering, we tested RA domain functions by individually deleting RA1 or RA2 (Cno Δ RA1 and Cno Δ RA2, respectively), and by creating mutants with RA1RA1 or RA2RA2 configurations. Strikingly, Cno Δ RA2 and CnoRA1RA1 flies are viable through adulthood. However, when we stress the system, reducing the maternal dose of Cno Δ RA2 by placing it over the null allele of *cno*, the zygotic

null phenotype is substantially enhanced. Thus, RA2 is not fully dispensable. In contrast, CnoΔRA1 flies die before adulthood, and maternal/zygotic mutants are embryonically lethal. To examine defects during morphogenesis, we analyzed junctional protein localization throughout early embryogenesis and examined phenotypic defects in apical junctions at later stages. This work, in combination with structural predictions and biochemical analysis, is helping us define the mechanisms by which Cno and Afadin are regulated by Rap1.

Identifying genomic patterns that underlie life history in a widespread lizard

Matthew McTernan, Christopher Parkinson, Michael Sears

Widespread species often exhibit variation in life history across populations, but the genetic mechanisms that underlie this variation are poorly understood. Most attention has been paid to documenting phenotypic patterns of life history, but genomic approaches may provide new insight into the genetic, cellular, and physiological pathways that produce these patterns. The Eastern fence lizard (*Sceloporus undulatus*) has long been a model for the study of life history evolution, and thus provides an ideal system for these investigations. Life history varies predictably in this species, with both body size and age at maturation increasing with latitude. Here, we utilize whole-genome sequences from 30 individuals across three populations to identify genomic variation associated with life history. We use PCA to create a composite life history variable that represents average adult size, size at maturity, age at maturity, and monthly survivorship. We then use three models to identify variants associated with population structure and life history: PCAdapt, BayeScEnv, and LFMM2. Genes harboring variants identified by all three models were then used in GO enrichment analyses to identify putative biological functions that underlie patterns of life history in *S. undulatus*. Our approach identified 13 candidate genes associated with five GO terms related to growth, development, and heart function. Our study provides one of the first examples of using genomics to understand life history patterns in a *Sceloporus* system.

Variations in Starling flight behavior during wind tunnel experiments

Noah Medina, Mareesa Islam, Siyang Hao, Ronan Gissler, Kenny Breuer

Understanding the patterns of flight that birds choose is fundamental to the study of avian flight. However, characterization of individual variation within a species remains unexplored in the literature. As part of a detailed study on bird flight mechanics, we collected time-resolved (25 Hz) position and velocity data of European Starlings, *Sturnus vulgaris*, flying in a wind tunnel at a mean wind speed of 11 m/s. Each flight lasted tens to hundreds of seconds during which each bird exhibited markedly different styles of flight - some maintaining a steady position in the wind tunnel section, some flying in a repeatable loop alternating between flapping and gliding, and others flying along a more varied trajectory. With the goal of characterizing variation between these individual flight trajectories, we used a series of statistical tests and tools to analyze our position and velocity data. In this work we present characteristics of these flight trajectories, including clustered measures of unsteadiness and repeatability, flight-classification commonalities, and comparisons between estimated energetic costs. The trajectories of birds flying in a clean flow versus flying in a “dirty” flow - generated using a flapping wing placed in the test section - are also compared.

Exposure to long-wavelength light modulates recovery of retinal integrity in *Drosophila melanogaster*

Michael Meece, Diego Zagazeta, Shubham Rathore, Elke Buschbeck

It has previously been shown that near-infrared light can positively affect the physiology of damaged tissue. This is likely mediated by the modulation of metabolic activity via cytochrome c oxidase (COX) and the rate of ATP production and generation of reactive oxygen species. This process is wavelength dependent, with different wavelengths either increasing (maxima at 670 and 810 nm) or decreasing (minima at 750 and 950 nm) the activity of COX. The impact of these effects on retinal health is not yet well understood. To answer this question, we first induced photoreceptor damage in the eyes of white

mutant *Drosophila melanogaster* through prolonged bright light exposure. We then investigated the recovery of retinal health following exposure to different wavelengths of near-infrared light (670, 750, 810, 850, and 950 nm) over the course of 10 days. Retinal health was assessed through electroretinograms, fluorescent retinal imaging, and transmission electron microscopy. We found that all treatments except for 950 nm light facilitated the recovery of the electroretinogram response in previously light-damaged flies. All near-infrared exposed groups showed at least some improvement in retinal structure and auto-fluorescence compared to an untreated recovery control. Our findings support the notion that fine-tuning the ATP production mechanism can positively affect damaged eyes and highlight how modulation with specific wavelength bands can have different effects.

Expression and evolution of antimicrobial peptide genes in flies with expanded immune repertoires

Richard Meisel, Danial Asgari, Dana Nayduch

Antimicrobial peptides (AMPs) are crucial components of the host immune defense against pathogens by preventing the proliferation of bacteria and other micro-organisms within the host. As such, AMPs are important for many aspects of the ecology and evolution of immune systems and host-microbe interactions. Despite this critical role, many aspects of the regulation and evolution of AMP genes remain poorly resolved—including how and why AMP genes evolve constitutive versus induced expression and expanded copy numbers via duplication. We aimed to address these unresolved questions through comparative and functional genomic analysis of AMP genes amongst flies in the family Muscidae, which include the house fly (*Musca domestica*) and stable fly (*Stomoxys calcitrans*). These species are well-suited models to study the regulation and evolution of AMP genes because they inhabit septic environments and have evolved expanded repertoires of immune genes relative to most other flies. Our work has identified separate classes of induced and constitutively expressed AMP genes, and we identified candidate novel mechanisms for their transcriptional regulation. By comparing across species, we identified how AMP repertoires diverge in both their regulation and amino acids sequences. We specifically found evidence for differential selection pressures acting on AMP proteins with divergent regulation. Altogether, our work has shown that the evolution of AMPs involves species-specific correlated changes in gene regulation and protein sequences.

Chemical properties of target odors affect search kinematics of detection dogs

Daniel Mejia, Lydia Burnett, Nicholas Hebdon, Lauryn DeGreef, Lindsay Waldrop

Trained dogs detect many chemical cues (odors) such as explosives. To locate an odor source, detection dogs follow complex odor plumes created by diffusion of the chemical from its source into the air. Air currents then mix odor filaments into intermittent signals that the dog must learn to interpret. These signals are affected by the physical properties of the target chemicals. How these physical properties, such as diffusivity and vapor pressure, affect search behavior is unknown. Here, we use two chemicals common to explosive-detection training: ammonia (NH₃, a byproduct of ammonium nitrate) and 2-ethyl-1-hexanol (2E1H, a byproduct of C4). These chemicals differ in vapor pressure by 55,000 times and diffusivity by 3.6 times, respectively. Unrestricted search behavior and motion of fourteen trained dogs were observed by video. In each run, dogs searched for one of the two target odors. Kinematic movements were analyzed using DLTdv8a, and behavior was analyzed through an ethogram using trained observers. While the movement when searching differed between the chemicals, the behaviors did not. Dogs exhibited wider kinematic search areas, further away during casting, and longer search durations during localization for NH₃. Dogs were more successful in correctly alerting to NH₃ than 2E1H. These differences suggest that the physical properties of target odors affect the kinematic patterns used by dogs to search for different target odors.

Effects of Plastic Ingestion on Blood Chemistry, Gene Expression and Body Condition in WTSH

Nicole Mejia, Flavia Termignoni-Garcia, Jennifer Learned, Jay Penniman, Scott Edwards

Plastic pollution is a global threat that affects almost every marine ecosystem. Seabirds, typically top predators in marine food chains, have been greatly affected by ocean plastic pollution, with 59% of species found to have ingested plastic. However, little is known about the sublethal effects — when organisms or populations survive exposure to a toxicant — of plastic on seabirds that otherwise appear healthy. Here we document the effects of plastic ingestion on Wedge-tailed Shearwaters (*Ardenna pacifica*, WTSH) in Maui, Hawai'i, using analyses of blood chemistry, gene expression, morphometrics and stomach contents. We aimed to understand possible effects of plastic ingestion in live specimens. Plastic pieces were found in 12 out of the 28 gut samples. There was a negative correlation between body weight and the presence of plastic. We found that genetic modules associated with body weight were up-regulated in birds that ingested plastic. Finally, this study provides baseline data for WTSH blood chemistry, and will guide future examinations of the impacts of plastics on this population. We hope that this research further encourages uses of genetics in conservation research.

Crab Walking Kinematics on Flat vs. Inclined Hemispherical Obstacles

Alex Melendez, Adin Sokol, Glenna Clifton

The crab bauplan has convergently evolved at least five times, including in species that have diverse lifestyles and movement strategies. Intertidal crabs are especially versatile in that they navigate rocky terrain in both terrestrial and aquatic environments. Crab locomotion has been previously studied, but these studies only tested crabs on flat surfaces. To better understand crab walking on non-flat terrain, we recorded 19 purple shore crabs (*Hemigrapsus nudus*) walking sideways on one flat substrate and three arrays of cylinders (with diameters corresponding to 37%, 62%, and 87% of the length of their second pereopod) at both 0-degree (level) and 20-degree inclines. Tracking points on the carapace, pereopods, and dactyls reveal that crab walking kinematics are more variable on substrates with larger obstacles and when walking on an incline. Crabs were unable to walk at angles above 20 degrees, even on the flat and smallest cylinder substrate. We also observed significant behavioral differences among sexes, with males being slower and more resistant to walking. These findings will enhance our understanding of how crabs walk on terrain, more like their natural environment, which is critical for contextualizing a crab's ability to mate, find food, and avoid predators. Further, biomimetic legged robots currently struggle on rough terrain and crabs may inspire improved designs and control strategies for legged robots.

Motoneuron soma size measurement optimization through analysis in ImageJ

Nicole Melendez, Nicole Moody, Matthew Fuxjager

Spinal motoneurons innervate vertebrate skeletal muscles to control how these tissues generate movement, such as for communicative behaviors. Biologists have long used retrograde tract-tracing techniques to map out motoneuron pools and evaluate the morphology of these cells. Here, we explored motoneuron morphology in woodpeckers, looking at the cells that innervate the muscles that actuate specialized neck movements that produce their characteristic "drum" signal. One problem we faced was how to process motoneuron images in order to obtain accurate morphological measurements. Thus, we tested the effect of different levels of image processing on cell area measurements, with an eye toward establishing the ideal contrast enhancement to most accurately measure cell size. We found that subjecting images to contrast enhancement of 0.40% or 0.50% saturation resulted in the most consistent measurements of all visible cells. Lower levels of contrast enhancement increased the likelihood of faintly stained cells being overlooked by the researcher, whereas higher levels of contrast enhancement often resulted in multiple cells combining into one ill-defined bright spot. Our results highlight the importance of conducting rigorous methodological testing at the early stages of analysis to refine protocols that optimize inter-researcher

repeatability and accuracy of measurements. Further, our findings suggest that contrast enhancement is an important processing tool in the ImageJ toolbox for visualizing and measuring cell soma size.

Gotta Fan 'Em All: Using computer vision based tracking to understand the impacts of heatwaves

Grace Melone, Acacia Tang, James Crall

Bumblebees are a diverse group of important wild and managed pollinators in temperate regions across the globe. Climate change, and particularly extreme heat events, are likely negatively impacting bumblebee populations, but the mechanisms underlying these impacts—and the secondary stressors that modulate them—are not well understood. Here, we investigate the impacts of multi-day heatwaves on collective thermoregulation within bumble bee (*Bombus impatiens*) colonies, specifically focusing on how heat wave progression and nutritional status affect thermoregulatory behavior and colony growth. We exposed bumble bee (*Bombus impatiens*) microcolonies to a 3-day heatwave (35°C) under varying nectar quality (25% sucrose vs 50% sucrose) and quantified impacts on both behavior (i.e., ventilating fanning behavior) and colony performance (survival of workers and brood). We quantified fanning behavior of individually tracked bees using a high-throughput, automated imaging system based on the Raspberry Pi. We integrated tag-based tracking of individual workers with deep learning-based identification of fanning behavior before, during, and after simulated heatwaves. The proportion of bees fanning declines throughout the heatwave, with no effect of nutrition on fanning behavior. However, nutrition reduces worker and brood survival and increases nectar consumption. Our results suggest reduced performance under low quality nutrition is not due to impaired fanning behavior, but that altered nutrition may decrease brood-care behavior or directly affect larval sensitivity.

Weighing the risks: Foraging barn swallows decrease flight speed as they approach a water surface

Laura Mendez, Amanda Li, Iris Reed, Victoria Yan, Tyson Hedrick

Barn swallows (*Hirundo rustica*) are insectivores recognized for their highly maneuverable and fast foraging flight. Flight speeds of up to 10 ms⁻¹ have been recorded while also performing fast adjustments to the flight paths and turns to hunt successfully. Barn swallows mostly forage at low altitude over open grass fields, but it is common to see them descent to drink and feed on insects at the surface of the water. To avoid negative interactions with the water that could lead to costly energetic and survival consequences, we proposed that the birds switch to a conservative flight behavior as they approach the surface by reducing their speed, but not sacrificing their maneuverability. We recorded and tracked barn swallows foraging over a medium size pond in North Carolina during the breeding season. In accordance with our hypothesis, barn swallows decreased their flight speed at altitudes below a wingspan at around 0.3 meters away from the water surface, with a steeper decline below an altitude comparable to the length of a wing. In contrast, flight altitude had no effect on turning radius. Our data suggest that barn swallows avoid the risks of interacting with water by decreasing flight speed allowing them to continue foraging at ease.

Eugenie Clark Fellowship: The Human Connection to Shark Sciences through Digital Storytelling

Aimee Mendoza

My research delves into the captivating convergence of digital storytelling and marine sciences. As a 2023 Eugenie Clark Fellow and with the invaluable support of Minorities in Shark Sciences, I am excited to share my exceptional journey through this esteemed program. Throughout this experience, I have been presented with countless opportunities to explore previously unimaginable concepts, fostering the development of my identity as a science communicator with an authentic and distinctive voice. My talk will explore the ways in which digital storytelling can be used to communicate shark sciences in an informative, meaningful, and impactful way. Recognizing the significance of representation, I proudly embrace my role as a representative for minorities,

people of color, single parents, and lifelong academics. Through my work, I aspire to inspire individuals like me, who may feel voiceless or doubt their intellectual aptitude for science. I firmly hold the conviction that our current society undervalues the importance of science communication, perceiving it as a trivialization of scientific concepts or a mere quest for popularity. Yet, in my perspective, science communication entails possessing the knowledge and skill to effectively convey complex scientific ideas in an accessible manner, thereby empowering us to make a positive impact in the world.

Genomic simulations to explore allelic exchange via reciprocal introgression

Devin Mendoza, Evan Forsythe, Darren Clavette

Hybridization is an evolutionary catalyst in eukaryotic organisms. Genetic introgression, stemming from interspecies hybridization followed by iterative backcrossing, orchestrates allele transfer between populations, profoundly influencing the evolutionary trajectory of man crops and livestock, as well as that of modern humans.

Despite the profound implications of introgression, current understanding of dynamics and genomic outcomes of introgression is restricted to a limited set of theoretical and statistical frameworks. Here, we use genomic simulation to explore the possibility that introgression events result in alleles being transferred in two directions simultaneously, a process we define as 'reciprocal introgression'. The current mechanistic model of introgression suggests reciprocal introgression is probable; however, this layer of introgression is not detectable within prevailing statistical frameworks, hindering evaluation of its prevalence in nature. Further, unaccounted-for reciprocal introgression may bias statistics that are widely used to quantify introgression, obscuring our understanding of the full scope of introgression. To address this problem, we (1) implement evolutionary simulation software to emulate genomes that have undergone reciprocal introgression, (2) apply existing introgression statistics to these data to evaluate their performance in the presence of reciprocal introgression, and (3) provide a roadmap for incorporating reciprocal introgression into our working model of the introgression process. This work paves the way for updated statistical frameworks, thus providing a more comprehensive view of the evolutionary impacts of introgression in nature.

Re-examining the functional morphology of bladderwort traps

Lukyon Mendrin, Ulrike Muller, Luz Gonzalez-Ponce

Utricularia, bladderworts, are a genus of carnivorous plants comprising more than 200 species. They occupy aquatic, terrestrial, epilithic and epiphytic habitats. Bladderworts use millimeter-sized underwater suction traps to capture plankton prey. Traps vary widely in their morphology, especially in the structures surrounding the trap opening. Entrance appendages include antennae, recurved spurs, dense bristle bundles, wings, and many more. Many aquatic species have antennae, which are long horn- or antler shaped appendages. A 1979 study by Meyers and Strickler hypothesized that the antennae structures attract prey and guide it toward the entrance, and found that the removal of the antennae resulted in a decrease in prey capture. In this project, we attempted to replicate and generalize the 1979 study of *Utricularia vulgaris* by using several different bladderwort species. Yet we found no significant effect of antennae removal on prey capture success in several bladderwort species. Our inability to replicate these findings in multiple bladderwort species raises questions about the function of the antenna structures. In future studies, we plan to perform experiments within a natural habitat to ensure that our observations are not the result of constraints imposed by laboratory experiments, such as effects of the experimental protocol on prey availability or prey behavior.

Uncovering the Population Structure and Genetic Diversity of the Spotted Lanternfly

Fallon(Fang) Meng, Anthony Snead, Gaia Rueda-Moreno, Kristin Winchell

The spotted lanternfly (*Lycorma delicatula*), an invasive species native to Asia, has caused significant agricultural and forestry damage in the northeastern

United States since its arrival in 2014. The invasive range spread from Pennsylvania to multiple other states, with the leading edge now spreading northward in Connecticut. With potential widespread economic impact, understanding its invasion history and global phylogeographic pattern is crucial. However, population genomics of spotted lanternfly is a key missing piece in understanding the invasion, and ultimately developing effective strategies to combat its spread. We aim to fill this critical knowledge gap by analyzing whole genome sequences of spotted lanternflies across the Northeastern United States. We focus on the northern range expansion in the New York metropolitan area since the invasion appears to be facilitated by urbanization. By investigating genetic diversity across the invasive range, evidence for population structure, and contemporary and historical demography, we gain insights into the origins of the invasion, demography of the invasive range expansion, and potential for local adaptation as the invasion progresses. We find signatures of reduced genetic diversity consistent with the recent invasion history, but also note local population differentiation suggestive of local adaptation. These results offer valuable insight into the eco-evolutionary dynamics of spotted lanternfly, emphasizing the critical need for further research into the underlying genetic structure of the invasion when developing intervention strategies.

A model of the cockroach antenna links tactile features to distinct motifs on a soft sensor

Lingsheng Meng, Parker McDonnell, Kaushik Jayaram, Jean-Michel Mongeau

The parsing of rich spatiotemporal information to guide decision-making is a hallmark of biological systems. However, the mechanisms behind how biological sensors process vast amounts of information remain poorly understood. A broad class of animals rely on touch sensation for perception. Among these, the American cockroach *P. americana* uses a pair of soft antennae for tactile exploration and guidance. We hypothesize that antenna mechanics is tuned to breakdown tactile features into a lexicon of spatiotemporal patterns (strain motifs). To test this hypothesis, we developed a mechanical model of the antenna (flagellum). Inspired by the morphology of the flagellum, our model included a sequence of rigid links interconnected by hinge joints. From mechanical testing data, we modeled the stiffness and damping of these joints using nonlinear optimization. We simulated the mechanical response of the flagellum using the MuJoCo physics engine and demonstrated the model's high fidelity to the actual antenna. Our simulation revealed how antenna mechanics represent a diverse set of tactile features into a lexicon of strain motifs. These motifs may assist in decision-making during slower tactile exploration and rapid course control. Our mechanical model is inspiring a soft, distributed robotic sensor whose shape and dynamics can be modulated.

Flight kinematics of common bird species landing on feeders

Kabir Menon, Jose Iriarte-Diaz

Landing is a critical phase for vertebrate flight that requires careful regulation of the direction and velocity of the flight. A successful landing requires controlled deceleration to prevent injury while maintaining a flight velocity capable of producing enough lift. In this study, we report the landing kinematics of common birds that visit feeders in the Southeastern US, such as Northern Cardinals, House Finches, Downy Woodpeckers, Tufted Titmice, and White-breasted nuthatches. We recorded the three-dimensional body and wing kinematics of landing behaviors of six passerine species, using four high-speed cameras, as they approached a feeder placed outdoors. We hypothesized that differences in landing kinematics would result from differences in body size and wing shape between species. Our findings revealed significant differences in flight velocities among species when the birds were positioned at 80 cm from the feeder. However, the flight velocities at landing were remarkably similar. Notably, the flight velocities of White-breasted Nuthatches and Downy Woodpeckers exceeded those of most other species during the approach, indicating that they decelerated rapidly as they neared the feeder. In contrast, Finches exhibited the slowest landing velocities. Our results suggest a significant difference in landing behavior across species that result from unique interspecific maneuvers.

The interactive effects of nitrate and road salt on benthic algal assemblages in artificial streams

Margaret Menso, Paul Moore, Christopher Ward

With increasing urbanization, impervious surface cover has increased worldwide. With this increase, urban surface runoff, which carries anthropogenic chemicals, has grown to levels that can create problems for freshwater habitats. In the northern climes of the United States, one of these chemicals is road salt. Urban surface water runoff also has high concentrations of nitrate due to nitrogen deposition on roadways. To understand the impacts of road salt and nitrate on benthic algal assemblages, we created artificial flow through streams that contained nutrient diffusing substrates (NDS) containing both salt and nitrate. The NDS were made from terracotta flowerpots, agar, and petri dishes; containing varying concentrations of both salt (0-7500 mg/L above ambient levels) and nitrate (0-5.9 mg/L above ambient levels) and were deployed in artificial streams at the University of Michigan's Biological Station. Diatom concentrations were significantly impacted by salt ($p < 0.05$), and the combination of salt and nitrate on sampling days 4 and 28 ($p < 0.05$). Cyanobacteria concentration was significantly impacted by nitrate on day 28 ($p < 0.05$). Total algae concentrations were significantly impacted by salt and nitrate ($p < 0.05$), and the combination of salt and nitrate on sample days 14 and 28 ($p < 0.01$). Overall, this study shows salt and nitrate, as well as the combined effects of the two, significantly impact benthic algae assemblages and community development.

Endocannabinoids vary with seasonal life-history transitions in a wild reptile

Lauren Merlino, Lin Lin, Faizy Ahmed, Daniele Piomelli, Deborah Lutterschmidt

The neuroendocrine mechanisms regulating seasonal transitions in behavior are not well understood. Endocannabinoids are a strong candidate for regulating such transitions because they interact with glucocorticoid hormones, are widely distributed throughout the vertebrate nervous system, and function as neuromodulators of both reproductive and feeding control centers. Using ultra-high-performance liquid chromatography tandem mass spectrometry, we asked if changes in brain endocannabinoids are associated with the seasonal transition from reproduction to migration and feeding in red-sided garter snakes (*Thamnophis sirtalis parietalis*). We further assessed the transition of males by measuring courtship behavior in combination with a Y-maze trial, where individuals were asked to choose between a reproductive cue (female pheromone trail) and a feeding cue (worm trail). Within the reptilian homologue of the hippocampus, 2-arachidonoylglycerol (2-AG) was significantly higher in female and male snakes captured at the breeding grounds compared to those migrating to summer feeding areas. Furthermore, males transitioning from courtship to feeding behavior (i.e., exhibited courtship but preferred a worm trail) had significantly more 2-AG in the brain than males that completed the seasonal transition to feeding behavior. Our results suggest a decrease in 2-AG is involved in the seasonal termination of reproductive behavior. Because all vertebrates have an evolutionarily conserved endocannabinoid system, these results are broadly applicable to understanding how behavioral output is altered in response to shifts between reproduction and self-maintenance.

Uncovering male and female mosquito contributions to seasonal differences in reproductive physiology

Megan Meuti, Carlos Esquivel, Chiristiana Arkorful-Bondzie, Lydia Fyie, Sarah Short

Females of the Northern house mosquito, *Culex pipiens*, transmit West Nile Virus during summer and overwinter in a reproductive diapause in response to short days. Diapausing females do not bite or lay eggs, instead keeping sperm viable within their spermathecae until they terminate diapause approximately three to six months later. Although male mosquitoes do not enter diapause, we hypothesized that they change the composition of their ejaculate in the fall to promote long-term sperm storage in females. Using RNAseq, we found that male *Cx. pipiens* reared in winter-like conditions downregulate several genes that promote host-seeking, bloodmeal processing and egg

maturation, while upregulating transcripts that protect sperm and enhance immunity. Additionally, we found that nondiapausing females that mated with "summer" males blood-fed at higher frequencies and laid more eggs than females mated with "winter" males, while mating with "winter" males enhanced longevity in diapausing females. Finally, diapausing, mated females of *Cx. pipiens* upregulate transcripts encoding for antioxidant proteins. Together, these results demonstrate that male mosquitoes alter the content of their ejaculate in response to daylength and influence female blood-feeding, fecundity, and longevity. Additionally, our data suggest that female mosquitoes also play an active role in long-term sperm maintenance. Future studies that examine the interplay between male and female-derived factors in mosquitoes will not only enhance our understanding of insect reproductive physiology, but may also offer novel means to control disease vectors.

Amphibious compound eyes: ontogenetic shifts in eye morphology and function in *Belostoma flumineum*

Tanner Mierow, Kate Feller, Alexandra Kingston

Belostomatids (Hemiptera: Belostomatidae), or giant water bugs, are insects that use vision to capture prey and navigate to new bodies of water. Belostomatids are fully aquatic as nymphs but transition to an amphibious lifestyle as adults. Since vision is ecologically important to belostomatids at all stages, we hypothesized that the visual system shifts, through changes to the corneal optics, to support the visual needs of aquatic nymphs and amphibious adults. To test this hypothesis, we examined compound eye morphology, optical performance, and visually-guided behaviors of *Belostoma flumineum* throughout ontogeny. We used SEM, TEM, and light microscopy to characterize changes in corneal morphology. To quantify the focusing power of the cornea throughout ontogeny, we used a modified hanging drop method to measure focal lengths of eyes in air and water. Finally, we used a modified optomotor assay to examine how observed changes in eye morphology throughout ontogeny affect visually-guided behaviors. We discovered differences in eye morphology and optical performance across ontogeny in *B. flumineum*. Nymphal instars have convex corneas that optimally focus light onto the retina underwater, supporting visually-guided behaviors best in water but not in air. Adults have flattened corneas with similar focusing power in air and water, providing similar optical performance for visually-guided behaviors in both aquatic and terrestrial environments. Here, we show the first example of an amphibious visual system in an invertebrate.

The feeding kinematics of a surgeonfish and the associated functional implications

Michalis Mihalitsis, Peter Wainwright

Benthic-feeding coral reef fishes display an impressive degree of morphological and functional diversity. While the ecological outcomes of such feeding have been studied in detail, the morphological adaptations and mechanisms required to carry out benthic feeding have only been sparsely studied. Here, we use high-speed video to show how a surgeonfish (*Acanthurus leucosternon*) uses its morphology during feeding (i.e., detachment of filamentous algae). We quantified cranial, body, and pectoral fin components, and how they function during a bite. After gripping mouthfuls of attached algae in its teeth, this species used quick dorsoventral rotation of the jaws to detach the algae, facilitated by an intra-mandibular joint. This motion occurred in conjunction with the retraction of ventral elements between the neurocranium, pelvis, and pectoral girdle, followed by a lateral head flick. This previously undescribed mechanism of removing algae from reefs, employs in novel ways the intra-mandibular joint, as well as cranial elevation and pectoral girdle retraction, two motions primarily employed during suction feeding by teleosts. Ventral rotation of the jaws may represent a more efficient method of detaching benthic algae, that does not require the same additional forces delivered by lateral body bending and a head flick. Overall, our results provide novel insights into functional traits related to cropping, a function with significant implications to the process of herbivory on coral reefs.

Characterizing Variation in Pectoral Fin Evolution and Development Using Single Cell Transcriptomics

Katelyn Mika, Julius Tabin, Cristian Molina, Atreyo Pal, Anindita Basu, Neil Shubin

Pectoral fins across fish species are easily identifiable, but differences in selective pressures from their environment and life history constraints result in vast morphological diversity across species. Additionally, mutations affecting developmental genes can result in morphological diversity within a species. For example, *hoxa13a* and *hoxa13b* knockout mutant zebrafish have shorter pectoral fin rays when compared to wild type. This phenotypic diversity can be characterized at various biological levels, such as gross morphology, cell type variation, or gene expression and gene pathway differences across development. With the use of single cell sequencing we can investigate directly how shifts in gene expression affect cellular differentiation and the resulting cell populations, which in turn determine the final morphology of the fin. To establish this methodology, we have developed a custom, high throughput, single cell sequencing assay to investigate two questions. 1) Can we detect the molecular changes underlying morphological differences between *hoxa13* mutants and wildtype pectoral fins of the same species, *Danio rerio*? And 2) can we detect shared and unique developmental pathways in different species (zebrafish, spotted gar, little skate, and chained catshark)? While additional validation still needs to be completed, initial analyses on time point data suggest that this method is sufficient to detect both known and novel developmental drivers within and across species.

Long-term data reveals a breakpoint in buffering capacity of thermoregulation

Donald Miles, Bryce Rager

Determining a species response to global warming often entails comparing contemporary data on thermoregulation with predicted future climates based on global circulation models. However, the use of long-term data collected during a period of increasing temperatures provides direct evidence for the ability of species to use behavioral buffering to cope with rising temperatures. We used a 37-year long record of field-active body temperatures (*T_b*) measured on tree lizards (*Urosaurus ornatus*) to assess temporal variation in the thermal quality of the habitat, effectiveness of thermoregulation and hours of restriction and hours of activity. We also measured thermal preference in a thermal gradient and quantified operative environmental temperatures (*T_e*) to whether selected temperatures changed over time. Finally, we obtained historical records for drought intensity. The mean and variance in *T_b*s remained showed remarkable consistency between 1987-2015. Despite substantial temporal variation in operative environmental temperatures, the effectiveness of thermoregulation showed limited variation in the same period mainly due to a 2 degree increase in preferred *T_b*. However, the buffering capacity of thermoregulation shifted after 2015 with the highest *T_b*s were recorded between 2016 – 2022. The effectiveness of thermoregulation declined during this second period. The fluctuation in hours of activity and restriction was explained by cycles of drought. Our data suggest limits in the ability of behavior to buffer lizards from future increases in environmental temperature.

The evolution of multi-component weapons in the superfamily of leaf-footed bugs

Christine Miller, Michael Forthman, Rebecca Kimball

Sexually selected weapons, such as the antlers of deer, claws of crabs, and tusks of beaked whales, are strikingly diverse across taxa and even within groups of closely related species. Phylogenetic comparative studies have typically taken a simplified approach to investigating the evolution of weapon diversity, examining the gains and losses of entire weapons, major shifts in size or type, or changes in location. Less understood is how individual weapon components evolve and assemble into a complete weapon. We addressed this question by examining weapon evolution in the diverse, multi-component hind-leg and body weapons of leaf-footed bugs, Superfamily Coreoidea (Hemiptera: Heteroptera). Male leaf-footed bugs use their morphological weapons to fight for access to mating territories. We used a large multilocus dataset comprised of ultraconserved element loci for 248 species and inferred

evolutionary transitions among component states using ancestral state estimation. Our results suggest that weapons added components over time with some evidence of a cyclical evolutionary pattern — gains of components followed by losses and then gains again. Further, we found that certain trait combinations evolved repeatedly across the phylogeny. This work reveals the remarkable and dynamic evolution of weapon form in the leaf-footed bugs. It also highlights that multi-component weapons may be helpful in providing insights into the evolutionary interplay of form and function.

Phylogenomics and micro-CT scanning reveal continuous innovation in a deep-sea radiation

Elizabeth Miller, Rose Faucher, Pamela Hart, Melissa Rincon-Sandoval, Aintzane Santaquiteria, Ricardo Betancur-R, Luke Tornabene, Kory Evans, Dahiana Arcila

The deep sea is thought to be a harsh environment that imposes many evolutionary constraints. Alternatively, the deep sea could also provide a release from usual constraints in the shallow realm, such as competition or visually-driven predation. It is unclear how the push-and-pull of these opposing factors influences macroevolution. Here we examine rates of evolution in body and cranial shape using the anglerfishes (Lophiiformes), an order containing lineages that inhabit continental shelf, slope, and bathypelagic habitats. In particular, the ceratioid anglerfishes are an iconic radiation containing half the family and species diversity of the order, all of which evolved in the extreme environment of the bathypelagic zone. We estimated a novel phylogenomic hypothesis for Lophiiformes based on 1,000+ exons. Our taxonomic sampling expands on previous studies and includes 10 of 11 families and 40% of all species, the largest tree yet. We measured body shape from linear measurements, as well as generated micro-CT scans of museum specimens, for ~75% of the species in our tree. In this presentation we will discuss the tempo and mode of phenotypic evolution associated with benthic-to-pelagic and shallow-to-deep-sea transitions within Lophiiformes.

Using Computational Fluid Dynamics to Understand Fluid-Organism Interactions

Laura Miller, Matea Santiago, Nick Battista

This talk will provide a concise primer for the use of computational fluid dynamics (CFD) to quantify biological flows and understand fluid-organism interactions. Our target audience includes students and postdocs from the biological sciences and

engineering, as well as interested faculty. To motivate the use of CFD as a tool, we briefly review relevant computational work that has been applied to questions in organismal biology by interdisciplinary teams. We then provide an overview of modeling fluid flows and the choices that can be made in terms of dimensionality, steady or transient flow, and laminar or turbulent models. We describe the implementation of three numerical simulations of biological flows, including 1) airflow around a fog desert cactus, 2) saltwater flow around an anchored seahorse, and 3) pulsatile flow through an elastic venous valve. We discuss common open-source and commercial options for performing computational fluid dynamics at the organismal level. Common challenges and errors are also addressed, including the difficulty in generating reproducible CFD results and problems related to numerical convergence and stability. Finally, we present modern approaches for incorporating data science, machine learning, and uncertainty quantification into the development of mathematical models and the analysis of CFD data.

Eavesdropping embryos: How does prenatal exposure to alarm calls influence offspring phenotype?

Taylor Miller, Kristen Navara

Inside the egg or womb, embryos listen to the external world. Through sound, embryos are tuned into a direct channel of information exchange from the postnatal environment. Researchers have demonstrated that sound is capable of signaling specific characteristics about the postnatal environment, such as predation or high temperatures. We designed a study to investigate this idea in a songbird species known for maternal programming, but for which it has

never been tested. Our objective was to investigate how eggs exposed to adult alarm calls during embryonic development influence offspring phenotype in Eastern bluebirds (*Sialia sialis*). We hypothesized that embryonic exposure to alarm calls would alter nestling behavior and make them more reactive to stress. We monitored 120 nest boxes supporting breeding pairs of free-living bluebirds at University of Georgia auspices. At the onset of incubation, we randomly assigned nest boxes to either treatment (acoustic stimulation) or control (silence) groups. Treatment boxes were exposed to playback of bluebird alarm calls for 10min, 4 times daily between 9:30 and 17:30 from d10 to d14 of incubation. We then took measures of growth, immunity, and stress-responsiveness from nestlings. These results will be discussed.

Population divergence in thermal reaction norms in an invasive butterfly, *Pieris rapae*

Madison Milotte, Tyler Pereira, Anna Parker, Joel Kingsolver

How rapidly can populations evolve in response to changing local climatic conditions across a species range? How does this vary across life stages? Answering these questions is important for understanding whether species can adapt to recent and future climate change. To address this, we are studying the invasive butterfly *Pieris rapae*, which colonized many areas of North America within the past 170 years. We used laboratory experiments to quantify thermal reaction norms for key life history traits of *P. rapae* populations from three distinct climatic regions: Seattle, WA; Chapel Hill, NC; and Davis, CA. We measured survival, development rate and body size for the egg, larval and pupal life stages at five ecologically-relevant mean rearing temperatures from 16 to 34 C. Preliminary analyses indicate substantial differences in thermal reaction norms between the three populations, but these differences vary among life history traits and between life stages. Our results suggest that evolutionary responses of populations to local climatic conditions can involve changes in thermal sensitivity of multiple life stages. The consequences of such population and life stage differences for predicting range shifts and adaptation to future climate changes remain to be explored.

Rapid acclimation of heat tolerance in crayfish as a model for a CURE for first-year undergraduates

Patrick Mineo, Paul Arriola

Following calls for improvement of undergraduate STEM education like Vision and Change in Undergraduate Biology Education (AAAAS, 2011), the Department of Biology at Elmhurst University developed a pair of course-based undergraduate research experiences (CUREs) for the introductory biology sequence. Here we present the experience from the second semester which focuses on data collection, analysis, and writing a scientific paper. Our approach centers on guiding students in reading and analyzing primary literature to facilitate the construction of a scientific paper. This project involves a scaffolded approach where students collect data on the effects of short-term thermal acclimation on heat tolerance in crayfish. This experiment allows students to collect data in one lab period and relies on a field of literature that is appropriate for first-year students. Once the experiment is complete, the remainder of the semester is dedicated to data analysis and the process of writing. Students are guided on how to read and write each different section of a scientific manuscript. For each section (introduction, methods, results, and discussion), students read and critique a section from a publication before drafting their own. We provide formative feedback on each draft so that students make the appropriate revisions on the final draft of the paper. The students then have a completed manuscript as an artifact for their professional portfolio that is compiled in the senior capstone experience.

Lymphocyte Inhibition by Cell Wall Components of *Batrachochytrium dendrobatidis* (Bd)

Ria Mirchandani, Kaitlyn Linney, Louise Rollins-Smith

The spread of *Batrachochytrium dendrobatidis* (Bd), a chytrid fungus, has been linked to global amphibian population declines. Bd causes the lethal disease chytridiomycosis when it evades amphibian immune defenses. Bd infects amphibians as swimming zoospores, and once attached to the mucus

of the skin, the zoospores encyst, form a cell wall, and invade epidermal cells. Disruption of the host skin results in death. Although immune defenses such as antimicrobial peptides have been found to reduce the number of encysting zoospores and limit the infection, susceptible species are not fully protected. The ability of the fungus to evade the immune defenses has been attributed to the release of inhibitory factors. Previous studies have revealed three small metabolites released by Bd that inhibit lymphocyte responses. We hypothesized that another molecule associated with the fungal cell wall is also inhibitory for lymphocytes. Here we show that components of the chytrid cell walls, lacking other soluble factors, will inhibit lymphocyte proliferation. Treatment of mature Bd sporangia with a mixture of carbohydrase enzymes resulted in reduced lymphocyte inhibition by the cell wall fragments suggesting that the active factor is a carbohydrate or a protein-carbohydrate complex in the cell wall. Ongoing studies aim to further understand the nature of the lymphocyte inhibitory factors associated with the chytrid cell wall. Support: Vanderbilt SyBBURE Searle Undergraduate Research Program and NSF IOS-2147467.

Waiting and balancing acts: the evolution of reproductive delays in bats

Deblina Misra, Janet Young, Ching Chang, Harmit Malik, Theresa Luktisch, Teri Orr

Reproductive pauses, commonly referred to as delays, play a crucial role in key stages of the reproductive process in various organisms. These delays occur in three distinct developmental stages: between mating and conception (sperm storage), from conception to implantation (delayed implantation), and during the period between implantation and parturition (delayed development). We studied the evolutionary and biological basis of these important transitions using three approaches. First, using a comprehensive study of these delays in an evolutionary framework of 42 bat species, we described key transitions that led to each of these delays. Second, to focus on gene expression changes that may accompany such transitions, we investigated Jamaican fruit-eating bats (*Artibeus jamaicensis*), belonging to the family Phyllostomidae, which annually alternate between a non-delayed (ND) pregnancy and a pregnancy with delayed development (DD), allowing us to compare these two pregnancy types in the same individual. Third, to specifically investigate whether developmental delays during sperm storage may have affected the evolution of sperm chromatin proteins, we conducted an evolutionary analysis of protamines, which package chromatin in mammalian sperm. Despite rapid evolution of protamine genes, we found general phylogenetic concordance between protamines and species trees in bats. Our three-pronged approach offers an unprecedented molecular and evolutionary insight into the distinctive gestational adaptability observed within this bat lineage.

Distinct network-level strategies for different forms of non-associative learning in *Tritonia*

Viral Mistry, Evan Hill, William Frost

The marine mollusk *Tritonia diomedea* performs a vigorous escape swim consisting of full-body ventral/dorsal flexions in response to an aversive stimulus. This escape swim undergoes two forms of non-associative learning with repeated stimulation, sensitization and habituation, which can be observed in both the intact animal and the isolated CNS. Further, large-scale voltage imaging can be used to identify learning-induced changes in the escape swim network. These features make the *Tritonia* escape swim an excellent model for exploring how memories are encoded in neural networks.

Here we report that sensitization and habituation learning employ different strategies to encode information in *Tritonia*'s escape swim network. Sensitization involves two key network modifications: it increases network size by adding neurons to the active network, and it increases the firing strength of a particular group of efferent neurons, the ventral flexion neurons (VFNs). Interestingly, habituation is not the inverse of this. While the VFNs do fire less in each motor program as habituation develops, the number of active neurons is not altered in habituation the way it is in sensitization. These two forms of learning thus appear to employ different network-level strategies to encode

memory: one involves changing both network size and neuronal firing, the other only involves a change in neuronal firing. These results underscore the value of investigating network-level strategies for different memories.

Exploring Form-Function Relationships in *Euplectella Aspergillum* Using Computational Modelling

Yash Mistry, Swapnil Morankar, Nikhilesh Chawla, Oliver Weeger, Clint Penick, Dhruv Bhate

The Venus flower basket (*Euplectella Aspergillum*) has been the subject of significant study due to its hierarchical, lattice-like structure. While various hypotheses have been proposed for the basis of the macrostructure of the basket and its sequential addition of features during growth, validation of these hypotheses remains elusive. In this work, we examined the complex network of lattice members using X-ray microtomography, selecting three fundamental design principles for further study: (i) the selectively interwoven nature of the foundational base lattice grid, (ii) the diagonal weave that overlays it, and (iii) the helical ridge that juts out from the cylindrical base structure. These three principles were abstracted and modelled computationally, which then served as inputs for additive manufacturing and simulation, and then studied under different mechanical loading conditions: compression, bending, and torsion. Our experimental results show that each of the three design principles represents a significant and sequential improvement in mechanical performance in each of the three conditions evaluated. Simulation reveals some mechanisms for these findings, primarily on account of the manner in which stresses are distributed within the structure. Taken together, these insights provide a structural basis for the morphogenesis of the Venus flower basket, and also serve as inspiration for engineering design.

Ionotropic receptor and neuropeptide expression in chemosensory appendages of the nudibranch *Berghia*

Gianna Misuraca, Cheyenne Tait, Kelsi Watkins, Paul Katz

The molecular basis of chemosensation is relatively unknown in molluscs compared to other taxa. It was recently shown that the chemosensory ionotropic receptors (IRs) of insects are ancient across protostomes. Neuropeptides also play roles in the sensory periphery across invertebrates. We investigated the intersection of these molecular systems in the chemosensory appendages of the nudibranch mollusc *Berghia stephanieae*. We predicted that the rhinophores (distance chemosensation) and oral tentacles (contact chemosensation) would exhibit different patterns of gene expression. Two putative chemosensory IRs as well as the molluscan neuropeptides APGWamide and FCAP (feeding circuit activating peptide) were identified from brain transcriptomes. In-situ hybridization chain reaction (HCR) for the four genes showed that in the rhinophore, APGWamide-expressing cells were the most abundant, with IR-D restricted to cells only at the base of the appendage. FCAP and IR25A showed significant coexpression in an intermediate number of rhinophore cells. In the oral tentacle, there were fewer IR25A, FCAP, and APGWamide expressing cells, and more IR-D expressing cells than in the rhinophore. In conclusion, labeling with a mere four molecular markers revealed complex, differential patterns of expression between the rhinophores and oral tentacles of *Berghia*. Each appendage likely contains an even wider diversity of sensory neuron types that will be revealed as we use additional receptor and peptide markers. This diversity of cell types suggests a similar diversity in function.

Dissecting the wound response from the onset of regeneration in the ctenophore *Mnemiopsis leidyi*

Dorothy Mitchell, Allison Edgar, Joseph Ryan, Mark Martindale

The regulatory mechanisms that initiate regeneration and how they may have changed over animal evolution remains enigmatic. Species capable of whole-body regeneration (WBR) provide the opportunity to isolate the differential responses underlying different injury contexts. Control of injury responses is vital, as wound healing is required across all injuries, but unregulated regrowth can result in the formation of ectopic structures. *Mnemiopsis leidyi* is a comb jelly capable of WBR and belongs to the phylum Ctenophora which is thought to be the earliest diverging position in the animal tree as sister to the rest

of Metazoa. Similar to planarians and Hydra (Wurtzel et al 2015, Cazet et al 2021), using whole-mount in situ hybridization, we show that *M. leidyi* exhibits a generic response to injury in which ML_Fos3 is expressed around the wound edge in both injuries that require regeneration (r-wounds) and those that require healing alone (h-wounds) (Owlarn et al 2017). However, we found that the expression of ML_Fos3 in h-wounding is initiated faster and down-regulated earlier than that in r-wounds, suggesting that early response genes are tightly coordinated according to the severity of injury. In addition, using hybridization chain reaction, we characterized the spatial distribution of cells expressing ML_Fos3 in r-wounding and h-wounding events, focusing on the role of migrating cells to the wound site. We are currently perfecting techniques to knock down injury response genes during regeneration in effort to determine how generic injury responses can determine regenerative outcomes.

Shark skin denticle diversity

Gianna Mitchell, Ella Nicklin, Steven Byrum, Gareth Fraser

Sharks, skates, and rays have tooth-like, skin appendages called dermal denticles. Denticles function as armor and help reduce drag as sharks swim. Owing to this function, denticle morphology varies greatly not only within individuals of the same species, but also across species and groups. Using a combination of whole-mount clearing and staining, and micro-CT imaging techniques, we investigated the spread and diversity of denticles in eight different locations across the body of the embryo and adult Bonnethead (*Sphyrna tiburo*; a live-bearing viviparous species) and Small-spotted catshark (*Scyliorhinus canicula*; an oviparous species). These two species have seemingly contrasting lifestyles; the Bonnethead shark is a continuously swimming open-water shark, while the small-spotted catshark is a benthic species that spends extended periods as a bottom-dweller in shallow water and moves in short bursts. Comparing embryos to their respective adults, we observed multiple morphological changes, including differences in size, shape and pattern suggesting a functional shift in denticle ontogenetic morphology. Between species, we also observe drastic morphological variation between denticles from equivalent regions of the body. We suggest that such morphological diversity in denticle type may be due to the functional needs of occupying alternative habitats. Furthermore, drastic shifts from the first embryonic denticles to adult morphology appears to be characteristic of some species, not all, which might be a response to the variety of gestational modes in sharks.

Ecological & morphometric traits are linked to recovery of bottlenecked populations in birds

Matthew Mitchell, Amanda Trask, John Ewen, Ryan Felice

As vertebrate biodiversity becomes increasingly threatened, understanding the ability of the most endangered species to recover is becoming extremely important. Recent evidence shows that species that exhibit certain physical and ecological traits may be more or less likely to be at risk of extinction than others, but little is known about how these traits may then be linked to population recovery. Here we aim to determine which phenotypic or ecological traits may be linked to potential post-bottleneck population recovery in endangered species of birds. Using the IUCN Red List, the recently published AVONET database and additional primary material, we investigate how phenotypic traits predict conservation outcomes in species that have experienced extreme population bottlenecks (< 50 individuals) since 1950. Employing Bayesian ecological modelling techniques, we find that ecological traits are linked to the probability of bird species recovering from a population bottleneck, with birds that primarily feed aquatically less likely to recover, as well as those that feed primarily on insects. We find that birds exhibiting certain morphometric traits, such as shorter, thinner beaks are also less likely to recover. Our results indicate that in a constantly-changing world where population sizes are at risk of falling to extremely low levels, birds that exhibit these traits are more likely to be at risk of extinction, thus providing information on which future conservation efforts can be planned.

Deconstructing a Complex Lens

Amartya Mitra, Shubham Rathore, Annette Stowasser, Ruby Hyland-Brown, Augusta Jester, Elke Buschbeck

For eyes to function properly, they need to be correctly focused. This process requires coordinated development of the eye's components such as the lens and retina, to receive a clear image. Establishing this state of correct focusing and maintaining it through growth is challenging, as the distance between the lens and retina, and the optical properties of the lens are subject to change. In vertebrates, this challenging process is achieved partly through active regulation in the form of neural feedback from visual input. However, the process of establishing this state in arthropods remains unclear. Here, we examined vision in larvae of the sunburst diving beetle, *Thermonectus marmoratus*. Using RNAi to knockdown *Lens3* (a widely expressed lens protein) during post-embryonic growth, we aimed to defocus the principal eyes of these visually guided predators. Although we did not detect significant changes in the focal length of lenses, *Lens3* knockdown resulted in the formation of 'cataracts', causing the projection of blurry, low contrast images. Further, using a custom-built microphthalmoscope, we did not observe systemic changes in where eyes were focused. This is unlike vertebrates, in which form-deprivation, or attenuation of image contrast, results in the dysregulation of eye growth, causing refractive errors such as myopia. Our results are consistent with previous findings that arthropods do not require visual input to establish correctly focused eyes and point to *T. marmoratus* as an informative biological model to explore the poorly understood etiology of refractive errors.

The Tail End of Things: Comparative muscular morphology and function of mammalian tails

Juri Miyamae, Talia Moore

Mammalian tails have diversified alongside changes in locomotion as mammals radiated into the world's terrestrial, aquatic, and aerial environments. However, the intricate and challenging anatomy of the muscles and tendons comprising the tail has rendered this section of the axial skeleton relatively unexplored. Our survey of tail muscular anatomy collected from dissections and diceCT scans in a phylogenetically diverse sample of mammals has found that caudal musculature is organized into longitudinal tracts that include bilateral dorsal and ventral tracts of intrinsic muscles as well as dorsolateral and ventrolateral tracts of extrinsic muscles. Anatomical data collected from the dissection of unfixed specimens revealed an unusual morphology for these extrinsic muscles as a sheet of fused muscle segments with associated tendons that span multiple joints to effect movement of distal caudal vertebrae. We noted diversity in relative tail length, number of caudal vertebrae, relative integration of the caudal and hindlimb musculature, relative size and shape of the extrinsic musculature, and tendon branching patterns. In addition, we introduce preliminary data from material testing of tendons and physical models to understand the biomechanics of this unconventional muscle-tendon system as a foundation towards building form-function relationships across mammal clades and locomotory types.

Runaway chromosome evolution in a clade of freshwater fish

Brendan Mobley, Andrew Anderson

Freshwater fishes display remarkably conserved chromosome counts, with over half of all karyotyped species having a diploid chromosome number (2N) of 48 or 50. Most species in the family Osphronemidae share follow this trend, but within the subfamily Luciocephalinae, two species have been noted for their low chromosome number ($2N \leq 20$). Large disparities within a family are extremely rare, and with little cytogenetic information on Luciocephalinae available in the literature, we aimed to determine how, when, and why these shifts in chromosome count occurred. We collected data on eight of nine species within Luciocephalinae, establishing novel karyotypes for five. We find the subfamily Luciocephalinae has a karyotype evolution pattern characterized by a rapid series of inter- and intra-chromosomal rearrangements, and a large reduction in chromosome number is implicated in the early evolutionary history of the clade. We propose several possible explanations for this pattern.

Thermal Limitations in Slimy Salamanders: Influenced by Latitude

Aida Mohd-Khairi, Martha Munoz, Nathalie Alomar

All ectotherms have critical thermal limits, the upper and lower thresholds of temperatures where a loss of locomotion occurs. With the rate of climate change increasing, understanding how ectotherms' critical thermal limits vary can provide insight into how vulnerable they are to extreme temperatures. Our work focuses on slimy salamanders, a sublineage of woodland lungless salamanders (Plethodon). We tested to see if their critical thermal limits vary across species and if those limits correlate to the environments where they are found with respect to their latitude location ($^{\circ}$ N/S). We conducted critical thermal limit experiments on slimy salamanders collected from the southeastern United States. We hypothesized that their critical thermal limits would vary and that northern species would have lower CTmins/maxes in comparison to southern species. We found that critical thermal limits do vary across the Plethodon glutinosus sublineage. The temperatures ranged from -1.2°C to 0.7°C for CTmin and from 29.9°C to 33.8°C for CTmax. We found that the critical thermal limits of this sublineage weakly correlated to the latitude where they were found. CTmin values were found to be lower in species found more north; however, CTmax did not show a relationship with latitude. While the critical thermal limits do vary within this sublineage, more factors than just latitude seem to be influencing this variation.

Experiments and modelling of swarming induced fluid velocity

Nina Mohebbi, Joonha Hwang, Matthew Fu, John Dabiri

Zooplankton in the ocean make a synchronized daily vertical migration through hundreds of meters of the ocean water column. This migration may be the largest migration on earth by mass and a primary source of biologically generated turbulence. Thus, this migration potentially impacts climate modeling, solute mixing, and gas exchange. However, estimates of the magnitude of mixing from field and laboratory measurements span several orders of magnitude.

We utilize brine shrimp (*Artemia salina*) as a model organism to investigate the effects of collective movement on the fluid environment. Leveraging the positive phototaxis of brine shrimp, we induce synchronized vertical migration and capture three-dimensional trajectories of individual swimmers within the swarm with a single camera and scanning laser. An analytical model estimates the mean convection velocity induced by the animals' combined wakes. Individual animal wake structures are derived semi-empirically and combined with captured animal trajectories to construct a theoretical fluid velocity field based on a momentum-conserving model of wake superposition.

Suburban Landscapes as Winter Habitat for Migratory Birds

Claire Molina

Since 1970, total bird populations in North America have decreased by 2.9 billion birds. Habitat loss and urbanization are leading causes of bird decline, and with the reduction of natural habitat, migratory songbirds can be found using suburban areas for over-wintering grounds. This research asked how suburban areas compare to natural habitats in supporting the White-crowned Sparrow, a migratory bird. We studied sparrows in two areas in Claremont, California: a natural reserve with native habitat and suburban neighborhoods. We used mark-recapture and automated cameras to collect data on locations of birds and collected fecal and blood samples. Between the two environments, we compared return rates to the wintering ground, infection rate of gastrointestinal parasites and white blood cell counts. We predicted that when compared to natural habitat, birds caught residing in suburban areas for the winter will have higher infection rates of gastrointestinal parasites and a higher Heterophil to Lymphocyte ratio based on previous research that shows suburban areas disseminate disease among birds at higher rates. However, we predict that the return rate will be higher for birds in suburban areas because there are more consistent sources of food provided.

Mitogenomic rearrangements and phylogenetic relationships among *Pristimantis achatinus* and relatives

Toriann Molis, Utpal Smart, David Rodriguez

Species identity and phylogeny based primarily on morphology has presented challenges due to cryptic speciation in hyper diverse assemblages. The use of molecular data for species identification (i.e., barcoding) and phylogenetics can help circumvent these issues. In this context, the mitochondrial genome (mitogenome) has been extensively leveraged, given its rapid rate of evolution (relative to the nuclear genome), lack of recombination, and conservative gene order. Several studies have revealed changes to the typical vertebrate gene order within the mitogenome across a multitude of taxa. These rearrangements can make sequencing mitochondrial loci challenging, as Sanger and most short-read technologies are unable to recognize structural variants (SVs). These challenges can be overcome by using long-read sequencing. In this study, we used Oxford Nanopore Technologies (MinION) long-read sequencing to characterize the mitogenome of *Pristimantis achatinus* from two geographically distinct populations in Ecuador. Previous attempts to sequence informative mitochondrial loci for this putative species complex have been unsuccessful due to suspected SVs. Additionally, we sequenced close relatives of *P. achatinus* in the *P. conspicillatus* species group to trace the evolutionary history of the SVs to their most recent common ancestor. We uncovered mitogenomic rearrangements unique to the *P. achatinus* mitogenome for both populations. Our discovery of SVs implies that phylogenies and species delimitation need to be revisited in *Pristimantis*, the most speciose terrestrial vertebrate genus in the world.

Aedes japonicus phytophagy of fly poison (*Amianthium muscivictim*) and analysis of floral volatiles

James Moloney, Irving Upshur, Chloe Lahondere

Although it is a popular belief that all mosquitoes consume blood for energy, only females of some species of mosquitoes are known to consume blood meals. Moreover both male and female mosquitoes primarily get their energy from plant carbohydrates. Mosquitoes use a combination of olfactory and visual cues to locate nectar meals. At Mountain Lake Biological Station (Pembroke, VA), the invasive *Aedes japonicus* mosquito was observed landing on the native fly poison plant, *Amianthium muscivictim*. To better understand this plant-insect interaction, we first released lab-reared *Ae. japonicus* mosquitoes into enclosures containing one fly poison inflorescence and collected them after 16 hours. Second, we screened each mosquito for pollen grains on its body and nectar consumption using the anthrone assay. Finally, we collected the volatile organic compounds that make up the floral scent using porapak traps and analyzed them using GC/MS. We found that 29% of the mosquitoes tested positive for fructose. Pollen was likely primarily collected during sugar feeding, but not all positive pollen mosquitoes were positive for fructose. Nonanal and α -pinene make up the majority of the volatiles emitted by the fly poison inflorescence. This study provides critical insights into the development of new baits to control populations of *Ae. japonicus* which will become increasingly important as the climate warms and this mosquito species range increases.

Leveraging individual power to improve racial equity in academia

Patrick Monari, Emma Hammond, Candice Malone, Amelia Cuarenta, Lisa Hiura, Kelly Wallace, Linzie Taylor, Devaleena Pradhan

Academia in the United States continues to grapple with its longstanding history of racial discrimination and active perpetuation of racial disparities. Universities and academic societies must grow in ways that reduce racial minoritization and foster racial equity. What are the effective and longlasting approaches academics should prioritize to promote racial equity in our communities? To address this, the authors held a diversity, equity, and inclusion (DEI) panel during the Society for Behavioral Neuroendocrinology 2022 annual meeting. Here, we synthesize the panelists' recommendations for fostering racial equity in US academic communities.

Chronic intranasal oxytocin impairs behavioral synchrony between California mice

Patrick Monari, Candice Malone, Emma Hammond, Yiru Chen, Lillian Li, Zhimin Hu, Sumanth Karnati, Zhiwei Xue, Catherine Marler

Chronic oxytocin administration has lasting effects on affiliative behavior in monogamous mammals, such as prairie voles and titi monkeys, likely through long-term structural plasticity within social brain networks. However, the influence of chronic oxytocin over cooperative behavior in such species is unclear. Using opposite-sex California mice, monogamous and jointly territorial rodents, we investigated the role of chronic oxytocin in pair coordination and communication. Adult females and males received a daily intranasal dose of 0.5 IU/kg oxytocin or saline over 7 days, a treatment regime we previously found to alter neuroplasticity after 3-weeks. Following a similar 3-week delay, we introduced males and females to examine courtship and pair bond development. One week after introductions we exposed pairs to playbacks of aggressive vocalizations, as we previously observed both pairing and acute oxytocin to create similarities in their joint approach of this stimulus. We investigated behavioral synchrony throughout pair introduction and bond development using markerless pose estimates during undisturbed observations and playback tests. Time-lagged cross-correlations of these estimates revealed that chronic oxytocin pairs were less synchronous throughout courtship and bonding. Additional analyses will be described. Our results suggest that chronic oxytocin may have negative long-term impacts on pair coordination.

Variable warm temperature elevates thermal tolerance but impairs freshwater tolerance in killifish

Michelle Monette

Climate change is increasing both mean temperature and temperature variability in aquatic environments. Therefore, it is critical to understand if organisms can acclimatize to new temperature regimes, and whether this limits physiological flexibility in response to other stressors. We investigated the effects of constant and variable warm temperature on thermal and freshwater tolerance of killifish (*Fundulus heteroclitus*). Killifish were acclimated to control (20°C), constant warm (28°C), and variable warm (20-28°C, shifting every 2 days) conditions for 4 weeks. We then used critical thermal maximum (CT_{max}) assays and freshwater challenges (transfer from 30 to 0 ppt for 24h) to examine thermal and freshwater tolerance, respectively. Indices of whole-animal physiology (hematocrit, osmolality, glucose) were also examined. Acclimation to constant and variable warm temperature resulted in a 2.6°C and 1.8°C increase in CT_{max}, respectively, however, this was associated with impaired freshwater tolerance as indicated by reduced plasma osmolality after freshwater challenge. Hematocrit was not identified as a mechanism underlying improved thermal tolerance. Overall, we show that acclimation to both constant and variable warm temperature improves the thermal tolerance of killifish and that this is associated with a cost to osmoregulation in freshwater. We suggest that acclimatization to new temperature regimes imposed by climate change may reduce the osmoregulatory flexibility of some organisms. Future work will examine gill transcriptomics and morphology to investigate the mechanisms underlying this physiological tradeoff.

An engineering perspective to unravel adaptive organismal locomotion

Jean-Michel Mongeau, Kaushik Jayaram

Robustness and adaptive control of locomotion arise from both physical (i.e., body design) and computational (i.e., brain) intelligence. Robustness can be achieved by well-tuned sensory feedback and smart mechanical design. Thus, robustness can enable animals to maintain performance without any learning. Alternatively, animals can learn or adapt over time to maintain adequate performance. While robust and adaptive control processes are not mutually exclusive, i.e., organisms can use both strategies, teasing out their relative contribution is of great relevance to understanding the flexibility of locomotion. It can also inform the development of bio-inspired fault-tolerant strategies, integrating intelligent control and robust mechanical design. In this concluding talk to our symposium, we highlight grand challenges for the field and present promising mathematical frameworks to unravel principles

of adaptive organismal locomotion. In particular, we will discuss how control theory can serve as a useful mathematical framework for theoretical and experimental decomposition of adaptive locomotion across taxa. Throughout, we will highlight ongoing opportunities to extract principles from biology to build physically intelligent systems.

Residual force enhancement along the descending limb in mouse muscles with different titin genotypes

Jenna Monroy, Siwoo Jeong, Madhusudhan Venkadesan, Kiisa Nishikawa

Residual force enhancement is the increase in the steady-state force after stretch compared to the isometric force at the same final length. Recent work suggests that this definition of RFE overestimates the active component of extra force due to stretching when passive tension is large. We investigated RFE along the descending limb of the force-length relationship in soleus muscles from mice with different titin genotypes (WT, mdm, and Ttn Δ 112-158) characterized by differing passive tension. Muscles were activated maximally, stretched by 5% L0 at a velocity of 1 L0/s, and maintained at the final length until an apparently steady state was reached. RFE was calculated at each length. Similarly, we measured the stress after passive stretch relative to the passive stress at the same final length (DPS) to estimate slow-decaying forces that persist after stretch. Results showed that RFE was greatest in mdm, intermediate in WT, and lowest in Ttn Δ 112-158 muscles. After accounting for passive stretch, RFE was negligible in mdm, and negative in Ttn Δ 112-158 muscles. In addition, DPS was highest in mdm and negative in Ttn Δ 112-158 muscles. Our results suggest that slow-decaying viscous forces contribute to RFE. When passive force is high, the extra force due to stretching is likely due to both viscous and elastic components. Measuring RFE along the descending limb provides additional evidence for a role of titin in active muscle force.

A Dynamical Model of Growth and Maturation in *Drosophila*

Amirali Monshizadeh, John Tyson, Stanislav Shvartsman, Alexander Shingleton

The decision to stop growing and mature into an adult is a critical point in development that determines adult body size, impacting multiple aspects of an adult's biology. In many animals, growth-cessation is a consequence of hormone release that appears to be tied to attainment of particular body size or condition. Nevertheless, the size-sensing mechanism animals use to initiate hormone synthesis is poorly understood. Here we develop a simple mathematical model of growth cessation in *Drosophila melanogaster*, which is ostensibly triggered by attainment of a critical weight early in the last instar. Attainment of critical weight is correlated with synthesis of the steroid hormone ecdysone, which causes a larva to stop growing, pupate and metamorphose into the adult form. Our model suggests that, contrary to expectation, the size-sensing mechanism that initiates metamorphosis occurs before the larva reaches critical weight; that is., the critical weight phenomenon is a downstream consequence of an earlier size-dependent developmental decision, not a decision point itself. Further, this size-sensing mechanism does not require a direct assessment of body size, but emerges from the interactions between body size, ecdysone and nutritional signaling. Because many aspects of our model are evolutionarily conserved among all animals, the model provides a general framework for understanding how animals decide to transition from their juvenile to adult form.

The effect of food quality and water availability on resource allocation in *Diploptera punctata*

Isabella Montavon, Thomas Gerth, Agus Munoz-Garcia

Basic biological tasks like maintenance, growth, and reproduction, need an allocated amount of resources to be completed. During times of limited resources, some tasks are prioritized over others, leading to biological tradeoffs, adjustments that impact fitness of individuals. In this research, pregnant females of *Diploptera punctata*, a viviparous species of cockroach, were used to test the effect of food and water availability on resource allocation. We used two dietary regimes: high-quality food (HQ) and low-quality food (LQ), and two environmental conditions of water availability: restricted and ad libitum. We

measured body mass, food consumption, food assimilation, total evaporative water loss, and oxygen consumption, on days 0, 15, 30, 45, and 55 of pregnancy for each female. After day 30 of pregnancy, females were assigned to one of these experimental groups: Control High quality (C-HQ) females, given water ad libitum and HQ food. Control-LQ, given water ad libitum and LQ food. Water deprived females given HQ food (WD-HQ); and WD-LQ females. Results suggest that pregnant females change their resource allocation patterns when eating low-quality food and when deprived of water. Understanding the adjustments of physiological variables in animals exposed to different environmental conditions is important to predict changes in the distribution of species in our current biodiversity crisis.

Evolution of thermal sensitivity across levels of biological organization

Kristi Montooth, Ibrahim ElShesheny, Omera Matoo, Maathir Basi, John DeLong

Temperature accelerates biological rates. Yet, our understanding of temperature-dependent declines in organismal function and population growth remains fragmentary. We characterized the thermal sensitivity of traits across levels of biological organization including mitochondrial respiration, metabolic rate, life history, and population growth in an outbred population of the fruit fly *Drosophila melanogaster*. The thermal optimum for mitochondrial respiration coincided with the thermal optimum for fecundity at 22C, the temperature at which this population was reared in the lab. While metabolic rate and development rate continued to accelerate with temperatures beyond this thermal optimum, mitochondrial leak increased, and survivorship and fecundity declined. The thermal sensitivity curve (TSC) for population growth rate emerged from the thermal sensitivity of underlying life-history traits, and from temperature-dependent integration of these traits. For example, the relationship between parental age and offspring survivorship was itself thermally sensitive. Activation energy increased, TSCs got narrower, and trait declines became steeper across increasing levels of biological organization. This multivariate approach identified the thermal sensitivity of the mitochondria as a critical component underlying the cost of accelerating the pace of life in ectotherms. I will also present data on the evolution of traits and TSCs after replicate populations derived from this base population were evolved at 17C and 28C, including early embryo thermal tolerance, a trait that is linked to maternal provisioning of eggs.

Effects of post-weaning food texture modification on chewing behavior and kinematics in pigs

Stephane Montuelle, Susan Williams

As young mammals transition from liquid to more solid foods, they are exposed to a more challenging range of food properties that must be transformed into a swallowable bolus. This exposure is essential for the maturation of motor systems and sensorimotor control of feeding movements. Infants and children encountering difficulties with swallowing may benefit from a more gradual transition where solid food texture is mixed with liquids to make the bolus easier to process and swallow. However, long-term use of texture modified diets may delay or impede the development of mature chewing behavior. We assess the effects of post-weaning diet on chewing dynamics by comparing two groups of pigs maintained on regular hard chow (control group) versus chow mixed with water to generate a softer and thicker food texture (texture modified group). We found that post-weaning texture modification alters feeding behavior on novel foods by increasing the time between two successive swallows, and reducing the number and frequency of ingestion cycles to allow for more chewing cycles per swallow. X-ray Reconstruction Of Moving Morphology also shows that texture modification reduces chewing cycle duration, the duration of tooth-food-tooth contact, and the amplitude of jaw movements. These effects all suggest that the lack of post-weaning exposure to solid foods affects intraoral food management and kinematics necessary to modify the food bolus during chewing. Funding: NIH grant #1R15HD101984-01.

Neuromotor specialization for gestural communication in the downy woodpecker

Nicole Moody, Nicole Melendez, Matthew Fuxjager

Spinal motoneurons initiate skeletal muscle contractions to produce communicative behaviors, including vocalization and gesture based signals. Motoneuron structure and specialization for producing complex vocalizations have been described in songbirds; however, the motor pathways underlying gestural communicative displays are relatively unknown. One such gestural signal is the woodpecker “drum,” which occurs when individuals rapidly hammer their beak on a hard substrate. Drums require highly precise muscle contractions, with species-specific patterns of beat production that are differentiated by milliseconds of timing. Such precision sustained at a rapid rate likely requires specialized neural structures to control the relevant neck musculature. Here, we compare properties of motoneurons innervating neck muscles (longus colli dorsalis and ventralis) in drumming (downy woodpecker) and non-drumming (house sparrow) species to determine how the neuromotor system might be specialized for the production of drum signals. We hypothesize that motoneurons controlling the woodpecker neck musculature will be more abundant and larger when compared to house sparrow motoneurons. Additionally, we describe the location of longus colli motor pools in the spinal cord, setting the stage for future neuroanatomical work in this system.

Swimming, terrestrial, and land-to-water locomotion in an aquatic caterpillar

Amalia Moore, Beckett Socha, Makenna Moore, Josh Taylor, Yohan Sequeira, Ignacio Moore, Jake Socha

Numerous insect groups exhibit aquatic larvae, ranging from mosquitoes to dragonflies and mayflies. For lepidoptera, the vast majority of species exhibit terrestrial larvae, and only a few are facultatively or obligately aquatic. How do caterpillars swim, and how do their swimming and terrestrial modes of locomotion differ? Here, we recorded active swimming in water, crawling on land, and transitioning from land to water in one species of neotropical aquatic caterpillar (probable genus: *Paracles*) in Peru. Caterpillars ($n=12$) were first recorded swimming in natural conditions in a pond using a GoPro 4 videocamera, and then collected and tested under laboratory conditions. In-lab recordings were conducted with two synchronized GoPro 4 cameras presenting top and side views of caterpillar movements in a plastic container. When fully submerged in water, caterpillars swam using side-to-side undulation, with the posterior end of the body at the surface and the anterior body tilted slightly downward. On land (a flat board), caterpillars used stereotypical inchworm-like movements, with dorsoventral waves in the vertical plane. In transition (board tilted downward into the water), caterpillars inched toward the water and only began lateral undulation once the entire body was submerged. This pattern of movement differs from that of terrestrial caterpillars as well as all other previously reported aquatic larvae, suggesting that these caterpillars have evolved specialized behaviors for aquatic-dominated phases of development.

Sex, size, & body condition influence exploratory behavior in wild kangaroo rats (*Dipodomys deserti*)

Katrina Moore, Anahita Sadrossadat, Oceanus Zhang, Craig McGowan, Monica Daley

Animal behavior and movement is diverse, and individuals may vary in their behavioral strategies according to age, mass, sex, and personality. Correlated behaviors can reveal a spectrum of risk-taking propensity within a population and drivers of different responses to environmental change. We aimed to understand how body size and condition contribute to risk-taking behavior and hypothesized that since larger individuals may have greater muscle power capacity, they exhibit more exploratory behaviors and faster speeds when exposed to a novel environment. We evaluated 10 behaviors and measured locomotor performance of wild desert kangaroo rats (*Dipodomys deserti*; $n=16$) within an enclosure (~5m²) in the species' natural home range. We used a principal component analysis (PCA) to examine correlations among exploratory and risk-avoidant behaviors and ran linear mixed-effects models to assess relationships between PCs, anatomical measurements, and locomotor performance. We found correlations in behaviors that differentiate

between risk-tolerant/exploratory and risk-averse behavior. Additionally, we found that sex, foot length, and relative body condition influenced risk-taking propensity. While results indicate that some risk-taking behaviors are informed by body size, there was much unexplained variance in behavior. This suggests that life experience, hormonal and nutritional status, age, and personality may influence behavior. Future studies examining correlated behaviors could track behavioral variation within populations and across generations as human influence and climatic states vary, thus informing management and conservation practices.

Effects of Lead on Performance Measures in the Common Wall Lizard (*Podarcis muralis*)

Maya Moore, Emma Foster, Ali Amer, Logan Fraire, Alyssa Head, Annelise Blanchette, Alex Gunderson, Eric Gangloff

Living in urban environments presents many challenges to wildlife, including exposure to potentially toxic pollutants. For example, the heavy metal lead introduces numerous health problems to various urban organisms, including humans. The little work that has been conducted on lead toxicity in reptiles suggests that lizards may be extraordinarily resilient to very high levels of lead pollution, by either avoiding or mitigating the toxicity. To assess the impact of lead exposure, we related performance and levels of lead in the blood in adult male common wall lizards (*Podarcis muralis*, $N = 41$) – a small reptile particularly capable of thriving in urban environments. We captured lizards from various locations across Cincinnati, Ohio, USA and quantified the concentration of lead in blood samples. We then tested two aspects of lizard performance important for survival: (1) Balance, a cognitively-demanding task, to assess the effect of lead on cognition, and (2) Running endurance, a type of aerobic exercise dependent on oxygen, to assess the impact of lead on blood oxygen-carrying capacity. We then used correlation analyses to quantify the relationship between lead levels and these ecologically-relevant performance measures. Understanding the effects of lead levels in a particularly resistant animal could help us better respond to public health and environmental pollution concerns.

Socially mediated reproductive development in incipient queen bumble bees

Meghan Moore, Sarah Woodard

Animals that live in proximity can exhibit social regulation, where social cues influence the development, behavior, and physiology of group members. Research on social regulation in eusocial insects has focused heavily on how it is mediated by pheromones. In relation to reproduction, pheromones play a prominent role in queen-mediated inhibition of egg laying by workers. Here, we examined the reverse scenario: how queen reproduction is regulated by workers. We explored how the premature addition of workers and males during an early nest stage influences queens and further explored the specific sensory cues that mediate social regulation of queen reproduction. Queens were either provided workers, males, or were otherwise allowed to initiate nests alone and then further subdivided into two groups in which queens they were or were not separated from added nest mates. We further explored the importance of tactile cues through stimulus exposure via an automated system designed to directly deliver a tactile stimulus to queens. Our data suggest that control of queen reproductive development is mediated by multiple, caste-specific sensory signals. This study provides insights into the sensory cues provided by the presence of perceived helpers in mediating social regulation within bumble bees.

A mating innovation facilitates niche expansion and buffers species against climate change

Michael Moore, Sarah Nalley, Dalal Hamadah

One of the main drivers of life's diversification has been the emergence of “evolutionary innovations”: the evolution of novel traits that grant access to underused ecological niches. Since ecological activities can occur separately from mating, traits that facilitate mating have not traditionally been considered as factors in niche evolution. However, in order to persist in their environment,

animals need to successfully mate just as much as they need to grow and survive. Evolutionary innovations that facilitate mating activity may therefore be an overlooked determinant of species' ecological limits. Here, we show that species' historical niches and responses to contemporary climate change are shaped by an evolutionary innovation involved in mating—a waxy, UV-reflective pruinescence produced by male dragonflies. Physiological experiments demonstrate that pruinescence reduces heating and water loss. Phylogenetic analyses show that pruinose species are more likely to breed in exposed, open-canopy microhabitats. Biogeographic analyses reveal that pruinose species occupy warmer and drier regions in North America. Citizen-science observations of *Pachydiplax longipennis* suggest that pruinescence can evolve to match local conditions. Finally, temporal analyses indicate that pruinose species have been buffered against contemporary climate change. Overall, these historical and contemporary findings show that successful mating can shape the evolution of species' niche limits in the same way as growth and survival.

Running away or running to? Do prey incorporate both fear and safety in their decision making?

Paul Moore

Predator prey interactions are a key part of ecosystem function, and non-consumptive effects fall under the landscape of fear theory. Under the landscape of fear, the antipredator responses of prey are based on the spatial and temporal distribution of predatory cues in the environment. However, the aversive stimuli (fear) are not the only stimuli prey can utilize when making behavioral decisions. Prey might also be using attractive stimuli that represent safety to guide decision making. Using a novel, orthogonal design, we were able to spatially separate aversive and attractive stimuli to determine if prey are utilizing safety cues to navigate their environment. Using a crayfish-bass system, we examined the relative role of safety and fear cues in decision making. Crayfish responded more strongly to alarm cues than fear cues, with only alarm cues significantly impacting habitat utilization. When responding to alarm cues, crayfish used safety cues as well as fear cues to relocate themselves within the arena. Based on these results, we argue that crayfish are utilizing a landscape of safety in conjunction with a landscape of fear when navigating their environment.

Forelimb Muscle Properties of Geomyid and Heteromyid Burrowing Rodents

Lexi Moore-Crisp, Jordan Fain, Madeline Stears, Michael Butcher

Pocket gophers have hypertrophied forelimbs with large mechanical advantage (MA) for burrowing, whereas those of semi-fossorial pocket mice and kangaroo rats are smaller in size and lack MA, yet these taxa dig elaborate burrow systems similar to gophers. To understand the functional capacities of their respective forelimb musculature, dissections of Botta's pocket gopher (*Thomomys bottae*; N=10), desert pocket mouse (*Chaetodipus penicillatus*; N=5), and Merriam's kangaroo rat (*Dipodomys merriami*; N=3) were conducted to quantify limb MA, muscle architectural properties, and myosin heavy chain (MHC) isoform content. As expected, metrics indicating MA were significantly larger in *T. bottae* than in both *C. penicillatus* and *D. merriami*. Except for the limb (humeral) retractors, the latter two taxa generally have longer, parallel fascicles and shorter moment arms in the power stroke muscle functional groups than pocket gophers. The limb retractors of *C. penicillatus* and *D. merriami* have large force per unit mass capacity by physiological cross-section area to muscle mass (PCSA/MM) ratios nearly 2x those of *T. bottae*. MHC content is also notably fastest in *C. penicillatus* and slowest in *T. bottae*. All three species, however, mainly expressed fast MHC-2A and -2X in proportion to body size. The findings provide evidence that pocket mice and K-rats compensate for their lack of MA by their forelimb muscles being capable of both high intrinsic contractile velocity and size-scaled force production.

Warming Waters Hinder Burying within the Common Sand Dollar, *Echinarachnius parma*

Journey Moore-Prewitt, Terence Leach

As climate change drives global temperature increases across marine ecosystems, it is necessary to study how warming waters may impact the basic and obligatory behaviors of ecologically relevant organisms. In coastal environments, the burrowing behavior of sand dollar species is crucial to nutrient cycling and generally maintaining a healthy, balanced marine benthos. In this study, the impact of temperature on this neuromuscular function (burying and movement) was evaluated by measuring rate of burying within the common sand dollar, *Echinarachnius parma*. Sand dollars were acclimated to two temperature treatments for 16 days, either: 16°C (n=4), representative of their current, ambient temperatures in summer months, and 22°C (n=5), representing summer temperatures projected by the end of the century. Throughout the acclimation period, sand dollars from the 16°C group buried themselves in significantly less time than those from the 22°C treatment. However, this difference in burying time between treatments was diminished when individuals from the elevated temperature treatment were allowed to recover at 16°C for four days. The results from this study suggest that rising temperatures may have an impact on the burying rate of sand dollars. As temperatures continue to rise, it could become increasingly difficult for sand dollars to perform the necessary functions that preserve both their own health as well as that of their coastal ecosystem.

Role of vision in dynamic camouflage of adult summer flounder (*Paralichthys dentatus*)

Vanessa Moreno, Lorian Schweikert

Dynamic camouflage is the capacity to rapidly change skin color and pattern, often for the purpose of background-matching camouflage. Summer flounder (*Paralichthys dentatus*) have an exceptional capacity for dynamic camouflage, and with eyes that face away from the substrate, it is unknown if they use vision to mediate this behavior. Past studies have shown that summer flounder can match to the pattern (i.e., spatial detail) of substrate with a high degree of precision, and for that to be achieved using sight, one testable assumption is that the resolution of vision must match the degree of detail produced in color-change performance. To test this, we used approaches in morphology and behavior to estimate visual acuity, the capacity of the visual system to resolve static spatial detail. Using image processing techniques, we then compared the spatial complexity of relevant substrates with what may be detectable by flounder spatial vision. We found that flounder visual acuity fell within a range previously determined across other flatfishes 2 - 7.64 cycles per degree (CPD) and is likely adequate for detecting the spatial information required for camouflage over short distances. These data provide new knowledge about the sensory information that is critical to flounder survival.

Guide RNA Design and Delivery for CRISPR/Cas9 Editing in Annual Killifish

Keria Moritsugu-Vandehy, Isabel Henkes, Yekaterina Chmykh, Amie Romney, Jason Podrabsky

The CRISPR-Cas9 genome editing tool has shown to be successful in knocking out genes in model organisms such as zebrafish, turquoise killifish, and cichlid fish. CRISPR-Cas9 genome editing has been demonstrated in many species of fish, but this technology has not been verified in the annual killifish, *Austrofundulus limnaeus*. We hypothesize that targeted editing of the tyrosinase gene in embryos of *A. limnaeus* would lead to the development of fish without the ability to produce melanin, the black/brown pigment molecule. Early embryos (1-cell stage) were injected with a Cas9 cocktail containing a mix of guide RNA molecules that target the genomic sequence of the tyrosinase gene and either an mRNA coding for the Cas9 protein, or Cas9 protein. Guide RNAs were designed using ChopChop, and two guides were selected for injection based on a high predicted percent efficiency for binding with low probability for off-target effects. Many injected embryos developed without expressing black pigment. We found for the first time in this species that Cas9

can be successfully used to knockout the tyrosinase gene. In the future, we plan to establish a breeding line of non-pigmented killifish to aid in embryological studies of this species.

Investigating octopus arm recruitment during blind manipulation of prey

Halia Morris, Ashley Gendreau, Camille Boucaud, Sayre Rooney, Kendra Buresch, Roger Hanlon

Octopuses are soft-bodied creatures that exhibit intricate behaviors and refined chemotactile sensory skills using their eight identical arms. These arms possess elaborate neural connections, enabling remarkable flexibility, coordination, and environmental exploration. Prior research indicates that during visual attacks, anterior arms are preferred for interaction, while neighboring arms assist in manipulating prey. However, many octopus species adopt a “blind exploration” strategy, pouncing on potential prey with arms and suckers. To emulate this behavior, we conducted an animal behavioral experiment using nine *Octopus bimaculoides*, presenting them with the following objects placed within a 3D-printed dome: a frozen crab, a live crab, a 3D-printed rock shape, a crab-infused agarose disk, and a 3D-printed crab shape. Sequential arm recruitment was observed and scored for contact and exploration. Results reveal equal use of recruitment types A+1, A+2, and A+3 when encountering frozen crab, the 3D rock shape, and the crab-infused disk. Similarly, A, A+1, A+2, and A+3 were equally employed when engaging live crab. Adjacent arms (A+1) were frequently recruited for dome exploration. With the 3D crab shape, all recruitment types except A+3 were equally used for contact, while A and A+1 dominated dome exploration ($n = 4$). Remarkably, regardless of the object, all arms were similarly engaged for dome exploration and object contact. Understanding these complex recruitment patterns has significant implications for soft robotics and material sciences, driving innovative engineering solutions and advancements.

Multiple biases distort biodiversity records of freshwater aquatic invertebrate indicator species

Lee Morris, Travis Wendel

Freshwater invertebrates are crucial indicators of water quality and environmental changes, necessitating a robust understanding of their distribution. Limited sampling of these organisms due to resource constraints has led to gaps in species range and abundance data, evident in both scientific research and government-collected information. Citizen science initiatives have surged recently, contributing substantial biodiversity data—exemplified by iNaturalist’s 100+ million observations—beneficial to researchers and organizations once validated. However, these contributions are prone to biases – geographical, taxonomical, observer-based, and habitat-based. Addressing the under-observation of freshwater invertebrates is vital even as casual observers often overlook these aquatic organisms. We are investigating two arthropod taxa, Astacidae and Odonata (Anisoptera), utilizing citizen science databases to analyze distribution and abundance. While both taxa have aquatic life stages, Astacidae (crayfish) are predominantly aquatic, and Odonata (dragonflies) are aquatic only in nymphal stages. We hypothesize that adult Odonates are overrepresented in these databases compared to nymphal Odonates and Astacidae. We are also conducting field studies in the Piedmont region of South Carolina, addressing observation bias in crayfish species. Discrepancies in species presence between adjacent counties with similar geological, hydrological, and ecoregional traits highlight the need for targeted surveys to enhance local biodiversity records.

A geographic comparison of gut microbial flexibility in tree swallows (*Tachycineta bicolor*)

Natalie Morris, Jennifer Houtz, Cédric Zimmer, Conor Taff, Daniel Ardia, Maren Vitousek

Organisms can flexibly shift their phenotype to match environmental demands. In the face of both unpredictable challenges like severe weather, and predictable challenges like the energetically demanding periods of reproduction, appropriate phenotypic flexibility may result in higher fitness. Microbial

flexibility, the ability to dynamically restructure the gut microbiome in the face of environmental change, may mediate phenotypic flexibility in fitness-related traits such as body mass. To explore whether the gut microbiome can act as a mediator of phenotypic flexibility, I analyzed the gut microbial diversity and flexibility of female tree swallows (*Tachycineta bicolor*) during incubation and nestling provisioning from four populations breeding across a geographic range in Alaska, Wyoming, Tennessee, and New York. In the New York population, earlier breeders were heavier and had more diverse microbiomes during mid-incubation. Birds that laid earlier lost more mass and decreased microbial diversity more from mid-incubation through early provisioning. Cloacal microbiome samples are currently being analyzed from the Alaska, Wyoming, and Tennessee populations to determine if our findings persist across a geographic range and to determine the role of environmental unpredictability in microbial flexibility. A better understanding of the relationship between the gut microbiome and the phenotypic flexibility of traits, such as body mass, can provide insight into the mechanisms through which organisms cope with changing environments and their ability to cope with increasing climatic variability.

Hanging on by a thread: how substrate friability affects *M. galloprovincialis* byssal attachment

Mimi Morrison, Ali Cramer

The interaction between sessile marine organisms and the substrate to which they attach influences their survival and persistence within a habitat. Many studies have been done concerning mussel attachment strength as a result of environmental factors, but few are based on the qualities of hard substrate. The aim of this study was to characterize *Mytilus galloprovincialis* byssal attachment to model substrates of varying friability to investigate the influence of physical substrate qualities in the attachment mechanisms of sessile marine organisms to hard substrate. A significant difference was found between the number of byssal threads attached to ($p = 0.006$) and preference for the substrate of lower friability versus higher friability ($p = 0.002$), though the force of removal was not significantly different between substrates ($p = 0.14$). Understanding mussel attachment has implications for mussel behavior, classifying ecosystem structures, and material usage for aquaculture, so these findings provide insight into the interaction between mussel byssal threads and hard substrate friability within many spheres of influence.

Disentangling causes of microbiome variation using reciprocal transplants of *Schizoporella* sp.

Jose Moscoso, Robert Thacker

Microbiomes, composed of symbiotic microorganisms like bacteria, archaea, and viruses, influence host physiology, fitness, and adaptation, and exhibit variation across diverse environments. High-throughput sequencing facilitates microbial community analysis, driving studies on microbiome responses to environmental shifts. However, disentangling the influence of microbiome-environment interactions and host genetics during microbiome assembly remains challenging. Reciprocal transplants, an ecologically-rooted technique, offer a strategy for dissecting the impacts of changing environments on microbiomes. In the Summer of 2022, we translocated *Schizoporella* sp. colonies via reciprocal transplants at four locations on Long Island, NY to examine changes in microbial community diversity and composition due to a rapid environmental change. We characterized the host’s microbiome by sequencing bacterial 16S rDNA using an Illumina platform. We initially identified host taxonomy visually, and later validated by Nanopore sequencing of a CO1 marker gene of the host, cross-referenced with BLAST. Results reveal variations between sites in *Schizoporella* sp. microbiomes, reflecting genetic or environmental differences. However, translocated hosts’ microbiomes shifted to match local conspecifics, thus suggesting a relatively stronger influence by the environment on microbiome composition. Reciprocal transplants in marine invertebrate microbiome research advances knowledge of microbiome-environment interactions during environmental disturbances. These insights have broad implications spanning ecology, evolution, conservation, and biotechnology, guiding mitigation of environmental change impacts on hosts and their microbiota.

Inverse dynamics model for suspensory locomotion in brown-throated three-toed sloths

Angela Mossor, Andrew McKamy, Melody Young, Michael Granatosky, Michael Butcher, Jesse Young

Long bone loading in upright animals has been well-studied across many tetrapod taxa. Overall, these studies show that limb elements experience greater bending loads than axial loads. However, few studies have evaluated bone loading in animals that employ below-branch arboreal locomotion. Tree sloths are among the rare mammalian taxa that demonstrate near obligatory suspensory habits as part of their arboreal lifestyle. Consequentially, limb bone loading in tree sloths may then indicate higher levels of axial (i.e., tensile) loading and reduced levels of bending. To test this hypothesis, we used an inverse dynamics model to assess *in vivo* bone loading during suspensory walking in brown-throated three-toed sloths (*Bradypus variegatus*; N=4). Bone loading patterns show that both proximal and distal elements (femur, tibia) of the hindlimb experience predominantly axial loading, whereas bone loading in the forelimb distal elements (radius, ulna) is mainly axial but primarily bending in the proximal element (humerus). The predominance of axial loading suggests that limb loading patterns in suspensory species differ from those of upright mammalian taxa. Further material testing of tree sloth long bones is needed to verify how the limb bone structural properties may adapt to this unusual loading regime.

Running on a rocky road: the impacts of substrate rugosity and movement cue on sea urchin locomotion

Andrew Moura, Caleb Mast, Austin Garner

Sea urchins are ecologically and economically important herbivores in near-shore marine ecosystems. Their success as herbivores is largely dependent upon the coordinated action of their spines and tube feet, which interact with the substrate to generate friction and adhesion, respectively. The coral and rocky reefs that many species of sea urchin inhabit are inundated with substrates that likely vary drastically in their micro- and macroscale topography. The impacts of microscale surface roughness on organismal attachment and locomotion have received considerable attention, while similar investigations involving macroscale surface roughness (i.e., rugosity) are less common. Furthermore, recent studies examining sea urchin locomotor performance in the laboratory have employed artificial cues (e.g., dilute bleach, hot freshwater) to induce locomotion for biomechanical analysis, yet their efficacy relative to natural movement cues has not been examined. Here we studied the individual and interactive effects of substrate rugosity and movement cue on the locomotor performance of the Pacific rock-boring urchin, *Echinometra mathaei*. Sea urchins were coerced to move on all combinations of three different substrates (smooth glass, 0.5 cm glass rocks, and 2.5 cm glass rocks) and two movement cues (hot freshwater and extract from crushed conspecifics). Our findings will enhance our understanding of how these dominant herbivores navigate their complex and heterogeneous environment and improve our ability to assay their locomotor performance in the laboratory.

Running up that hill: Locomotion of sea urchins on variable inclines and surface roughness

Andrew Moura, Austin Garner, Aria DiLeo, Maria Garcia

Sea urchins are dominant herbivores in coastal marine habitats and the results of their grazing and associated movements have profound effects on community organization and structure. These habitats are three dimensionally complex and consist of a variety of living and non-living surfaces which may limit sea urchin mobility. Sea urchins navigate these habitats using a combination of friction-inducing spines and adhesive tube feet. Both substrate incline and rugosity of marine habitats individually influence sea urchin locomotor performance and movement, with reduced performance at steeper inclines and greater rugosity. However, the potential interactive effects of incline and surface roughness on locomotor performance are unknown. We assessed the effect of varying surface roughness and substrate incline on the locomotor performance of the sea urchin, *Echinometra lucunter*. Mechanistic explanations for performance differences were explored by assessing the individual performance of sea urchin tube feet and spines on the range of test

substrates. Our findings will refine our understanding of how these important herbivores navigate variable substrates in complex habitats and identify the relative contributions of spines and tube feet during locomotion in ecologically relevant circumstances.

Integrating genetics, phenotype, and physiology in a hybrid population of *Plethodon*

Kyle Moxley, Nathalie Alomar, Martha Munoz

Woodland salamanders in the genus *Plethodon* are considered an example of a nonadaptive radiation, consisting of 56 species with low morphological variation and a tendency to hybridize. These features make the study of their evolution and current-day interactions both particularly relevant for understanding the formation of new species and particularly difficult. This study synthesizes genetic, morphological, and physiological techniques in the study of a recently-formed hybrid population between two woodland salamanders, *Plethodon shermani* and *P. teyahalee*, in order to understand population dynamics and assess the accuracy of using coloration to predict hybrid status of the salamanders. Salamanders were collected along a transect known to include the hybrid zone for the two parent species, and experiments were done on living salamanders to determine thermal limits and preferences. Skin pattern scoring and linear morphometrics were completed on photos of specimens and DNA was extracted to perform a triple-enzyme RADseq analysis. When complete, this will tell us the hybrid status of the collected salamanders and show what links, if any, exist between the hybrid status of an individual in this group and its morphology, physiology, and coloration. These results will clarify previous studies of *P. shermani* and *P. teyahalee* that only used coloration to determine hybrid status and will aid in a greater understanding of what drives speciation and hybridity in *Plethodon* salamanders as a whole.

Bioprospecting British Columbia's Intertidal Zone for Novel Ice Binding Proteins

Nathaniel Moyes, Allan Bertram, Katie Marshall

Intertidal invertebrates are exposed to the high and low extremes of both air and water conditions throughout the year. During the winter, low tides often occur in the middle of the night, resulting in rapid declines in temperature over a short period of time. Being exposed to these temperatures leaves animals at risk of freezing. Many intertidal invertebrates are freeze tolerant, meaning they can survive either full or partial freezing of their body's tissues. There is genetic evidence that these animals possess proteins that initiate ice formation at high-subzero temperatures, allowing them to freeze in a gradual, controlled manner, rather than uncontrolled spontaneous ice growth. Eleven invertebrate species were collected from the intertidal zone in Vancouver, British Columbia with the goal of characterising novel proteins with ice binding potential, in addition to investigating macroevolutionary variation of ice binding proteins. To assess the presence of these proteins, total protein was extracted from whole-body tissue. These extracts were either boiled to denature proteins or left untreated. To assess ice nucleating potential, droplets of the homogenate were put on a cooling stage and the temperature gradually lowered until droplets were frozen. Other activities of ice-binding proteins, including thermal hysteresis and ice recrystallization inhibition, were also examined. The results of this project expand on our understanding of how animals manipulate ice growth to survive in extreme environments

Predicting harmful algal blooms through high-resolution metaproteomics of a bacterial microbiome

Miranda Mudge, Emma Timmins-Schiffman, Mike Riffle, Gabriella Chebli, Julia Kubanek, William Noble, Brook Nunn

The San Juan Islands, Washington, USA border a dynamic coastal ecosystem that is unique to the world in its predictable occurrence of biannual harmful algal blooms (HABs). To fully characterize this ecosystem and harness its potential for revealing clues to HAB initiation, a high-resolution temporal study was conducted to identify microbial community interactions and their molecular-level controls on large scale algal bloom events. In June of 2021 we sampled coastal waters in Eastsound by collecting the bacterial microbiome

every 4 hours for 22 days, with additional time-matched samples for environmental and water chemistry analyses. The metagenome was sequenced and a quantitative metaproteomic analysis performed on the last 6 days of the timeseries during a period characterized by low environmental disturbance preceding a *Chaetoceros* bloom. The microbiome revealed a distinct shift in overall peptide abundance pattern by NMDS prior to the initiation of the HAB, characterized by an increase in peptides attributed to bacterial class Verrucomicrobiae. These bacteria, previously documented for their potential to degrade complex HAB biproducts, were primarily engaged in translation, followed by chromosome condensation, protein folding, and protein peptidyl-prolyl prior to the HAB. Our time series of a marine bacterial community is unprecedented in its scope and resolution and has revealed that some bacterial taxa are metabolically linked to the formation of a HAB, yielding potentially specific and predictive biomarkers of bloom formation.

Thermal developmental plasticity varies across geography in green anoles (*Anolis carolinensis*)

Morgan Muell, Kendall Jackson, Christian Cox, Daniel Warner

Developmental plasticity in response to temperature is thought to arise in thermally heterogeneous environments. Therefore, populations evolving in environments with high thermal heterogeneity should exhibit larger plastic responses than those in environments with low thermal heterogeneity. However, canalization may also enable organisms to deal with local environmental conditions, where phenotypes are adapted to local conditions but are not shaped by immediate environmental factors. Alternatively, non-adaptive plasticity may evolve if selection for an optimum trait value is inconsistent or weak. We examined patterns of thermal developmental plasticity at embryonic and juvenile stages in green anoles (*Anolis carolinensis*). We bred adults from 7 populations spanning their latitudinal range. We incubated eggs under 3 temperature treatments, with fluctuating sine waves to mimic natural conditions. We measured heat tolerance (CT_{max}), cold tolerance (CT_{min}), and thermal preference in hatchlings, and heat tolerance in embryos. We find developmental plasticity in CT_{min}, but not CT_{max} or embryonic heat tolerance after controlling for body size. Population effects were strong for all phenotypes, including most comparisons between latitudinal replicate populations, meaning we did not identify a strictly latitudinal gradient in degree of plasticity. Soil temperatures will assess relationships between thermal heterogeneity and plasticity. Our study highlights the complexity of potential adaptive change among populations and suggest that adaptation to local environments may occur via numerous routes that do not always conform to theoretical expectations.

Species-specific Variation in Noseleaf and Pinna Movement of Echolocating Bats Using Deep-Learning

Putri Muhd-Haidzir, Ulmar Grafe, Samuel Kramer, Syafi'ie Su'eif

Colocalization and aggression-induced neural activation of androgen receptors in an African cichlid

Kathleen Munley, Sarah Gawlik, Beau Alward

Androgens are essential in regulating physiological mechanisms and behaviors associated with social rank. Prior work from our lab has demonstrated that the two androgen receptors present in teleost fishes, AR α and AR β , control distinct traits of dominant social status in male *Astatotilapia burtoni*, a highly social African cichlid: AR α is necessary for the expression of dominant-typical behaviors (e.g., aggression, mating), whereas AR β is necessary for the expression of dominant-typical physiological characteristics (e.g., testes growth, bright coloration). The specific cells in the brain that modulate these phenotypes, however, have yet to be investigated. Here, we identified cells expressing ar1 and ar2 (genes that encode AR α and AR β , respectively) in the *A. burtoni* brain using multiplexed in situ hybridization. Then, we performed resident-intruder assays with dominant males and examined which ar1- and ar2-expressing cells in the hypothalamus are activated following an aggressive interaction using the immediate early gene *egr1*. We found that ar1 and ar2 are expressed in brain regions that regulate social behavior, decision making, reproduction, and sensory perception and processing. Moreover, we

determined that ar1- and ar2-expressing cells colocalize in several regions associated with behavior or reproduction, including the ventral telencephalon and preoptic area. Data will also be presented on aggression-induced ar1, ar2, and *egr1* expression and colocalization in dominant males. Collectively, these findings provide novel insight into the androgenic control of aggression in teleosts.

Be Aware: Native Bee Diversity on a California Community College Campus and Local Reserve

Briana Munoz, Luis Arredondo, Mariel Dawson, Katie Goldstein, Lisbeth Nicolas-Lopez, Esveidy Rodriguez, Erin Krier, Alicia Fox

Allan Hancock College, a California community college, has participated in an ongoing multi-campus study on native bee diversity in California using the college campus as a study site. Three 90-minute surveys were done at each of five locations. Two locations at each of the college's two campuses and Sedgwick Reserve, a local reserve in Santa Ynez, CA were included in the study. Surveys were done in the spring/summer and fall of 2022 and 2023. Comparisons of native bee genera were made across seasons and years. Throughout the study a total of 12 bee genera were collected. There was overlap of several bee genera (e.g. *Bombus*, *Halictus*, *Lasioglossum*) that were found between seasons and years, while other genera (e.g. *Xeromelecta*, *Augochlora*) were only found in a single season or a single year. Additionally, some genera (e.g. *Osmia*, *Colletes*) were unique to specific locations. Meaningful conclusions regarding native bee diversity on campus have led to suggestions for future studies, including surveying the same locations to look for weather anomalies, adding a central location between our campuses and Sedgwick Reserve, and implementing better landscaping protocols to sustain native bee diversity. Furthermore, as this study continues, we hope to develop outreach methods to educate our campus and surrounding community to help increase and protect our local native bee populations.

Lizards in the Trees: Substrate Integrity and Behavioral Responses in Arboreal Lizards

Victor Munteanu, Richard Blob, Savannah Swisher, Trevor Brewington

Animals that live in arboreal (tree-based) habitats must be able to respond to perturbations of branches to avoid a high-risk injury or death upon falling. This study evaluated two lizard taxa, *Chamaeleo calyptratus* (veiled chameleon) and *Gastropholis prasina* (green-keel bellied lizard), respectively tall and short-bodied lizards with prehensile tails, to better understand how arboreal animals with different morphologies resist unexpected perturbations in their habitats. Individual lizards were placed on a laterally sliding perch to conduct trials that simulated branch perturbations common in arboreal habitats. Trials were filmed with high-speed video, with a proxy for their center of mass (CoM) and key points on the limbs marked for landmark tracking. Results show that, after a perturbation, chameleons returned their center of mass relatively closer to the pre-perturbation position than the *Gastropholis* lizards. This suggests that although chameleons have a taller body, they may be better equipped to resist perturbations through different abilities to anchor using the prehensile tail. In contrast, *Gastropholis* may have evolved a different stabilizing behavior that uses forward locomotion to contend with inconsistencies in an arboreal environment.

WildPose: A Long-Range 3D Motion Capture System for Wildlife

Naoya Muramatsu, Sangyun Shin, Qianyi Deng, Amir Patel

Measuring 3D animal motion in the wild will provide new insight into ecology, neuroscience and evolutionary biology. However, whole-body motion capture of wildlife presents a challenge as to existing methods' intrusiveness or limited range. To address this issue, we introduce WildPose, a long-range motion capture system specifically tailored for wildlife observation. This system synergistically combines a solid-state LiDAR with a zoom-lens camera to capture both 2D color videos and 3D point cloud data, thereby allowing researchers to observe animal behavior without affecting their natural behavior. Unlike traditional data collection methods, our solution provides 3D animal data previously

only possible in controlled laboratory settings. We conducted extensive field collections in South Africa's Kgalagadi Transfrontier Park, successfully reconstructing several species. The outcomes of our study not only validate the efficacy of WildPose but also pave the way for innovative applications in wildlife monitoring and research. By enabling non-intrusive data collection from long distances, WildPose represents a significant advancement in the field, with potential implications for conservation and animal welfare, broadening the horizons of interdisciplinary research.

Mom, is that you? A northern elephant seal pup's ability to vocally recognize its mother

Molly Murphy, Caroline Casey, Jenna Camargo, Gita Kolluru, Maddie Schroth-Glanz, Heather Livanag

Mutual parent-young recognition is important in species with high densities at breeding sites, including pinnipeds (seals, sea lions, and walruses). Vocal recognition has been studied extensively in pinnipeds that leave their pups to forage, but is understudied in seals that remain with their pups throughout nursing, like northern elephant seals (NES). Despite not intentionally separating during the nursing period, NES pups can still become separated from their mothers, and the resulting chance of pup mortality is high. One recent study showed that NES females can recognize their pup days after birth, but it was unknown if the recognition is mutual. We tested this through auditory playback (PB) experiments of adult female attraction calls (ACs) at the Piedras Blancas NES rookery in San Simeon, CA. PBs included ACs of the pups' own mother and ACs of a female from a nearby beach. We recorded pup responses to each PB, including vocalizations and phonotaxis. PBs were performed four times per pup to examine changes as the pup ages. We found that pups can vocally recognize their mother around 24 days old, where the likelihood that they respond to their mother's vocalizations increases significantly. This study is the first to examine phocid pup vocal recognition abilities, and suggests that mothers are primarily responsible for maintaining contact during the nursing period to ensure pup survival.

Multi-tissue examination of gene expression in the Gulf killifish during hypoxia

Taylor Murphy, Bernard Rees

Low dissolved oxygen (hypoxia) is prevalent in aquatic environments, and when faced with this stressor, many fish show changes in gene expression that are thought to improve hypoxia tolerance. This study addressed two questions in the estuarine fish, *Fundulus grandis*, the Gulf killifish. How does acute exposure to hypoxia affect gene expression across multiple tissues? Does variation in transcript abundance among individuals correlate with the abundance of the transcription factor, hypoxia-inducible factor 1 α (HIF1 α), in the same tissues? Female fish were exposed to 1 mg/l O₂ for 0, 6, or 24 h. RNA was extracted from the liver, skeletal muscle, gills, and ovary, and sequenced by Tag-sequencing. Modules of genes with similar expression were determined by weighted gene co-expression network analysis. Liver exhibited the largest number of differentially expressed genes. One module was up-regulated during hypoxia and included genes involved in carbon metabolism and glycolysis. Five modules were down-regulated and contained genes involved in RNA and protein processing. An additional four modules were positively correlated with HIF1 α protein abundance, and they were enriched with genes involved in cell signaling and metabolic pathways. Skeletal muscle showed fewer changes in gene expression with no modules significantly affected by hypoxia, although one module was positively correlated with HIF1 α protein abundance. Future analyses will examine the gill and ovary to better understand tissue-specific responses to hypoxia in *Fundulus grandis*.

Multigenerational plasticity to hypoxia and acidification in Atlantic silversides (*Menidia menidia*)

Christopher Murray, Matt Long, Neel Aluru

Multigenerational plasticity may play a vital role in enhancing organismal resilience to anthropogenic climate change. Environmental stress experienced by parents could trigger adaptive phenotypic changes in offspring through

mechanisms involving inherited gene regulation. Here, we explored the potential for multigenerational plasticity in the forage fish species, Atlantic silverside (*Menidia menidia*) under the dual stressors of hypoxia and ocean acidification (hereafter HypOA). Mature adult silversides collected from an estuary (Connecticut, USA) were acclimated to either control (100% dissolved oxygen [DO] / 550 μ atm pCO₂) or HypOA conditions (35% DO / 2200 μ atm pCO₂) for 11 days before spawning. We reared their offspring in a factorial experiment to investigate how parental environment influenced offspring responses to HypOA conditions. Offspring from control-acclimated parents and reared under HypOA showed significantly lower survival. Gene expression profiling revealed 1,596 differentially expressed genes (DEGs) relative to control larvae. Functional enrichment analysis of DEGs suggests altered biological processes including oxygen delivery, synaptic signaling, neurotransmitter transport, and ion homeostasis. In contrast, offspring from HypOA-acclimated parents showed a modest reduction in survival under HypOA. Only 3 DEGs were observed between control- and HypOA-reared groups. These results suggest that parental exposure to HypOA primed the transcriptional patterns of their offspring, in ways that resemble direct responses to HypOA. However, we documented physiological tradeoffs in these offspring that could offset the advantageous effects linked to multigenerational plasticity.

Antifeedant/chemical defensive properties of a sea pen & a nudibranch against crab predation.

James Murray

Nudibranchs possess chemical defense mechanisms to deter predation. However, no study has assessed the chemical defensive potential of the sea slug *Tritonia exsulans* Pallas, (1788) [a.k.a. *Tritonia diomedea* Bergh (1894)], or the chemical ecology of the slug and its sea pen prey *Ptilosarcus gurneyi*. A series of antifeedant experiments were designed using two species of crabs, the subtidal *Metacarcinus magister* and intertidal *Hemigrapsus oregonensis*. We measured feeding rates when offered tissues of *Tritonia* or *Ptilosarcus*, or artificial diets infused with extracts of either species. Diet with *Tritonia* body wall tissue was only 13% consumed by *Metacarcinus* but control diet was consumed 3.5X more (46%; p500X difference). Results were similar when tissue was replaced by gelatin-flavored with extracts of *Tritonia* or *Ptilosarcus*. A comparison of feeding probability between crabs that were offered *Tritonia*-flavored gelatin and *Ptilosarcus*-flavored gelatin indicated that *Ptilosarcus* extracts are ~7X less attractive than that of *Tritonia* (p=0.04). Results support a chemical defense that makes both species unpalatable to potential predators.

Vision and flight behavior trade offs for undersized Greater Wax moths

Veronica Muzio-Crego, Jamie Theobald, Yash Sondhi, Elina Barredo

Nocturnal flying insects rely on vision for vital activities, such as foraging, navigation, and mate selection, behaviors that require them to efficiently collect the limited light available at night. But this becomes problematic with small eyes, that lack the surface area to collect much light. Although lab-reared insects tend to develop eyes that uniform and large, natural populations show much more variation. To understand the visual capabilities vary with eye size in nocturnal insects, we reared Greater Wax moths (*Galleria mellonella*) with a diminished diet, producing a range of adult body and eye sizes. We examined the trade-off between limited eye size and visual performance in these moths using an infrared, dual-camera, optic tracking system to quantify insect activity and flight paths. Our findings reveal that smaller individuals exhibit compromised visual acuity and reduced sensitivity in dimmer light. Additionally, we observed alterations in the moths' phototactic behaviors, suggesting a potential link between nutritional status and their ability to navigate in their environment.

Leveraging coral disease 'omics datasets for disease classification and predictive modeling

Laura Mydlarz

Stony Coral Tissue Loss Disease (SCTLD) and White Plague are two of the most destructive diseases to affect Caribbean coral reefs. We have conducted

two parallel transmission experiments with 8 different coral species to study the susceptibility of these diseases and generated several data sets that not only describe the responses to disease but can be used comparatively to delineate the two diseases. Here we present large scale transcriptomics data that shows differences in the host and symbiont response to SCTLD and White Plague. Specifically, differences between the two diseases involve protein trafficking and symbiophagy. We are also applying novel machine learning algorithms to these data to determine if we can predict disease in healthy coral and provide disease classification at the gene level. We find that a small subset of genes can predict the disease fate of a coral genet and a set of several hundred genes are uniquely associated with SCTLD over White Plague. Host gene expression from disease transmission experiments are a rich dataset that can be used to assess and predict the onset and progression of diseases.

Does a lack of phenotypic variation reflect genomic variation in a widespread butterfly species?

Tanner Myers, Angelo Ruggieri, Riccardo Papa, Brian Counterman

Heliconius butterflies are renowned for the repeated adaptive radiation of wing warning colorations and have emerged as models for genomic studies of adaptation and speciation. Notably, certain Heliconius species are composed of 25+ geographic populations that differ wildly in wing coloration. This adaptive divergence in wing colors is reflected in patterns of genomic divergence, with populations distinguished by wing color pattern often exhibiting differentiation across the genome. Although many Heliconius species are characterized by striking phenotypic variation and genomic divergence, some species of Heliconius exhibit minimal phenotypic variation in wing color patterns across large geographic ranges. Here, we ask if the lack of color pattern variation in the zebra longwing (*Heliconius charithonia*) mirrors a lack of genomic differentiation. The zebra longwing is among the most widespread Heliconius species with a range spanning much of the Caribbean, North, Central, and South America. We hypothesize that zebra longwing populations show patterns of genomic divergence that reflect its phylogeographic history, with the most divergent lineages being at the range limits in the Caribbean, North America, and South America. Using a reference pan-genome and whole genome resequencing, we have begun to reconstruct the evolutionary history of the zebra longwing and test specific hypotheses of lineage divergence. Further, we will test for potential admixture between divergent Caribbean and mainland lineages where they seasonally come into contact in the Southeastern United States.

Innovation and diversification of and through sexual dimorphism: insights from horned beetles

Erica Nadolski, Armin Moczek

Sexual dimorphism represents one of the most significant dimensions of intra-specific variation, with the potential to constrain or facilitate the diversification of novel traits and functions. Yet, rather than exhibiting equal sexual dimorphism across all traits, organisms are mosaics of tissues that vary in the degree to which they exhibit dimorphism. While the ecological and fitness relevance of sex-dependent development is often well characterized, the genetic and developmental mechanisms underlying sexual dimorphism and its evolution are not. Using horned beetles, my work assesses the regulatory mechanisms that instruct sex-biased development along two critical axes of diversification: divergence across different body regions and macro-evolutionary divergences across species, with the ultimate goal of identifying genomic mechanisms by which sexual dimorphisms originate and diversify. Among others, I aim to test the hypotheses that (i) evolutionary elaboration of sexual dimorphism is made possible through the rapid acquisition of novel cis-regulatory elements and/or the elaboration of sex- and trait-specific transcriptomes, and (ii) that the evolution of novel secondary sexual traits is facilitated by the evolution of novel regulatory interactions, rather than the re-use of preexisting regulatory machinery. I discuss my results in light of fundamental questions regarding gene regulatory network evolution: are the networks that instruct the formation of a trait the same as those that facilitate its diversification, or is context-responsiveness decoupled from trait formation in development and evolution?

Evolutionary novelties, segmental boundaries, and unexpected Hox gene expression in the insect head

Erica Nadolski, Armin Moczek, Isabel Manley

From the cephalic horns of scarab beetles to the weevil snout and the stalks of stalk-eyed flies, the dorsal insect head constitutes a hot spot of evolutionary innovation and diversification. Yet how such morphological novelties originate as well as integrate within ancestral trait complexes without disruption, remains largely unclear. Growing evidence points to the recruitment of modular developmental genetic components into new ontogenetic contexts as a mechanism enabling integration of novel traits into ancestral contexts, including the re-functionalization of embryonic patterning genes in post-embryonic developmental processes. Motivated by preliminary data documenting the unexpected expression of anterior Hox genes in the non-segmental anterior region of the heads of horned beetle larvae, my work investigates the potential roles of anterior Hox genes in patterning the post-metamorphic dorsal heads of horned beetles. Specifically, I seek to test the hypotheses that anterior Hox genes gained novel functions in the initiation, positioning, and/or patterning of head horns, a text book example of evolutionary novelties which have undergone one of the most dramatic radiations of secondary sexual traits in animals. I discuss my results in light of proposed models for head development that incorporate a Hox-free non-segmental anterior region and use my results to inform our understanding of the segmental boundaries of the adult beetle head.

Interfacial Run-and-Tumble Dynamics of the Water-Strider Rhagovelia

Nithil Nagappan, Ishant Tiwari, Jacob Harrison, Saad Bhamla

Run and tumble motion is characterized by a series of persistent darts or “runs” separated by random reorientations or “tumbles.” The motion is documented in various microorganisms, such as *E. coli* and *C. reinhardtii*. In this study, we explore this intriguing motion pattern in *Rhagovelia*, a water-striding insect, which exhibits a similar “run-and-tumble”-like locomotion pattern. We have observed and characterized its locomotion through metrics such as instantaneous speed and turning angle distribution. Subsequently, we investigate how these metrics differ with various environmental conditions, such as flowing water or the presence of conspecifics. Our findings offer a macroscopic perspective on “run-and-tumble” motion, revealing how environmental factors shape this intriguing pattern of movement. This research could yield insights into adaptive movement strategies utilized by organisms that occupy the 2-D landscape between air and water.

Optimal kinematics for individual and group-swimming using deep reinforcement learning

Aishwarya Nair, Alejandro Alvaro, Siddhartha Verma

It has been shown that various fish species can utilize the velocity field generated in the wakes of obstacles, and in the wakes of other swimmers, to reduce their energy expenditure. Here, we explore the hydrodynamic benefits of group swimming using two-dimensional numerical simulations of self-propelled anguilliform swimmers, coupled with multi-agent reinforcement learning. These artificial swimmers utilize a sensory input system that allows them to detect the velocity field and pressure on the surface of their body, which is similar to the lateral line sensing system. Deep reinforcement learning is used as a tool to discover optimal swimming patterns at the group level, as well as at the individual level, as a response to different objectives and flow fields. This can be useful in distinguishing various swimming patterns and their role in achieving higher speeds or efficiency, which are desirable objectives in different scenarios. The adaptations in response to changes in the surrounding flow field are also examined by training the swimmers in stationary flow, as well as uniform flow. These flow fields are representative of conditions encountered by fish in lakes and oceans (stationary flow), as well as during long-distance migration and in rivers (uniform flow). The physical mechanisms revealed can be helpful in understanding the motivation behind different swimming behaviors from a hydrodynamic and energetics standpoint.

Molt-imposed constraints on hummingbird migration ecology

Nadje Najjar, Chris Clark

Migration is well-known to impose major constraints on avian molt, but there is growing evidence for the opposite relationship: molt pace, pattern, and initiation may constrain migratory behavior. I tracked the molt pattern of individual captive-held migratory ($n=11$) and sedentary ($n=11$) *Selasphorus* hummingbirds over the course of two molt seasons. Molt, especially primary molt, was strongly associated with very low body fat ($<10\%$) and highly reduced activity in all birds. Individuals varied in molt initiation date and molt pace (# days to complete molt), but within-individual molt characteristics were repeatable from the first season to the next. Residents only completely ceased any molt during the breeding season, but otherwise had a relatively slow pace of molt with individuals molting throughout the rest of the year. Migrants showed the opposite pattern, with a restricted window available to molt (i.e., during non-breeding, non-migration time) with a consequently fast molt pace and less inter-individual variation. Rufous hummingbirds, the species with the longest migration of any hummingbird, had the most extreme molt with two individuals replacing 7 of 10 primary feathers simultaneously in two different seasons. This precarious molt, where any feather damage would render a bird flightless, may represent an upper limit to molt pace and consequent constraint on the distance between the breeding and non-breeding range.

On cell type diversity, connectivity, and developmental origin of cnidarian mechanosensory neurons

Nagayasu Nakanishi

The mechanosensory neuron of cnidarians (e.g. sea anemones and jellyfishes) is characterized by its apical sensory apparatus consisting of a single cilium surrounded by a collar of stereovilli, and is referred to as the concentric hair cell. Hair cells occur in the ectoderm of sensory structures such as sea anemone tentacles and jellyfish statocysts, but are absent in free-swimming planula larvae, implying their postembryonic birth at life cycle transition. Yet, the timing of when hair cells commence postmitotic differentiation has not been experimentally defined. Moreover, relatively little is known about their cell type diversity and neuronal connectivity. Here we investigate the anatomy and early development of concentric hair cells in the sea anemone *Nematostella vectensis*. First, we establish two transgenic reporter lines - one in which a *pou-iv* promoter drives the expression of photoconvertible fluorescent protein Kaede in hair cells and other cell types during postmitotic maturation phases, and another in which a polycystin-1 promoter drives Kaede expression specifically in hair cells at the polyp stage. By microscopy we uncover morphologically and molecularly distinct types of concentric hair cells that innervate other tentacular ectodermal cells including epitheliomuscular cells. Furthermore, photoconversion-mediated cell lineage tracing experiments demonstrate that early postmitotic precursors to concentric hair cells emerge during planula development. This study provides a framework for future studies concerning developmental genetics, function, and evolution of cnidarian mechanosensory neurons.

The brittle stars of the northeast Pacific with abbreviated development

Nicole Nakata, Richard Emlet

Brittle stars are a diverse class of echinoderms that are abundant in many benthic marine environments. Yet for most species, we know little about aspects of their basic biology, including larval development. We present data accumulated over 20 years on the taxonomic and developmental diversity of brittle stars from the northeast Pacific. We used DNA barcoding to identify embryos, larvae, and juveniles from wild plankton samples, resulting in a dataset encompassing 18 species from seven families. The ophiuroid fauna of the northeast Pacific is rich in developmental diversity; we were surprised to find that a large portion of the larvae we observed (8 spp.) have abbreviated development, an ecological category characterized by rapid development and small post-larvae produced from moderate numbers of yolk eggs. This category includes reduced plutei, vitellaria larvae, and other nonfeeding planktonic forms, all of which we observed in the NE Pacific. To

better understand the selection for such intermediate larval forms, we used feeding experiments to show how one species with a reduced pluteus utilizes facultative planktotrophy to benefit from larval feeding on development time, percent metamorphosis, juvenile size, and juvenile time to starvation. Finally, we used phylogenetic analysis and ancestral state reconstruction in two families, Amphiruridae and Ophiuridae, to evaluate the evolutionary history of this unusual developmental mode in ophiuroids.

A new multi-scale frog-axolotl chimeric model system to study the algorithms of anatomical control

Vasilios Nanos, Gabriel Zimbler, Michael Levin

Organisms can be understood as complex systems built of modular subunits organized in a nested multi-scale hierarchy, spanning from the molecular level to the cell, tissue, organ, and whole organism level. Classic tissue and organ grafting experiments have utilized this modularity with the goal of better understanding cell and organ-intrinsic vs. -extrinsic mechanisms of pattern formation, such as the regulation of scaling relationships between appendages and the body during development. Here we introduce for the first time a new synthetic model organism, a chimera of the frog *Xenopus laevis* and the axolotl *Ambystoma mexicanum* called frogolotl. The chimera is created via xenoplastic transplantation and replacement of the frog embryo's tail bud by the tail bud of the axolotl, resulting in the development of a functional tail. Our analysis reveals that the grafting procedure results in a multi-scale chimera. At the organ and tissue level, we show, first that the frog nervous system can innervate the axolotl donor tail. Second, the vasculature of the donor and host are able to connect and build a functional circulatory system. At the cellular level, we demonstrate that *Xenopus* macrophages can invade and navigate in the axolotl tissue. Furthermore, we combine morphometric measures of the grafted tail with RNAseq analysis to acquire a more comprehensive understanding of how organs adapt to their new environment on the molecular level.

Why so blue? The potential roles of ventral coloration in the western fence lizard

Kaitlyn Napier, Guillermo Garcia-Costoya, Akhila Gopal, Noa Ratia, Karla Alujevic, Madison Glenwinkel, Shea McKendree, Cody Chapman, Allison Dorny, Gillian Moritz, Michael Logan

Reptile coloration is employed in crypsis, thermoregulation, and signaling, among other functions. Nonetheless, the eco-evolutionary forces that mediate the evolution of coloration are poorly known in most systems. For instance, western fence lizards (*Sceloporus occidentalis*) possess vibrant blue coloration on their bellies, the functional significance of which is not fully understood. Previous research indicates that this coloration might aid in thermoregulation, or it could be used for communication between conspecifics. To explore the potential roles of ventral coloration in western fence lizards, we studied the color patterns and spectral reflectance of two populations along an elevational gradient in the Great Basin desert. We combined morphological, behavioral, and life-history data (collected through intensive mark-recapture) with the analysis of standardized photographs of animals in the field and spectrophotometer measurements in the lab to understand how coloration differs by thermal environment, sex, life stage, and social status. We used these data to determine the possible evolutionary contexts in which blue ventral coloration may have emerged in this species.

Effect of climate change on the adhesive performance of the sea urchin *S. purpuratus*

Carla Narvaez-Diaz, Daniel Okamoto

Adhesive systems are common among marine benthic organisms and are critical for key functions such as locomotion, food capture, and withstand hydrodynamic forces. Sea urchins are key members of benthic communities that adhere strongly to the substrata by coordinating the use of hundreds of adhesive organs, called tube feet. The epidermis of the tube foot disc has a duo-gland system that releases adhesive and de-adhesive secretions allowing for temporary adhesion. In this study, a fully crossed factorial experiment was conducted to assess the effect of multiple stressors associated with climate

change, namely increased water temperature (10, 13, 16, 18, 20 °C) and acidification (pCO₂ 1200 and 600 µatm), on the adhesive performance of the purple sea urchin. Adhesive performance was assessed as: whole animal adhesive force, tube feet mechanical properties (maximum disc tenacity and stem breaking force), and behavior (number of tube feet used for adhesion). After 3 months of exposure, we found an overall detrimental effect of increased temperature on whole animal adhesive force and disc tenacity, and a significant, but counterintuitive effect, of pH and temperature on stem-breaking force. Understanding the effect climate change will have on organisms' ability to adhere is needed to accurately predict its effect on marine communities.

Evolution of substrate use across global assemblages of the goatfishes (Family Mullidae)

Chloe Nash, Linnea Lungstrom, Mark Westneat

Tropical marine fishes offer a spectacular case study on how the evolution of specialized ecological niches can effect rates of phylogenetic, morphological, and distributional change. The goatfishes (Mullidae) are diverse in body form and foraging behavior, yet remain relatively unexplored in their evolutionary ecomorphology and biogeographic patterns. The synapomorphy that unites the goatfishes is the presence of chemosensitive hyoid barbels, which enable goatfishes to access otherwise inaccessible food within benthic substrates. Goatfishes preferentially forage on different substrate types, which results in habitat partitioning across reef systems. In this study, we integrated a recently published phylogeny of the goatfishes with a robust morphometric dataset, ecotype trait analyses, and global assemblage delineations to test hypotheses about the evolution of ecomorphological and biogeographical associations with substrate preference. We find evidence that body shape, head shape, and fin profiles are significantly associated with substrate preference across the family. In particular, we show that preferential foraging on hard substrates evolved relatively recently within the genus *Parupeneus* and that rates of morphological diversification are significantly faster in hard substrate species. Additionally, we find that the presence of hard substrate species is consistent across Indo-Pacific, although there is species turnover within this niche. In conclusion, we find robust evidence for an evolutionary relationship between substrate preference, morphological diversity, and global distribution of the goatfishes.

Beyond categories: predicting multivariate diet from phenotypic data in extant and extinct taxa

Jonathan Nations, Anna Wisniewski, Graham Slater

direct observations, such as fossils. In comparative analyses, ecological traits, like diet, are often treated as categorical, which may aid prediction and simplify analyses but ignores the multivariate nature of ecological niches. Furthermore, methods for quantifying and predicting multivariate ecology remain rare. Here, we ranked the relative importance of 13 food items for a sample of 88 extant carnivorous mammals and then used Bayesian multilevel modeling to assess whether those rankings could be predicted from dental morphology and body size. Traditional diet categories fail to capture the true multivariate nature of carnivorous diets, but Bayesian regression models derived from living taxa have good predictive accuracy for importance ranks. Using our models to predict the importance of individual food items, the multivariate dietary niche, and the nearest extant analogs for a set of data-deficient extant and extinct carnivorous species confirms long-standing ideas for some taxa but yields new insights into the fundamental dietary niches of others. Our approach provides a promising alternative to traditional dietary classifications. Importantly, this approach need not be limited to diet but serves as a general framework for predicting multivariate ecology from phenotypic traits.

Trophic tinkle: Predator pee fails to frighten foragers and conifer chemistry depends on deer damage

Gum Nau, Hannah McSwain, Kenzie Garrett, Sophie Heisner, Baine Craft, Eric Long, Ryan Ferrer

Removal of top predators can initiate trophic cascades that impact primary producers by coupling or decoupling plant-herbivore interactions. Although

decoupling plant-herbivore interactions by herbivore predators has been studied extensively, few studies have examined potential changes in plant chemical responses to chronic herbivory when predator-naïve herbivores are confronted with historic predator chemical cues. In this study, we examined interactions between blacktail deer (*Odocoileus hemionus columbianus*) and four common coniferous species in an island habitat where deer predators have been absent for over a century. We video recorded free-ranging deer behavior with trail cameras at sites conditioned with either wolf urine (historic predator) or cow urine (control) as an indicator of predation risk to assess deer vigilance and foraging behaviors. In addition to monitoring browsing intensity and conifer species preference at sites with and without predator cues, monoterpene chemistry was measured with GC-MS in both caged and uncaged saplings. Our results indicate that deer exhibit no behavioral shift in the presence of predator urine, possibly as a consequence of relaxed predation pressure over numerous generations. Conifer saplings, however, responded to deer browsing by increasing the production of some, though not all, foliar monoterpenes.

A new model of sensory feedback for regulating dynamic color change

Lydia Naughton, Laura Bagge, Sonke Johnsen, Lorian Schweikert

Dynamic color change is the ability of certain animals to rapidly alter the color of their skin by aggregating and dispersing pigment within chromatophores. One commonality among color-changing animals is the presence of a light-sensing system in the skin and its apparent coupling to color change; however, the exact role of this sense remains poorly understood. Previously, we localized a short-wavelength-light-sensitive opsin (SWS1) to a distinct cell type underneath chromatophores of the Caribbean hogfish (*Lachnolaimus maximus*), a color-changing fish. We hypothesize that these putative photoreceptor cells may detect light passing through overlying chromatophores, where light transmittance may differ through aggregated versus dispersed pigment. In this way, the photoreceptors may monitor the current state of chromatophores to provide sensory feedback that could help regulate color appearance. Using microspectrophotometry and transmission electron microscopy (TEM), we tested whether light transmittance differs through aggregated versus dispersed chromatophores. We found that the average light transmittance within the range of SWS1 sensitivity decreased from the aggregated to dispersed state by approximately 30% and 50% for black and red chromatophores, respectively. Further, we used TEM and immunohistochemistry to investigate potential cellular connections for the communication of sensory information between putative photoreceptors and chromatophores. These findings provide initial evidence in support of the hypothesis that color-changing animals may use skin photoreception to detect current color state based on differential light transmittance through chromatophores.

Comparing Morphology, Kinematics, & Swimming Performance in Two Ctenophore Species

Randi Navarro

Ctenophores are predatory marine zooplankton characterized by their gelatinous bodies and rows of cilia, termed ctene plates, which they employ for locomotion through metachronal beating. Ctenophores are ecologically important carnivores in oceanic food webs, and their ability to impact plankton populations depends in part on swimming speed. In this comparative study, we used high-speed videography to examine the relationship between body size and ciliary kinematics and swimming speed of *Bolinopsis infundibulum* and *Beroë* spp. We found that body length has no significant effect on the distance traveled over a single ctene beat in both *Bolinopsis infundibulum* ($p=0.37$) and *Beroë* spp. ($p=0.32$). Comparisons of body length and swimming speed in both *Bolinopsis infundibulum* and *Beroë* spp. showed weak correlations suggesting other aspects of morphology may affect swimming across varying body plans. *Bolinopsis infundibulum* had a stronger and significant relationship ($p=0.02$) between swimming speed and ctene beat frequency, than *Beroë* sp. ($p=0.63$), indicating there may be differences in beating patterns or ctene characteristic of lobate and spherical ctenophores. Our findings suggest that other aspects, such as a ctene morphology, play a role in swimming performance in spherical ctenophores.

Phylogeographic variation in the standard metabolic rates of dusky salamanders (*Desmognathus*)

Emily Naylor, Jonathan Huie, R. Pyron, Sandy Kawano

Salamanders are excellent models for studying phenotypic and functional diversification across landscapes. Dusky salamanders (*Desmognathus*) are a particularly intriguing system in this context because they are broadly distributed across a range of aquatic and terrestrial habitats in the eastern U.S., with different patterns of range expansion and ecomorphological adaptation. Moreover, recent phylogenomic analyses have revealed a surprising amount of cryptic speciation in this clade, with some species splitting into different phylogeographic lineages. As dispersal often depends on physiological capabilities, quantifying metabolic requirements can help identify factors that limit or facilitate range expansion and gene flow, and subsequently diversification. We hypothesize that there are interspecific differences in metabolic rates related to variation in primary habitat use within *Desmognathus*, including elevation and water flow. Our initial set of experiments used terrestrial respirometers to compare standard metabolic rates (SMR), the minimum energetic requirement to maintain essential processes during rest, between three semi-aquatic species. Preliminary results suggest that SMR - both oxygen consumption and carbon dioxide production - in a primarily lowland creek/swamp species (*D. lycos*), was lower than one species found in montane oxygen-rich streams (*D. amphileucus*), but comparable to another (*D. mavrokoilius*). Further investigation of energetic demands in *Desmognathus* is currently underway and will contribute towards understanding how metabolic physiology varies across microhabitat and geographic scales.

A 3-Dimensional Reconstruction of the Arm Nerve Cord of the Octopus, *Octopus bocki*

Diana Neacsu, Robyn Crook

Cephalopods have the most complex peripheral nervous system structure of any invertebrate, which allows them to manipulate their arms and suckers to perform a variety of precise tasks including handling objects, capturing prey, and exploring their environment. Earlier studies have used dye tracings and serial sectioning to better understand the macroanatomy of the nervous structures in octopus arms. However, very little is known about the ultrastructural details of the cellular structures within the major arm nerve cord, a structure consisting of a series of ganglia spanning the length of each arm. This study aims to bridge this gap by creating the first 3-dimensional reconstruction of the major features of the arm nerve cord of the Pygmy octopus, *Octopus bocki*. Using blockface electron microscopy, 500 serial sections of the whole arm were imaged at 30x30x1000 nm (x,y,z), capturing two complete axial ganglia, the intramuscular nerve cords and two sucker ganglia. Structures were traced by hand using the software program Reconstruct and stitched together digitally to create a 3D model of nerve tracts, blood vessels, individual neurons and glial cells, axons, and all connections between the major neural structures in the arm. This study provides an anatomical foundation to better understand the sensory-motor circuits involved in controlling this intricate system and provides insight into the parallel evolution of a highly complex peripheral nervous system.

Gene regulatory networks underlying notochord development and evolution

Lenny Negrón-Piñero, Yushi Wu, Sydney Popsuj, Alberto Stolfi, Anna Di-Gregorio

Tissue-specific gene expression is fundamental in development and evolution, and is mediated by transcription factors (TFs) and by the cis-regulatory regions that they control. TFs and their respective cis-regulatory regions are essential components of the gene regulatory networks (GRNs) responsible for development of tissues and organs. Even though numerous TFs have been characterized from different organisms, the knowledge of the cis-regulatory regions responsible for their tissue-specific expression remains fragmentary. Using Ciona, we have identified and characterized through mutation analysis the cis-regulatory regions associated with ten TFs expressed in the notochord, an evolutionary hallmark of the chordate phylum. Our results illustrate how two evolutionarily conserved TFs, *Brachyury* and *Foxa2*, coordinate

the deployment of the notochord GRN. The integration of the results of detailed cis-regulatory analyses with those of trans-activation assays yielded a high-resolution view of the notochord GRN, and a reference for studies of notochord TFs and their roles in development, disease, and evolution.

Longer days, larger grays: lasting effects of photoperiod on eastern gray treefrogs, *Hyla versicolor*

Troy Neptune, Michael Benard

Carryover effects are prevalent among organisms with complex life cycles, but the carryover effects of photoperiod and temperature, as well as their additive and interactive effects, remain poorly understood. We experimentally manipulated photoperiod and temperature to examine their direct and carryover effects on gray treefrog (*Hyla versicolor*) life history in outdoor mesocosms. Photoperiod and temperature had substantial effects on amphibian traits that carried over well past metamorphosis. The long photoperiod increased size and age at metamorphosis, while the warmer temperature treatment decreased age but increased size at metamorphosis. Larval growth rates were thus affected by temperature but not photoperiod. However, juveniles from warmer larval conditions exhibited the opposite relationship by growing slower during a 10-day post-metamorphic growth period. Yet photoperiod, not temperature, affected juveniles approximately 56 days post-metamorphosis; juveniles from long photoperiods exhibited nearly double the growth rate of juveniles from the shorter photoperiods. Photoperiod also affected 56-day juvenile thermal selection such that juveniles from the late-season (short) photoperiod selected cooler temperatures. Importantly, we did not detect any interactions of photoperiod and temperature; photoperiod and temperature acted additively on amphibian traits with photoperiod having more comprehensive effects across traits than temperature. These results suggest that photoperiod can have lasting effects on organisms with complex life cycles, which may affect adult fitness and be important to understanding how species respond to global change.

Behavioral changes facilitate the spread into urban environments in odorous house ants

Kevin Neumann, Liam Hoeflerlin, Saieshwar Chikoti, Grzegorz Buczkowski, Andrew Suarez

Urbanization has altered ecosystems across the globe. While many organisms flee in search of familiar habitat, others adapt to these novel urban conditions. By understanding the behavioral traits that are present in city-dwelling organisms, we can predict how other species might respond to urbanization. Here, we studied the behavior of the odorous house ant (*Tapinoma sessile*). *Tapinoma sessile* can be found in "natural", forested areas, typically in smaller colonies (~1-5 queens), but have recently spread into urban areas, typically in massive colonies with many queens. While this colony structure is well studied, it is less clear to what extent behaviors also differ across environments. To assess the importance of behavior in the colonization of urban environments, we first asked if natural and urban colonies differ in their exploration, aggression, and nest relocation. Next, to investigate the potential function of these behaviors, we asked if the individual behavior of a worker correlates with the task it is performing. Finally, we explore a possible mechanism of behavior by asking if there is a relationship between these behaviors and octopamine, an important hormone in many ant species. We found that natural colonies are more exploratory and faster to relocate their nest following a disturbance. Finally, both natural and urban colonies tend to allocate more aggressive workers to colony defense and more exploratory workers to foraging.

Variation in Yolk Carotenoid Concentrations of Carolina Chickadees in Response to Human Disturbance

Jennifer Newbrey, Michael Newbrey, Jaleesa Clarke

Human disturbance negatively affects avian reproduction, with birds breeding in areas with high levels of disturbance typically experiencing lower reproductive success than those in less disturbed sites. A novel way to assess the effects of human disturbance on the reproduction of birds is to measure variation in concentrations of yolk carotenoids (i.e., biologically active yellow to

red pigments) from females breeding in habitats with different levels of disturbance. We studied variation in yolk carotenoid concentrations, egg metrics, female characteristics, and reproductive success of Carolina Chickadees (*Parus carolinensis*) breeding at four sites in west-central Georgia, USA. Yolk carotenoids had never previously been studied in Carolina Chickadees, so we first identified the carotenoids for the species: β -carotene, β -cryptoxanthin, lutein, and zeaxanthin. We found significant differences in total carotenoids and β -carotene across sites, with chickadees breeding at the site with the lowest level of human disturbance having the highest concentrations of carotenoids in their eggs. Surprisingly, chickadees that bred at Columbus State University, a high disturbance site, also had high yolk carotenoid concentrations, suggesting that other habitat features also influenced carotenoid allocation. No significant differences were found in egg metrics, female characteristics, or reproductive success across sites. In conclusion, our novel yolk-carotenoid approach allowed us to identify impacts of human disturbance on chickadee reproduction, which we failed to detect using traditional methods for quantifying variation in reproductive success.

Long-term anti-predator learning and memory across populations and sexes of an intertidal snail

Isabelle Neylan, Emily Longman, Eric Sanford, Jay Stachowicz, Andrew Sih

Anti-predator behaviors in response to predator cues may be innate, but they may also be learned through prior experience and potentially remembered over time. The duration and strength of this fear retention (i.e., continued anti-predator behavior after predator cues are no longer present) and fear memory (i.e., an enhanced response when re-exposed to predator cues later) are less well-studied and could account for observed variation in anti-predator responses. We explored these questions in the marine snail *Nucella canaliculata* from six populations distributed over >1,000 km of coastline and compared male and female snail responses. We exposed lab-reared, predator-naïve snails to cues associated with a common crab predator or seawater control in two serial experiments separated by over 7 months. Responses were population and sex dependent. In two populations, we found evidence of fear retention whereas in another two populations we found no evidence for fear retention (in fact prior exposure to predator cues caused higher activity in control conditions) and instead evidence of fear memory, which suggests a higher capacity for learning. Male snails showed a strong capacity for fear retention while females had more capacity for fear memory. These findings suggest enhanced learning may be more adaptive in some contexts than others.

Using RNAi to Understand Insect-Microbe Interactions

DiemQuynh Nguyen, Younghwan Kwak, Yumary Vasquez, Gordon Bennett

The leafhopper insect, *Macrostelus quadrilineatus* (ALF), is a plant-feeding pest that relies on vertically inherited intracellular bacterial symbionts, *Sulcia*-ALF and *Nasuia*-ALF. These bacteria collaboratively provide essential nutrients absent in ALF's plant-based diet. The intricate relationship between ALF and its endosymbionts poses considerable challenges. Bacterial symbionts are prone to genetic drift that causes widespread loss of genes essential to free-living bacteria. There are known experimental limitations to fully understanding how highly integrated symbiosis function. Here, we develop an approach to functionally test the role of host genes predicted to support symbiotic bacteria. RNA interference (RNAi) has emerged as an effective tool for non-lethally silencing gene expression to test gene function. We designed double-stranded RNA (dsRNA) to target genes horizontally transferred (HTGs) from environmental bacteria to insect genes. These HTGs compensate for specific gene losses within the essential cellular functions of each bacterial symbiont. Through injection, dsRNA was delivered to silence two HTGs *frr* and *ribD*, involved in bacterial transcription and nutrition synthesis. The effect of gene silencing was then investigated in bacterial and host fitness and phenotype assays. This study developed RNAi gene-silencing approaches, to better understand how bacteria and hosts fused into a single functioning biological system.

Fall forward, spring back: Mechanical drivers of the sea star bouncing gait

Brady Nichols, Graham Lucas, Hank Marriott, Olaf Ellers, Mary Lou Zeeman, Dale Syphers, Amy Johnson

Sea stars transition from a slower crawling to a faster bouncing gait as their podia transition from relatively uncoordinated to more synchronized movement. An open question is what mechanism(s) allow sea stars to synchronize podia and complete the crawl-bounce gait transition without a central brain. Many mechanisms that could drive the gait transition have been identified in terrestrial walkers, including inverted pendulum mechanics and the relationship between kinetic and potential energy in humans, and critical musculoskeletal force in horses. Here we focus on similar mechanical drivers of the sea star gait transition. Applying theories of terrestrial locomotion to underwater walkers is not often done, partially because buoyancy complicates the relationship between kinetic and potential energy. However, there are kinematic similarities between terrestrial and underwater legged locomotors, especially between organisms that move at similar Froude numbers (the ratio of kinetic to potential energy), such as sea stars and walking flies, and generally in the in-phase relationship of kinetic and potential energy in the fastest legged gait of many terrestrial organisms. Here we investigate how modifications of some models of terrestrial locomotion can be used to explain the sea star's bouncing gait kinematics and we explore how interactions between, and collective behavior of, podia contribute to podial synchronization, and the gait transition.

Time heals all wounds: the effect of wounding on skin denticle development in cartilaginous fishes

Ella Nicklin, Gareth Fraser

While sharks are widely recognized for their remarkable ability to heal wounds quickly, our understanding of the genetic mechanisms involved in the reformation of patterned skin after injury is still limited. The regrowth of denticles, tooth-like structures found in the skin of sharks, skates, and rays, in these healing areas provides valuable insights into the genetic basis of skin regeneration. Previous research by Reif in 1978 showed that skin wounding can impact denticle patterning and morphology in adult sharks. However, wounding the skin of embryonic sharks prior to denticle development and patterning has not been investigated. We ask whether wound healing prior to denticle development has a significant impact on the normal progression of denticle patterning.

We conducted puncture wound assays before and during denticle formation in shark (*Scyliorhinus canicula*) and skate (*Leucoraja erinacea*) embryos. Using live imaging, histological sectioning, and micro-CT scanning techniques, we analyzed embryonic skin healing and its impact on denticle patterning in both sharks and skates. Our findings indicate that embryonic shark skin takes approximately 30-35 days to completely heal with minimal scarring. Furthermore, the effects of skin wounding and healing on denticle patterning differ between sharks and skates. These results provide insights into the genetic differences underlying denticle patterning in these species. Additionally, the fast healing of shark skin presents a valuable model for future wound healing studies.

Discovering new populations of the Hawaiian Bobtail Squid, *Euprymna scolopes*, using eDNA analysis

Zachary Niedermaier, Yuya Nishida, Hannah Osland, Elizabeth Heath-Heckman

The Hawaiian Bobtail squid, *Euprymna scolopes*, and its bioluminescent symbiont, *Vibrio fischeri*, are used as a model to study animal-bacterial symbiosis. *E. scolopes* is endemic to the Hawaiian Islands, and two populations of *E. scolopes* have been studied on the coast of O'ahu. While the use of population genetics in this system is of interest to the community, population studies have been limited due to a lack of knowledge of the distribution of *E. scolopes*. Therefore, we are using amplicon-based environmental DNA (eDNA) sequencing to discover new populations of *E. scolopes* and to determine whether this technique might be useful in the study of other squid species. We collected sediment samples from ten locations around O'ahu, including both previously studied locations: Maunaloa Bay and Kane'ohe Bay. We then

extracted and quantified total DNA from sediment of each site, from which we will amplify sequences using CO1 primers and sequence them. We will then analyze these data to determine which and in what proportion different molluscan species are present at each site and generate a geospatial map of molluscan species distributions at these sites. Our initial goal is to identify new populations of squid around the island, yet future studies could focus on ecological relationships, predator-prey interactions, population genetics, and serve as a species distribution baseline for many additional studies.

Flight of the butterfly: Energetics of butterfly migration and dispersal

Kristjan Niitepõld

Flight is critical for insects, but also energetically costly. While resting metabolic rate (RMR) is one of the most commonly measured physiological parameters, the dynamics of flight metabolic rate (FMR) are less well known. My work has focused on energetics in butterflies in the context of dispersal and seasonal migration. Studies on the sources of variation in FMR in several butterfly species have revealed effects of genotype, environmental conditions, mitochondrial performance, and dietary stress. Using the Glanville fritillary (*Melitaea cinxia*), we found a positive relationship between FMR and dispersal (measured with a harmonic radar). Simply put: good fliers fly far. The relationship may be different in migratory species. Monarch butterflies (*Danaus plexippus*) from migratory populations had lower FMR than individuals from non-migratory populations. In the studies mentioned above, we found no correlation between RMR and flight performance. However, RMR was measured with a setup optimised for flight measurements. I measured RMR using two setups: one optimised for flight, the other optimised for RMR (early morning, room temperature, small chamber). Both methods yielded good results. Body mass explained 80% of the variation in RMR with the flight setup and 90% with the RMR setup. There was a significant positive correlation between the two measurements when correcting for body mass ($R=0.36$). However, neither measure of RMR predicted FMR. My recommendation is: When interested in flight, measure FMR.

A reduced representation sequencing technique for the Squid-Vibrio system

Yuya Nishida, Hannah Osland, Elizabeth Heath-Heckman

The Hawaiian bobtail squid, *Euprymna scolopes*, and its luminescent bacterium *Vibrio fischeri* forms a beneficial symbiosis that is an important model to explore host-microbe interactions. However, due to their large genomes, the population genomics of bobtail squid, such as *E. scolopes*, have been difficult to study. We therefore developed a cost-effective technique to study the population genomics and coevolution in the Squid-Vibrio system and are currently using it to assess genetic differences between three different sites across O'ahu. We have successfully tailored a double digest restriction-site associated DNA sequencing (ddRADseq) protocol for *E. scolopes*. The library is prepped by extracting DNA using the Qiagen Blood and Tissue Kit, digesting using *Mlu*CI and *Not*II restriction sites/enzymes, performing library preparation and then sequencing. We will then align the sequenced DNA fragments to our existing reference genome and identify single nucleotide polymorphism sites (SNPs) to be used for population studies. This includes determining the historical effective population sizes at the sites where *E. scolopes* are currently collected and integrating new populations of *E. scolopes* into our understanding of their distribution and population structure. Finally, we will further modify this protocol to be used in both host and microbe simultaneously and explore gene flow and host-symbiont coevolution in *E. scolopes* and *V. fischeri* to gain further insight of this host-microbe relationship.

Stress relaxation after passive and active stretch in mouse soleus muscles with titin mutations.

Kiisa Nishikawa, Jenna Monroy, Madhusudhan Venkadesan, Siwoo Jeong

Force enhancement is a long-known property of skeletal muscles that remains incompletely understood. Previous studies focused on residual force enhancement – the increase in steady-state stress after stretch of active

muscle compared to isometric stress at the same final length. We investigated stress relaxation after passive and active stretch in soleus muscles from mice with different titin genotypes (WT, mdm, and Ttn Δ 112-158). Muscles were stimulated to maximum isometric tetanus, stretched by 5% LO at a velocity of 1 LO/s, and maintained at the final length until a steady-state was reached. Total and passive stress were measured isometrically after the onset of stimulation and s after stretch from different starting lengths (100%, 105%, 110%, 115% and 120% LO). We measured peak stress, peak – steady state stress, and half-relaxation time during stress relaxation. Each trace was fit to a bi-exponential function, and time constants for fast ($\sim$ 15-50 ms) and slow (5 – 15 s) decay processes were estimated. All dependent variables differed significantly among genotypes, active and passive stretch, and starting lengths. Time constants increased with length for both active and passive muscles, and were lower in active compared to passive muscles. Differences among genotypes suggest a role for titin in stress relaxation. Residual force enhancement fails to account for viscous forces generally, as well as for differences in rates of stress relaxation which change with length and activation.

Variation in physiological stress between big brown bats roosting in different roost types

Lydia Nixon, Erin Gillam

Bat species in regions heavily affected by White-nose Syndrome are experiencing major changes to habitat and resource availability. One of these species, the big brown bat (*Eptesicus fuscus*, EPFU) is particularly valuable for the control of certain agricultural pests. A habitat generalist, EPFU roosts in a variety of locations, both man-made and natural. In this study, we examined differences in plasma cortisol levels between bats living in three roost types (attics, barns, bat boxes) and randomly assigned to treatment groups receiving one of three subcutaneous injection types (adrenocorticotrophic hormone, sterile saline, none). From each bat, two blood samples were collected to assess both baseline cortisol levels and concentrations following treatment. In addition, baseline plasma cortisol levels were measured for both male and female juvenile EPFU. The results of this study will be valuable in understanding how baseline stress levels of bats are impacted by their roosting environment. This information is particularly important given the many threats to bats in North America and the fact that continued habitat modification will continue to increase the number of bats living in anthropogenic structures.

Hormonal Plasticity in Cichlid Fish

Fiona Noble, Andrew Anderson, Suzy Renn

Mating pairs of the biparental fish, *Julidochromis transcriptus* exhibit behavior patterns determined by relative size. While each sex has a preference in natural settings to be either larger or smaller, behavior is plastic and both sexes can display either behavior pattern. What is unclear is whether hormonal mechanisms correspond to the behavior or to the sex. We manipulate pairings by altering relative partner size in a balanced design such that a medium sized individual is paired once with a smaller fish to exhibit a behavior pattern and then paired with a larger individual to exhibit another behavior pattern. Behaviors are recorded to confirm role within the pair and hormone levels are quantified after each pairing. Our results establish the relationship between behavior, gonadal sex, and hormone level. We focus on the four hormones estradiol, 11-ketotestosterone, progesterone, and testosterone in *J. transcriptus* in comparison to *J. marlieri*, which naturally pairs in the opposite size relationship. This work has important implications for understanding the mechanisms of cross-sexual transfer and the evolution of sex-biased behaviors.

Calculating Joint Torques in Axolotls during Underwater Walking with Aquatic Force Plate

Zachary Nopper, Kaelyn Gamel, Dan Bartlett, Henry Astley

Underwater walking was the evolutionary predecessor of terrestrial walking and represents a crucial step in the invasion of land. During walking, muscles generate torques and motion about joints which in turn generate substrate reaction forces to propel the animal as its appendages engage with the substrate. An animal that walks underwater, however, due both to the nature of being buoyantly supported and the much higher drag posed by water as a

medium, faces different mechanical demands than those needed for terrestrial locomotion. These differing mechanical demands, along with different limb kinematics, may require different patterns of torque at the limb joints. Using a custom-built underwater force plate and synchronous biplanar video, our lab has recorded substrate reaction forces and joint positions during individual footfalls of axolotls (*Ambystoma mexicanum*) performing underwater walking. By combining this data, we can calculate the torques at each joint during each footfall, as well as the work and power provided at each joint. Once completed, this dataset will provide a useful comparison to terrestrial walkers for examining the difference between walking on land and walking underwater, thereby providing insight into the water-to-land evolutionary transition of the earliest tetrapods.

Intrinsic variation in wild killer whale body condition indices evaluated using trained individuals

Dawn Noren, Jane Christopherson, Kristine Burtis, Tayler Kaplan, Lydia Staggs, Nick Tolimieri, Amy McCoy, Judy St-Leger, Todd Robeck

Monitoring individual health and impacts of stressors is important to the management of endangered Southern Resident killer whales. Body condition is evaluated using aerial photogrammetry of free-ranging killer whales and direct measurements of stranded individuals. Despite relying on morphometric measurements to evaluate changes in condition, no controlled studies have been conducted to understand the influence of age and sex on killer whale body condition indices (BCIs). To address this, we measured body mass, straight body length, and girth at four locations from 21 killer whales at three oceanaria monthly for one year. Relevant archived husbandry data were also obtained, resulting in four body length ratio BCIs (each girth/body length) and three eye girth ratio BCIs (each girth/eye girth) from 23 individuals (n=13 females, n=10 males, ages: 1.5-53 years old), monitored for 1-13 years. The relationship between length and mass in trained killer whales was similar to that for wild eastern North Pacific killer whales. Generalized additive mixed models (GAMMS) revealed that all seven BCIs varied by age and sex. Eye girth ratio BCIs typically increased nonlinearly with age. Relationships were more complex for body length ratio BCIs. These BCIs typically decreased with age in younger animals and increased with age in older animals. The influence of age and sex on killer whale morphometrics is important to consider when interpreting changes in free-ranging killer whale BCIs.

Turn on the Bright Lights: the Sea Urchin *L. variegatus* is not Uniformly Sensitive to Light

Julia Notar, Hazel Havens, Sonke Johnsen

Sea urchins are sensitive to light across their bodies and recent work has shown that they are also capable of spatial vision. Many animals' eyes possess areas of specialization or regions that respond differentially to light. The sea urchin visual system is hypothesized to be distributed throughout the skin, perhaps functioning as a whole-body compound eye. Here, we investigated the behavioral response of the sea urchin *Lytechinus variegatus* to light on different parts of the animal's body. We used a beam of 470nm (blue) light to compare two regions of the body (the ambulacrum and the interambulacrum) and three elevations (moving from the equator-line to overhead). In addition, we tested a range of brightness levels on the most responsive body region. Light on the ambulacrum produced a behavioral response when compared to the interambulacrum, which did not differ from a control (dark) response. The three elevations tested all produced significantly different responses from each other, but with no consistent trend. Finally, *L. variegatus* were able to detect as little as 10 lux, approximately the ambient brightness during civil twilight. Surprisingly, urchins were mostly positively phototactic and only moved away from the brightest stimulus (10,000 lux). These results clearly demonstrate that responses to light across the body of *L. variegatus* are not uniform and have implications for future studies of their visual sense.

Syntactic rules alone can account for song type matching in a songbird

Steve Nowicki, Lauren Chronister, Jill Soha, Susan Peters, William Searcy

Song type matching is suggested to be an aggressive signal in birds, but matching is not always associated with aggressive behavior. An alternative is that song type matching occurs simply as the consequence of syntactic rules governing song type sequencing. In song sparrows, we have shown that song sequences follow a complex set of syntactic rules that influence the order in which song types are produced over lengthy periods of singing. One such rule – the cycling rule – dictates that a bird cycles through its song types in a minimal number of bouts. Another rule – the bout length rule – dictates that a long bout of a song type is followed by a long interval before that type is repeated. In an eastern population of song sparrows, we tested these rules by challenging males at the end of a recording session with playback of one of their own song types. Matching probability increased with the length of the interval since the subject had last sung that song type, as predicted by the cycling rule. Matching probability decreased as prior bout length increased, as predicted by the bout length rule. Interval length and prior bout length together correctly predicted matching in 81.3% of cases. These results support the syntactic constraints hypothesis that matching can be a non-signaling by-product of internal rules governing the ordering of song type sequences.

Wound innervation coincides with cell proliferation and dedifferentiation in annelid regeneration

James Nowotny, Alexandra Bely

Annelids include some of the most remarkable regenerators of the animal kingdom. Like many other animals, annelids regenerate by forming a blastema, a mass of undifferentiated cells at the wound from which new structures develop. However, how their blastema is initially formed is poorly understood. Other animals with extensive regenerative properties such as flatworms employ migrating pluripotent stem cells to carry out regeneration, but the cellular source for annelid regeneration is poorly understood. To better understand where and when blastemal cells arise, we performed a fine-scale time series of cellular and molecular assays after head amputation in the freshwater oligochaete *Pristina leidyi*. We show that blastemal cell proliferation begins as soon as the ventral nerve cord extends into the wound site, immediately adjacent to neural termini. Also at the early wound site, we detect putative muscle dedifferentiation via actin filament shedding in close association with the severed end of the ventral nerve cord. RT-qPCR reveals a sharp *de novo* upregulation of pluripotency markers at the wound within just 3 hours of amputation, long before nerve cord extension or proliferation. These results imply that the same pre-existing cells at the amputation site transcriptionally take on a stem cell-like identity before dividing. Together, these data support the hypothesis that, like limb regeneration in vertebrates, head regeneration in annelids involves nerve-dependent dedifferentiation and proliferation of somatic cells.

Insect size responses to climate change vary across elevations and life histories

César Nufio, Lauren Buckley

Shrinking size has been termed the third-universal response to warming, mostly based on assembling data on endothermic organisms from museum collections. For the less examined ectotherms, the temperature dependence of development is more likely to shape size responses. Using grasshoppers historically and recently collected a part of a resurvey project, we detect size shifts that depend on population elevation and species' seasonal timing, consistent with evolved differences in developmental plasticity. Size shifts have been focused at low elevations with early season species getting bigger and later season species getting smaller. Size shifts are partially accounted for by seasonal temperatures, but additional size differences between time periods suggest a potential for longer term (evolutionary) responses. Warmer spring temperatures increase size (developmentally). For species that overwinter as juveniles, warmer temperatures the previous summer decrease size. These thermal responses are most pronounced at high elevations, where developmental plasticity is stronger. Grasshoppers, particularly of nymphal

diapausing or early season species, tend to capitalize on warm conditions by both get bigger and having early phenology. Our analysis further reinforces the need to consider physiology, and how it varies across elevations and life histories, when projecting organismal responses to climate change.

Resilience in a time of stress. Using proteomics to understand what makes some corals resilient and

Brook Nunn, Tanya Brown, Emma Timmins-Schiffman, Miranda Mudge, Mike Riffle, Jeremy Axworthy, Jesse Zaneveld, Lisa Rodrigues, Jacqueline Padilla-Gamino

Worldwide, corals are enduring an increased frequency of bleaching events. *Montipora capitata*, an abundant branching coral found in Hawai'i, demonstrates higher thermal tolerance than other species yet some colonies of *M. capitata* recover from local bleaching events (resilient) whereas others die (susceptible). As bleaching events degrade the symbiotic association between coral host and symbiotic algae, we explored how resilient corals maintain energetic demands after bleaching and determine if there are distinctive molecular signatures of resilience before bleaching starts. *Montipora capitata* was subjected to a simulated thermally-induced bleaching event by warming tank water to 30°C for 4 weeks, inducing bleaching, and then observing recovery over 8 months. Using an integrated-systems-biology approach that included quantitative mass spectrometry-based proteomics, 16S rRNA of the microbiome, total lipids, and symbiont density, we explored molecular-level mechanisms of tolerance in pre- and post-bleached colonies and found biomarkers that identify resilient and susceptible corals before thermal-induced bleaching events. Prior to thermal stress, resilient corals exhibited a more diverse microbiome and increased abundances of proteins involved in specific carbon and nitrogen acquisition strategies, symbiont retention, and pathogen resistance. Susceptible corals had early signs of symbiont rejection and utilized urea uptake pathways for carbon and nitrogen. Further, molecular signatures identified prior to bleaching were amplified after bleaching, suggesting these pathways may be deterministic in a colony's fate. Our results reveal molecular factors necessary for survival through bleaching events and provide diagnostic biomarkers for coral reef management.

Unveiling the Secrets of Seductive Smell: Comparison of female firefly extracts to male attraction

Katie O'Connor, Yiyu Zheng, Nathan Peot, Zhangyi Wu, Sean Halloran, Jocelyn Millar, Gregory Pask, Sarah Lower, Douglas Collins, Greg Fahrmer

Fireflies are renowned for their pretty shimmering light on a summer night, but most people do not know that their flashes are actually calculated to get a female's attention. However, some fireflies have lost the ability to shine bright, are day active, and likely use pheromones as a mating signal. Behavioral evidence suggests pheromone use, but only one firefly pheromone has been identified. In order to extract the pheromone, we tested two different approaches: "dunks" of female fireflies in hexane solvent and aerations of live female fireflies eluted with dichloromethane (DCM) for an unlighted species, *Pyropyga decipiens*. These processes gave us two different types of extracts that were bioassayed with male fireflies to compare which one has a greater ability to attract males. These two types of extracts were then run on a chemical instrument, gas chromatography mass spectrometry (GC-MS), and the chromatograms of negative and positive bioassay extracts were compared to see if any compounds varied between the two. Identifying a pheromone for this species would further the study of unlighted firefly populations for conservation, and also give us a glimpse of life on the "dark" side of fireflies.

Single nuclei sequencing reveals molecular basis of thermal acclimation in *D. melanogaster* embryos

Thomas O'Leary, Emily Mikucki, Sumaetee Tangwanchaoen, Sara Cahan, Seth Fietze, Brent Lockwood

Thermal acclimation is a physiological shift that allows organisms to tolerate extreme temperatures. This process often occurs over several days, while cells adjust the constituents of lipid membranes and the proteome. Here we demonstrate thermal acclimation occurring much faster, over just a few hours,

in developing *Drosophila melanogaster* embryos. Given the speed of embryonic acclimation, we hypothesized that chromatin-state-mediated changes in the transcriptome form the molecular basis of this trait. To test this hypothesis, we performed single nuclei multiome ATAC and RNA sequencing on embryos that were acclimated to different temperatures. We found coordinated changes in chromatin state and the transcriptome in response to thermal acclimation. Although many shifts were common across cell types, many significant changes were cell type specific. We discuss the implications of our results in the contexts of developmental and ecological physiology. Overall, our results indicate that chromatin modification is likely to be a heretofore underappreciated mechanism of thermal plasticity.

Fledgling on board: over-water offspring ferrying behavior in waterfowl, grebes, and loons

Juniper O'Leathlohair, Daniel Goldberg, Robert Jadin

Ferrying, the behavior in which waterbirds transport their offspring on their backs while in the water, is a captivating behavior that wildlife photographers and documentarians are no strangers to. In the avian research sphere, however, only a handful of studies have investigated this behavior, leaving much remaining to be understood about its evolution. One pattern that appears to emerge at a cursory glance is that the species that ferry tend to also exhibit diving ecology. We sought to test whether a true correlation exists between diving and ferrying, so we reviewed the published literature on the behavior, built a dataset of species that ferry, and ran statistical and phylogenetic analyses. To further assess ecological factors that may influence ferrying, we looked for correlations between ferrying and coloniality, territoriality, parental care type, and clutch size. Our initial analyses supported a strong correlation between diving and ferrying, though its significance diminished when accounting for phylogeny. Finally, we found that ferrying has originated at least 4 times within Anseriformes and has also been lost in 2 separate lineages of waterfowl. We suggest that a variety of avian life history factors may have influenced the evolution of ferrying behavior and should be the focus of future research on this topic.

Role of different legs in directionality and propulsion of *Microvelia* on water

Johnathan O'Neil, Pankaj Rohilla, Saad Bhamla

Microvelia uses the alternating tripod gait to walk on water by leveraging surface tension. In nature, these water walkers are occasionally found without limbs and tarsi due to predation, cannibalism, and competition for food and/or a mate. From experimental observation, we notice that the middle legs stroke at a higher amplitude than that of their other legs. Therefore, we investigate the role of the tarsi of different legs on their locomotion in terms of their directionality and speed by systematically ablating the tarsi to study their locomotion. High-speed imaging was used to record their locomotion on the water. DeepLabCut was used to track the joints of their limbs in addition to different parts of the body to estimate their kinematics. Through experiments, we show that different legs act as propellers and rudders and the body orientation is stable within a range of 2-5 degrees during locomotion regardless of the angular rotation of their legs during power strokes. We also quantified the strength of the vortices shed by the legs with and without tarsi using particle imaging velocimetry. Furthermore, we then explore how the *Microvelia* can adapt to the removal of tarsi over a 24-hour period. Through these ablation experiments, we then see how the alternating tripod gait is adapted to walking on water.

Delicious *Desmarestia*? *Pugettia producta* Dietary Preference Study

Lauren O'Rourke, Katie Dobkowski

Within the Salish Sea, bull kelp (*Nereocystis luetkeana*) is a major primary producer and structural species within kelp forest ecosystems. Another alga present in this habitat is acid weed (*Desmarestia herbacea*), which stores sulfuric acid in its cells. *D. herbacea* is a highly opportunistic species, often taking over after disturbances, and its acid is widely suspected to be a

herbivore deterrent. The Northern kelp crab, *Pugettia producta*, is a voracious consumer of bull kelp and can have a significant impact on kelp forest health; while they eat copious amounts of bull kelp, it is unknown whether or not they consume *D. herbacea*. I conducted choice and no-choice laboratory feeding experiments with *P. producta* collected near Friday Harbor Labs to determine whether or not the crabs could consume *D. herbacea*, and whether or not they prefer to consume it over *N. luetkeana*. Results suggest that *P. producta* does not consume *D. herbacea*, even when given no other food choice, and exhibits a strong preference for *N. luetkeana* over *D. herbacea* when given a choice between the two. This indicates that *P. producta* does not utilize *D. herbacea* as a food source and is unlikely to exert top-down control over this species in nearshore subtidal kelp habitats. Further investigation is required to determine which consumers can exert this control, potentially reducing *D. herbacea*'s competition with bull kelp.

Convergent evolution and the three epochs of eye evolution

Todd Oakley

Eyes evolved convergently many times and comparing allows new insights into evolution. I discuss how light-induced stress may have had a direct and prominent role in early steps in the evolution of eyes by bringing together genes to repair and prevent damage, even before the origin of eyes themselves. I will briefly summarize a vast literature indicating most genetic components of eyes historically responded to stress caused directly by light, including UV-induced damage of DNA, production of oxidative stress, and production of dangerous aldehydes. I refer to this early stage as the "First Epoch" of eye evolution, when stress-repair and prevention genes were perhaps originally deployed where light was prominent. The advent of regulatory-genetic control marked the "Second Epoch" of eye evolution. At that time, light-interacting genes may have been expressed even before light stress appeared, evolving as modules of interacting genes that could be influenced by natural selection and result in simple, dispersed photoreceptors. Networks of interacting genes that respond to light not only became parts of eyes, but also parts of other light-interacting novelties, including light-producing organs and faux eyes that ward off predators. A focus on stress-induced origins complements the long-standing account of eye evolution that argues natural selection favors morphological variations that increase functionality for sensing light. I refer to the traditional, gradual-morphological model of eye evolution as the "Third Epoch", but I argue that the gradualism is often taken too far. Instead, I explain a 'pre-fixe menu' model where entire modules are added in graduated steps. The highly convergent evolution of eyes led to the multiplicity of histories that led to these insights.

Stable isotope ecology and conservation of cave-roosting bats in Jamaica

Phillip Oelbaum, Ronald Hall, Damion Whyte, Ronald Stewart, Susan Koenig, Elizabeth Dumont, Kenneth Welch

Bat faunas across the Greater Antilles are remarkable in their diversity and high levels of endemism. Of 21 species of bat in Jamaica, 7 are found only on the island, including the critically endangered *Phyllonycteris aphylla* and *Natalus jamaicensis*. Jamaica is also unique in that many species occupy the same trophic guilds (14 insectivores, 4 nectarivores, 2 frugivores, 1 piscivore) and share roost space occupying many of the same caves. In March-April 2023 we set out to survey seven caves known for their large bat communities with the purpose of better understanding how these communities partition resources outside of the cave chambers. We collected hair and wing tissue from frugivorous and nectarivorous species and used carbon and nitrogen stable isotope analysis to assess their dietary niche breadth and overlap. We sought to compare populations of the same species across the sites they were captured in, as well as two-dimensional niche partitioning among co-roosting species. We additionally noted reproductive *Phyllonycteris aphylla*, previously believed to be limited to a single cave, at two sites where they had never been documented. These capture records, in addition to our isotopic data, critically inform conservation efforts for this species. Understanding how bats interact with their environment is also critical to understanding and safeguarding the important ecosystem services they provide.

The role of kelp canopy detritus and food availability on zooplankton population growth

Emily Ogawa, Katie Dobkowski

Bull kelp (*Nereocystis luetkeana*) is the only canopy-forming kelp in the Salish Sea and creates habitat throughout the water column for a wide range of organisms. Beneath kelp canopies on the seafloor, detritus accumulates as organic matter such as fragmented kelp and fecal outputs from consumers like sea urchins and kelp crabs (*Pugettia producta*) accumulate. However, the importance of kelp crab feces to detrital subsidies is unknown. Using a resilient intertidal copepod species, *Tigriopus californicus*, as our model consumer in the lab, we compared two different diets: kelp crab feces and fresh bull kelp blades. We hypothesized that there would be a difference in copepod growth and life stage abundance in populations fed kelp crab feces as compared to those fed fresh bull kelp. We found that there was no significant difference in copepod population growth between diets and no significant interaction between life stages and diet. Regardless, both diets sustained population growth of the copepods, thus indicating both food options, fresh kelp and kelp crab feces, likely play a role in bull kelp forest food webs. Bull kelp populations are decreasing in some parts of the Salish Sea due to ongoing ocean change, which may cause a change in kelp crab diet that could alter benthic food webs and nutrient availability.

Environmental and community drivers of resilience to an amphibian pathogen in North America

Michael Ohmer, Emily Le-Sage, Mark Wilber, Allie Byrne, Brandon LaBumbard, Karie Altman, Nina McDonnell, Veronica Saenz, Ian Latella, Laura Brannnelly, Cheryl Briggs, Jamie Voyles, Louise Rollins-Smith, Douglas Woodhams, Corinne Richards-Zawacki

Temperature variability, in the form of daily, seasonal, or yearly rhythms, can be a strong driver of disease dynamics, and may influence the ecology and evolution of pathogens and their hosts. The disease chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), has been implicated in the declines of amphibians worldwide, but in North America, many populations are coexisting with the fungus in an enzootic state. Over three years, we surveyed year-round Bd infection prevalence and intensity within amphibian communities along a latitudinal gradient in the eastern/southeastern and southwest US. We found strong seasonal variation in Bd prevalence at mid-latitude sites, peaking in late winter/early spring, while prevalence at northernmost and southwest sites remained relatively high and low, respectively, year-round. In contrast to Bd prevalence, we found extreme seasonality in infection intensity in the southwest, primarily driven by leopard frog species. When accounting for seasonality, body temperature non-linearly predicted infection prevalence, with prevalence decreasing at body temperatures below 5°C and above 25°C, mirroring the thermal limits of Bd growth. Furthermore, water temperatures one month previously predicted infection prevalence at the site level, with temperatures lower than 15°C resulting in higher prevalence overall. By investigating the role of seasonality and community composition on disease prevalence and intensity, we can better predict the impact of seasonal shifts and climate extremes on host resilience to disease.

The Dual Dynamics of Gut Microbes: Unpacking Maternal and Environmental Determinants

Esther Okamoto, Jennifer Kovacs

The gut microbiome exerts a profound influence on host fitness attributes, including fecundity, lifespan, and species survival. The extent to which environmental or maternal factors shape this microbiome, however, remains an area of inquiry. Utilizing the red flour beetle (*Tribolium castaneum*) *p*— which undergoes its entire life cycle in flour, a primary microbial source — as a model, our study dives into these determinants of gut microbial content. In our investigation, we posed three hypotheses: (H1) environmental factors predominantly shape the beetle's gut microbiome, evident when offspring share gut content similarities with non-related beetles from identical flour environments; (H2) maternal lineage largely dictates the gut microbiome, as seen when offspring, irrespective of their flour environment, reflect their mother's gut content; and

(H3) both factors have a hand in shaping the gut microbiome, with offspring displaying gut content characteristics of both their maternal lineage and their immediate environment. To test these hypotheses, we conducted four trials across three generations, with control trials maintaining a single flour environment and experimental trials switching flour types between generations. This design aids in delineating the relative impacts of environmental and maternal influences on the beetle's gut microbiome.

The functional robustness of elastic recoil mechanisms

Jeffrey Olberding

The most powerful movements in biology are driven by energy from recoiling elastic structures. Movements using stored elastic energy are robust to changes in temperature that otherwise disrupt muscle-powered movements because the energy loaded into and recovered from elastic structures is independent of rate properties like muscle contractile velocity. I test the hypothesis that elastically powered movements should be similarly robust to any perturbation that diminishes muscle contractile properties. I used isolated muscle-tendon unit (MTU) experiments to examine the effects of muscle fatigue on the storage and recovery of energy from elastic structures and compare the results to the expected thermal robustness of MTU performance when exposed to low temperatures. Like the detrimental effects of low temperature, fatigue results in decreased muscle force, rate of force generation, contractile velocity, and power. However, storage and recovery of elastic energy is relatively unaffected by fatigue. This fatigue resistance is fundamentally similar to thermal robustness and suggests that the use of elastic recoil mechanisms may ameliorate the detrimental effects of muscle fatigue on movement performance. However, the robustness of such movements may still be limited when loading elastic structures with forces near maximum isometric tension. Additionally, elastic recoil requires a latch that prevents movement during loading then releases. Any effects that decrease muscle contractile properties could potentially influence latch function and still result in decreased performance.

A 3D geometric morphometrics approach to identifying regionality in the tooth rows of squamates

Savannah Olroyd, Suresh Singh, Adam Huttenlocker

Heterodonty allows for specialization of teeth for various functions. This term is typically associated with mammalian dentition, but there are many exceptions to this generalization among squamates. While many squamates sport fangs or bulky molariform teeth, subtle differences along the tooth row are often visible even in classically 'homodont' species.

In mammals, heterodonty is controlled by gene expression during development, but it is uncertain whether this is also true for squamates. Various other factors could cause squamate heterodonty, including response to forces during feeding, tooth replacement, and growth of the animal.

We used 3D geometric morphometrics to quantify regionality in squamate toothrows to explore the role of these potential mechanisms. We placed five landmarks and 20 semilandmarks on all upper teeth in 20 individuals each from two species. We used segmented linear regression to identify break points in the morphology of the teeth along the jaw. This method successfully identified visibly different regions. In *Agama agama*, regionality of the maxillary teeth corresponds with the permanent addition of larger teeth to the jaw during growth. A distinct premaxillary region is also present, possibly reflecting embryonic development. In *Varanus niloticus*, maxillary tooth regions were present despite constant tooth replacement. Overall, these preliminary results demonstrate that these methods can quantify subtle regionality in the toothrows of squamates and that multiple mechanisms underlie squamate heterodonty.

Silver Linings: Microplastic Exposure Study of American Robin Mass Mortality Event

E. Olsen-Hodges, Sara O'Brien, Katie Wheeler, Jack Crofton, Brian Walker, Karen Powers, Matthew Close, Jamie Lau

The spread of microplastic pollution across the world has put pressure on researchers to determine the extent of exposure to wildlife. These microplastic pieces can range in size and shape and turn up in the environment from a variety of sources such as industrial plastics manufacturing, cosmetics, poly-fiber clothing, lost fishing nets and lines, and broken-down pieces of larger plastic trash. Due to a mass mortality event of American robins, *Turdus migratorius*, we had the opportunity to sample individuals ranging from after hatch year and older, males and females, to collect tissue samples (GI tract, heart, breast muscle, liver, kidney) and gut contents to determine the presence, abundance, and identification of microplastics. Here we describe a novel approach to sample gut contents and tissue types in over 70 individual birds. These samples will be digested, suspended, and filtered to remove biological debris and highlight microplastics to be identified by number, shape, color, and type. The goal of this work is to better elucidate the degree of microplastic pollution experienced by a terrestrial migratory bird. This project also displays the benefits of partnering with researchers at local non-profit programs, such as the Southwest Virginia Wildlife Center of Roanoke, to answer important conservation questions.

Robotic model barnacle nauplii

Aaron Omadutt, Kit Yu Karen Chan, Carr Everbach

Like most crustaceans, barnacles have a naupliar form that has three pairs of limbs, which are used in both feeding and swimming. Furthermore, they all have the taxon-defining pair of frontal horns. Body extensions, as such, can influence the hydrodynamics of the larvae by acting as sea anchors. To better understand the role of body extensions and their interactions with limb motion in larval function, we set forth to develop a robotic system to analyze the swimming pattern of barnacle nauplii. To ensure the robot was lightweight and compact, Nitinol springs were used as a substitution for conventional motors. Springs were controlled with a microcontroller that could either enable the flow of electrical current through the springs, causing them to heat up and contract, or halt the current flow, allowing the springs to cool down and expand. Utilizing a series of springs, microcontrollers, and 3D-printed components, the cyclic motion of the nauplius's power and recovery stroke within their swimming pattern was successfully replicated. In future, this robotic model will undergo testing in a viscous fluid, and particle image velocimetry will be used to analyze the flow patterns during the robot's free swimming. The development of this robotic system helps inform the role of body extensions in low to intermediate Re swimmers and, more broadly, the evolution of planktonic larval form.

Dermal Bone of K/Pg Trionychids examined via Surface Topographic Analysis and Paleohistology

Nathan Ong

Pan-Trionychids show minimal gross anatomical change across the Cretaceous-Paleogene boundary, but is their bone histology and shell ornamentation equally unaffected? These soft shelled turtles mineralize the leathery dermis covering their shells into a metaplastic lappet which includes millimeter-scale pits and ridges on the external surface and underlying layers of coarse collagen fiber bundle layers called the Plywood-Like Structure. Surface Topographic Analysis (STA) and paleohistology sampling were applied to over 200 turtle shell fragments collected across Maastrichtian and Danian deposits, encompassing four formations, 11 degrees of latitude, 12 genera, and a range of sizes and depositional environments. 3D scans of shell ornamentation were captured, then the complexity, sharpness, and steepness of the ornamentation was quantified using R's MolaR package. Specimens were paleohistologically sampled and the resulting thin sections were used to collect 20 linear measurements like cortical thickness, nutrient foramina diameter, and suture morphology, which were then statistically compared against latitude, stratigraphic position, depositional environment, and formation. Preliminary data suggests that specimens from different depositional environments show no

consistent variance in paleohistology or STA measurements and stratigraphic position imparts negligible variance. Plastomenids exhibited greater cortical erosion, along with higher OPCr, DNE, and RFI values. Danian turtles overall had thinner cortices with more PLY remodeling, relative to their Maastrichtian counterparts, but more anatomical and stratigraphic control is still needed to discern deep time trends.

Urobatis halleri Strikes Back!: 3D Tail Kinematics of the Round Stingray

Sean Ono, Benjamin Perlman, Kambria Galindo, Jacob Sobol, Anthony McGinnis, Trinity Lozano, Hanna Adamson, Samantha Widdoss, Grace Armendariz, Justin Yip, Angela Velazquez

Around the coastal United States, stingray strikes account for nearly 2,500 emergency room visits on an annual basis, in addition to the several hundreds to thousands of less-serious injuries that do not warrant hospitalization. Along California beaches, the Haller's Round Ray (*Urobatis halleri*) in particular, is responsible for the majority of these interactions, with anywhere between 200 and 400 stingray-related injuries being reported each year from localities like Seal Beach alone. During summer months, *U. halleri* aggregate en masse in warm, shallow sandy-bottom beaches, often coinciding with the locations that beachgoers tend to frequent. While stingray strikes are generally non-life threatening, the morphology of ray barbs are capable of inflicting deep lacerations while potentially envenomating the victim as well. Despite the rate at which these encounters occur and the potential threat that they pose to public safety, very little is known about round stingray behavior or the biomechanical properties associated with tail-strike events; only one other study quantified the 2D kinematics of this behavior in a closely related species. In our novel study we use 3D high-speed videography and motion tracking software to describe strike behavior across the ontogeny of the species *Urobatis halleri*, quantify the kinematics associated with tail strikes, and use this information for applications relevant to public beach safety.

Environmental DNA to Guide the Re-wilding of Akagera National Park

Jada Onwuta, Rachel Meyer, Brenda Larison, Janvier Uwayezu, Drew Bantlin, Beth Kaplin, Antoine Nsabimana

For the purpose of shaping wildlife reintroductions in Africa's national parks, park managers are interested in keystone species and ecological processes that shaped thriving communities before human influence. Environmental DNA (eDNA) metabarcoding can potentially enhance the success of rewilding efforts in Rwanda Akagera National Park (ANP). ANP lacks mammalian diversity, such as the cheetah (*Acinonyx jubatus*), Zorilla (*Lctonyx striatus*), and the East African Potto (*Perodicticus ibeanus*). However, these mammals live in savanna habitats only 100km away, such as Seregenti, and it is unclear whether these absences are natural (e.g. unsuitable habitat) or solely human-caused (removal).

This project aims to provide a framework for eDNA metabarcoding to provide current and historical trends for mammals in the park.

Since 2022, 15 Rwandan scientists have been trained to collect soil, sediment, and water samples from Rwanda Akagera National Park. Extracted DNA (DNeasy PowerSoil Pro Kit) was amplified (CO1, 18S, Mammal16S, 16S, 12SVert, FITS, and PITS). We built libraries using the CALEDNA metabarcoding pipeline. We sequenced the libraries on an Illumina NextSeq 2000 at UC Santa Cruz.

Sequenced DNA is being analyzed via cloud computing using eDNA Explorer. In addition, sediment samples are being dated (PB-210) at University of Minnesota-CSD. Our primary result will be a species list of park inhabitants and their presence-absence patterns over 200–500 years. Publications, sequence data, and archival results will be open access.

Cross-Generational Place-Based Education to Facilitate Environmental Stewardship

Dara Orbach, Katie Doyle, Larisa Ford

There is a disconnect between planning for economic development, recreation, and conservation needs with the integration of ecological resiliency principles in the educational system. We showcase a partnership between a public primary/intermediate school, primarily undergraduate university, and non-profit conservation organization that maximizes utilization of resources in the district for enhanced environmental protection and community resilience. The overarching goals of our partnership are to: 1) connect multiple generations of community members and students to coastal resources, 2) implement an engaging, culturally responsive, locally relevant, and sustainable OCEANS themed curriculum in K-8th grade classrooms, 3) advance environmental literacy through place-based learning, and 4) empower students and local citizens in environmental stewardship. We disseminate project collaborations, skills used to maximize efficacy among minority populations, and lessons learned along the way. We discuss ways to engage varied community stakeholders in taking ownership of environmental conservation to ensure sustainability of natural resources, with broad actionable application to diverse communities.

Critiquing the carnassial: reconstructing body mass in fossil small carnivorans

John Orcutt

Accurately estimating body mass in fossil animals allows macroevolutionary and paleoecological analyses of body size and informs reconstructions of behavior and physiology in extinct taxa. Body size reconstruction presents a paleobiological challenge, though, as illustrated by the enigmatic Eocene-Miocene carnivoran *Palaeogale*. Size is a taxonomically and phylogenetically important trait in *Palaeogale* and one that has implications for the evolution of sexual dimorphism and feliform biogeography. However, body mass estimation in carnivorans relies on complete skeletons, skulls, or first lower molars, which are often unavailable. Furthermore, the equations used to estimate mass are based on a wide sample of carnivorans, including large-bodied species over an order of magnitude heavier than taxa like *Palaeogale*. Dental measurements of modern small carnivorans (musteloids, viverrids, and herpestids) in museum collections were used to create body mass regressions for each tooth to test the viability of reconstructing size based on incomplete fossils. Surprisingly, first lower molar length was not as strongly correlated with body mass as were the lengths of several other teeth, notably the upper canine and fourth lower premolar. This may be due to strong functional and phylogenetic signals in carnivoran carnassials. In any case, this does indicate that good dental proxies for body mass exist throughout the jaw, which has the potential to greatly expand sample size in future studies of carnivoran body size evolution.

Investigating the energetic cost of the distributed visual system of scallops

Ceren Ordas, Jeanne Serb

The high energetic cost to maintain neural tissues has been proposed to be the strong selective pressure on the evolution of eyes and their loss. In paired-eyed systems of insects and vertebrates, the cost of vision is between 15-20% of the resting metabolic budget. In distributed visual systems, where invertebrates have tens to hundreds of morphologically similar eyes that appear to be functionally redundant, eyes are also assumed to be energetically costly. To test this hypothesis, we are utilizing the natural variation in eye number among individuals in the common bay scallop, *Argopecten irradians* (Pectinidae). The distributed visual system of this species is composed of 30-60 similar eyes that have overlapping visual fields, indicating a high degree of functional redundancy, hence a possible high energetic cost to maintain eyes that carry out similar functions. We measured oxygen consumption as a proxy for metabolic rate. If eyes are energetically costly to maintain, we expect the metabolic rate will increase for individuals with greater eye numbers. This study is the first step to understanding energetic costs in distributed visual systems. Future work will examine functional redundancy in the scallop eyes.

Princes of Darkness: limb skeleton organisation and evolutionary dynamics in bats

Andrew Orkney, David Boerma, Brandon Hedrick

Powered flight has emerged independently at least 3 times in vertebrate evolution. Life on the wing grants access to novel ecological niches, resulting in taxonomically and ecologically diverse adaptive radiations that are distributed across a wide variety of body masses. The acquisition of this animal super-power is associated with a reorganisation of the skeleton in both birds and pterosaurs, facilitating independent evolutionary trajectories in the wings and legs. There are suggestions the same is true of bats, but no large sample has yet been appraised to confirm this. Moreover, bats possess a unique wing-suit that unifies the axial skeleton, fore- and hindlimbs into a continuous aerofoil, causing us to question whether bat limbs are constrained to evolve in unison, which may limit their evolvability and ecological disparification.

We assembled a phylogenetically and ecologically diverse dataset of 3D shapes and sizes of wing and leg bones across 70% of bat families and 2.5 orders of magnitude of body mass. We applied geometric morphometric and phylogenetic comparative methods, showing that bat fore- and hindlimbs tend to evolve in concert. We explore further, computing models of trait evolution in a phylogenetic context, and comparing bats with a dataset of 149 bird species. We show that bats evolve more slowly than birds and that there is significant convergence in their wing proportions, suggesting fundamental constraints upon their phenotypic evolution.

Hazard of an emerging pesticide on bumblebees

Sarah Orr, Michael Goodisman

More than 30% of human food crops require animal pollination. Farmers also depend on agrochemicals to control pest insects. However, these agrochemicals can have negative consequences on beneficial insect pollinators, such as bees. We investigated the effects of an emerging class of pesticides, sulfoxamines, on the highly social, common eastern bumblebee, *Bombus impatiens*. We performed a series of 96-hour toxicity tests on microcolonies of laboratory-reared *B. impatiens*. Our preliminary data show that sulfoxaflor is significantly less toxic than historically used alternative pesticides, such as thiamethoxam. Further, we found significant differences between caste sensitivity to sulfoxaflor; workers are more sensitive than drones. These findings are especially valuable since they demonstrate important sex differences in toxicity. Finally, ongoing chronic experiments will reveal interesting sublethal effects of sulfoxaflor exposure including colony development, gene expression, and foraging behavior. We posit that sulfoxaflor may be a better alternative to neonicotinoids but is not nontoxic to bees and should be applied in the environment cautiously.

A view from the shoulders of giants: trade-offs in context from Fisher to present day

Teri Orr, Chloe Josefson, Theodore Garland

How has our definition of trade-offs evolved over time? Growing far beyond a term from economics meaning 'missed opportunities', generations of biologists have employed this framework, but have we reached a consensus as to what a trade-off is and how best to study it? Here, we focus on the historical perspective of trade-offs as they relate to female reproduction. Providing a historical framework, we begin with Williams, Fisher, and Lewontin and wind through Lack, the seekers of invariant rules of life (Charnov, Whitehead) to those who've argued we must take a quantitative genetics approach (Roff, Fairbairn), a genome-to-phenome approach (Stearns, Reznick) with a special focus on just how trade-offs are discussed as integrative biologists (Arnold and others). What taxa are the mainstays, and which remain poorly studied? What sexes have been the focus of study is of particular interest whereby certain taxa (egg layers) are easier to study and measure reproductive output in. Additionally, we discuss trade-offs in a sociological context and discuss the impact of culture on how each scientist viewed trade-offs. We conclude by discussing the future of trade-offs with a focus on current definitions, unknowns, and problems with current approaches. Special emphasis is

placed on taxa and metrics that have been particularly well or poorly studied to highlight the utility of this framework for future studies as more tools become available.

Capillary origami oars allow water striders to skim turbulent streams in a nonstop journey

Victor Ortega-Jimenez, Dongjin Kim, Je-Sung Koh, Saad Bhamla

Rhagovelia bugs are unique among water striders because they have special fan-like structures located on their middle legs that allow them to glide on the surface of tempestuous streams, resembling animal fliers. It has been assumed that the fan actuation is exclusively under muscle command. Here we show that the fan spreading is actually triggered by capillary forces, resulting in a rapid microstructural self-spreading, which enables propulsion and locomotion control. An isolated fan placed in and out the water suddenly spreads and folds in less than ~10 ms, respectively. This rapid fan spreading facilitates quick maneuvers and extreme turning angles (~180°), that are similar to those performed by insect fliers during saccades. Moreover, a bioinspired self-morphing fan increased the rowing performance of an insect robot. Fluid dynamics of natural streams inhabited by Rhagovelia showed high turbulence levels. Interestingly, we discovered that these bugs kept rowing day and night resting obligated only during molt, thus covering a long journey. Therefore, Rhagovelias' self-morphing fan ensures a reliable and rapid fan spreading, fundamental to overcome and endure highly turbulent waters.

Running hot: Neotropical ant running speed is independent of surface temperature

Teresa Rose Osborne, Stephen Yanoviak, Alyssa Stark

Ants adhere to surfaces through a combination of the mechanical action of their claws and the physical and chemical properties of their adhesive foot pads. In some ant species, fluid-based adhesion has been shown to be temperature-dependent, with weaker adhesion to hot surfaces than to cooler surfaces. It is unknown whether these changes in adhesive performance at high temperatures result in similar reductions in locomotor performance. To assess locomotor performance, we measured running speeds of two ant species known to experience reduced adhesion at high temperatures, *Cephalotes atratus* and *Atta colombica*, across a biologically relevant range of surface temperatures. Neither species showed a clear reduction in running speed on hot surfaces. Interestingly, *C. atratus* showed a significant effect of ambient humidity on running speed, as well as a significant interaction between surface temperature and colony identification. Future research into the roles of humidity and inter-colony differences on locomotor performance are warranted. Our results indicate that ants experience no drop in locomotor performance when exposed to dangerously hot surfaces, adding to the body of evidence supporting the resilience of the insect tripod gait and adhesive system to environmental challenges.

The Effects of Sound and Carbon Dioxide on Invasive Bigheaded Carp Upstream Migration.

Ben Osei-Tutu, Michael Frett, Allen Mensinger

Silver (*Hypophthalmichthys molitrix*) and bighead carp (*H. nobilis*), collectively known as bigheaded carp, are invasive fish species that are currently threatening to establish resident populations within Minnesota waterways and the Great Lakes. Dams, along with their adjoining lock chambers, are potential strategic bottlenecks for restricting the movement of bigheaded carp within the Mississippi River and its tributaries. The development of a multimodal system incorporating bioacoustics and carbon dioxide (CO₂) has shown promise to act as an effective non-physical deterrent against the upstream movement of bigheaded carp through lock chambers.

The effects of CO₂ injection paired with broadband sound in a model lock and dam chamber was examined as a potential deterrent. Schools of bigheaded carp were conditioned to associate broadband sound (2-4 kHz) with the simultaneous injection of CO₂ within the lock chamber. Following conditioning trials, four experimental sound trials were conducted daily for one week. The conditioned fish avoided the lock chamber whenever the sound was active, which

prevented migration into the upstream pool. The carp showed no evidence of habituating to the sound after week-long trials. Subsequent trials will examine the efficacy of the multimodal deterrent system against food-motivated fish and unconditioned fish. Successful implementation of this non-physical deterrent system in the field could impede the upstream movement of bigheaded carp, protecting native fish populations and preserving the aquatic ecosystems in the Midwest.

State-dependence of neuronal gene expression for feeding-related peptides in a nudibranch

Kate Otter, Laurenzia Cairo, Cheyenne Tait, Paul Katz

Neuropeptides play important roles in regulating behaviors, such as food intake and foraging. In molluscs there are several neuropeptides that have been identified as modulators of feeding behavior including feeding-circuit activating peptide (FCAP) and buccalin. There are also several molluscan insulin-like peptides (MIPs) that are associated with growth, development and feeding. We have identified neurons in the brain of the nudibranch *Berghia stephanieae* that express FCAP, buccalin, MIPs and neuropeptide-F (NPF). Genes for FCAP, buccalin and NPF, were previously identified in our lab, here we identified the MIP genes. Neurons in all of the central-ring ganglia in *Berghia*'s brain expressed FCAP, buccalin and NPF as determined by in situ hybridization chain reaction (HCR). It is possible that neurons change their gene expression for these peptides in response to hunger state. Therefore, we compared gene expression in neurons in sated and food-deprived animals. When food-deprived, buccalin and FCAP were expressed in fewer cells. NPF expression did not appear to be grossly affected by hunger. In other gastropods, seven MIPs have been identified. Two of these, MIP-II and MIP-V have been found to be expressed in the brain and downregulated by starvation. Ongoing work will characterize expression of these MIPs. This investigation of feeding-related peptides will aid our understanding of how internal states are represented in the brain and affect hunger state.

Distinct phases of nudibranch predatory behavior are differentially affected by hunger and prey cue

Kate Otter, Paul Katz

Predation can be broken down into sequences of behaviors, whose transitions depend on internal and external information. The internal information includes satiety; hungrier animals are more likely to complete a predatory sequence. Essentially, predation is an approach-avoidance decision, especially in the context of dangerous prey. Unlike other animals, for predators of dangerous prey, the same stimulus can be both appetitive and aversive. The nudibranch, *Berghia stephanieae*, is a monophagous specialist predator, feeding on a single prey species, the anemone, *Euaiphtasia diaphana*. The anemone has stinging nematocysts, which *Berghia* can sense at close range, however *Berghia* can also sense anemones chemically at a distance. Here, we leveraged the unique ecology of *Berghia*, to understand how hunger-state modulates approach-avoidance decision-making. We found that hunger-state is a gradual process that shifts the balance between approach and avoidance. Furthermore, distance cues from the *Euaiphtasia* were initially attractive to all animals, sated animals avoided the prey after interacting at short-range, indicating contact cues are satiety-dependent. Intermediately hungry animals repeatedly re-evaluated their choice and showed specific behavioral motifs that more food-deprived animals did not show. Thus, we have defined a model where distance cues are appetitive regardless of state and the valence of close-range cues are satiety-dependent.

Using a facultatively symbiotic coral to investigate immune regulation of host-symbiont dynamics

Louis Oviedo, Erin Borbee, Lauren Fuess

Symbiosis and immunity have been linked in a number of biological systems. In corals in particular, the establishment of the coral-algal symbiosis is believed to be associated with suppression of the coral immune system. However to date, most studies investigating immune-symbiosis interplay in cnidarian systems have been conducted using obligately symbiotic corals. Facultatively

symbiotic species, which display immense natural variation in their symbiotic associations provide an ideal system for further investigating symbiosis-immune interplay, and the roles of immunity in regulation of symbiotic relationships. Here we used colonies of the temperate, facultatively symbiotic coral *Astrangia poculata*, which display intra-colonial variation in symbiont density, to investigate the relationship between immunity and symbiont density. Using a standard suite of biochemical immune assays which capture antioxidant activity, antibacterial activity, and melanin production, we characterized immune activity in high vs. low density regions of the same colony of coral. The data gathered from this study will contribute to an understanding of how symbiont density impacts coral immunity, and what mechanisms control symbiotic state within individual coral colonies.

Parental care and brain investment in a monogamous arthropod

Karmi Oxman

Families are basic units of most animal societies, but pair-based collaborative parental care is rare. Parental care in diverse vertebrates leads to changes in brain structure and function. Neural plasticity allows brains to accommodate novel cognitive requirements. Vertebrates with biparental care exhibit plasticity in care behavior in response to changes in brood size and the presence or absence of a partner with associated changes in the brain. We asked whether similar patterns in neural and behavioral plasticity hold in arthropods.

Hemilepistus reaumuri is a land-dwelling isopod that exhibits bi-parental care. *H. reaumuri* are single-bout reproducers, focusing reproductive resources on one clutch of offspring. Prior to the emergence of young, both parents feed only themselves. After the young hatch simultaneously, both parents shift their foraging behavior and provide food by bringing plant material back to the young.

We quantified parental care effort and associated brain tissue investment under different social environments. In the field, parents were paired or manipulated to be unpaired. Video monitoring was used to quantify foraging investment. Histological staining was used to describe the brains of these isopods before and after the offspring care season. We focused on quantifying the volume of the central complex because of its role in navigation and foraging. We provide a powerful new test of sex differences in brain plasticity and of parental care plasticity in arthropods.

Terrestrial locomotion of juvenile tidepool sculpins and the effects of size and substrate.

Cinnamon Pace

Juvenile tidepool sculpins (*Oligocottus maculosus*) are found in higher reaches of the intertidal than adults and have increased risk of emersion in an environment with a complex heterogeneous substrate. Additionally, within juvenile sculpins there is discrepancy among size in the same habitat. Thus, how similar is juvenile terrestrial locomotion to adults and how does substrate and fish size impact movements? Juvenile sculpins from 17.1mm–35.1mm long were filmed moving across three different substrates: paper towel, small rocks (1-2mm), and medium rocks (2-4mm). Locomotor sequences were digitized in order to calculate kinematic and timing variables of the body, caudal fin, and pectoral fins. Larger juvenile sculpins had longer and deeper bodies. Preliminary data suggests that their pectoral fins may also be relatively larger (%TL) than in smaller juvenile sculpins. Regardless of size or substrate juvenile sculpins could move over land by utilizing tail and trunk movements in conjunction with their pectoral fins in a fashion similar to adults. However, more complex substrates were harder for them to navigate. The smallest sculpins struggled the most and were the most likely to stop using their pectoral fins and to exhibit other behaviors (i.e. jumping). Examining interactions between size and substrate can help us understand how organisms cope with variable habitats throughout key developmental windows, challenges relevant to many organisms including early amphibious vertebrates.

Preparing the next generation of integrative organismal biologists

Dianna Padilla, Daniel Grunbaum

The use of modeling to understand organismal responses to both short- and long-term changes in their internal and external environments is increasing. Training students prepared to pursue the cutting edge questions in integrative organismal biology will need to include knowledge and use of various types of modeling, especially systems-type modeling, combined with integrative organismal biology. This means that in the future we will require teaching different approaches than we typically think of in training the next generation of organismal biologists. But it is unclear what the best approach is to such training, or when and how we start to train that next generation. To address this question we propose new ways of teaching and learning for that next generation of biologists. We provide one possible plan, including the development of training modules that will allow new scientists to approach this new way of imagining and conducting integrative organismal biology, using early life stages of invertebrates as an example. Such a teaching approach could be generalized to address a variety of questions at the future forefront of organismal biology.

The importance of life history strategies in the recovery of corals after thermal stress

Jacqueline Padilla-Gamino, Emma Timmins-Schiffman, Jeremy Axworthy, Tanya Brown, Callum Backstrom, Brook Nunn, Lisa Rodrigues

Discovering the mechanisms involved in tolerance and recovery of corals after thermal stress is crucial to predict the consequences of climate change. To date, most studies have focused on the potential of corals to survive and recover from stress. However, the persistence of corals will not only require the survival of adults but will also depend on corals' ability to continue sexual reproduction and produce viable offspring. In this study, we examined the reproductive capacity, symbiont and microbial transmission to eggs, and long-term (nine-month) physiological recovery in corals that were bleached naturally and experimentally. We found that bleached corals of *Montipora capitata* (a hermaphroditic spawner) were able to reproduce but that gamete development was delayed, and spawning occurred later in the summer. In *Porites compressa*, a gonochoric species, different sexes showed distinct physiological signatures after thermal stress, with male colonies exhibiting higher bleaching susceptibility. *M. capitata* allocated 10% more carbon to gametes despite bleaching by limiting the allocation of carbon to adult tissues, with 50–80% less carbon allocated to bleached compared to non-bleached colonies. Over the same period, *P. compressa* maintained carbon allocation to adult tissues, only allocating surplus carbon to gametes. Our study highlights the importance of autotrophy for carbon allocation from adult corals to gametes, and species-specific differences in carbon allocation depending on bleaching susceptibility.

Reconstructing cell type specification trajectories in the last common animal ancestor

Natalia Padillo-Anthemides, Fredrik Hugosson, Mark Martindale, Joseph Ryan, Brent Foster

One fundamental goal of evo-devo is to reconstruct ancestral cell type specification trajectories. Ctenophora, as the sister lineage to the rest of animals, are key to reconstruct the evolution of gene regulatory networks and developmental processes in all animals. We have sequenced RNA from more than 20,000 cells along nine timepoints between 4 and 24 hours in the ctenophore, *Mnemiopsis*. Using this dataset, we are generating single-cell trajectories of cell-state transitions during *Mnemiopsis* embryogenesis. Our results suggest that the majority of cell types have been specified by 24 hours with the following exceptions: certain neurons of the aboral organ, a subset of digestive cells, and a subset of comb-plate cilia. After comparing our data with similar data from bilaterian species, we have generated hypotheses that address the origin of several gene expression cascades underlying conserved cell types. The confirmation or refutation of these hypotheses will provide a significant advancement of our understanding of cell-type specification in the last common ancestor of animals.

Effects of fluctuating temperatures on fecundity and population growth in a widespread noctuid moth

Anchal Padukone, Kimberly Sheldon

Insect pest populations are generally forecasted to increase outside the tropics and decrease in the tropics under climate change. However, many predictive models of pest populations and impacts are based on laboratory studies that record insect performance under constant temperatures. These may not reflect the fluctuating temperatures insects experience in their natural environments. Our previous work on the widespread agricultural pest *Spodoptera frugiperda* shows that chronic exposure to realistic fluctuating temperatures alters juvenile survival and development relative to performance at a constant mean temperature. However, a key unanswered question is how temperature variability impacts fecundity and population growth in this species. We monitored survival and lifetime reproductive output in females reared from eggs under a wide range of mean temperatures and temperature fluctuations representative of conditions in the species' year-round range. We hypothesized that temperature mean and variability independently and interactively affect lifetime reproductive output and the distribution of egg laying over time. We used fecundity data to parametrize stage-structured matrix population models, allowing us to understand how expected population growth varies across temperatures and to determine what demographic processes may drive these differences. We hypothesized that increasing temperature mean and fluctuation would primarily affect population dynamics by impacting development rates and survival to maturity, which would also alter predicted crop losses from larval feeding.

Diet, Phylogenetic Signal, and Intra-specific Variation in the Musteloidea Carnassial Complex

Stephanie Palmer, Siobhán Cooke

Recent research suggests that dental topographic analysis (DTA) has utility in quantifying the dietary ecology of carnivorans, and in predicting diet in fossil taxa, particularly mustelids. However, our understanding of phylogenetic effects and the range of intra-specific variation present hinders interpretation of DTA results. We assess these factors in DTA values of the carnassial complex (m1 and P4) of 13 Musteloidea species, with sample sizes ranging from 1 to 6 individuals per species.

Dietary trends were observed in DTA values. Small-mammal and fruit feeders had lower DNE and OPCR values while hard-invertebrate and plant feeders had higher values with the exception of OPCR in maxillary p4. The North American river otter (*Lontra canadensis*) had degrees of intra-specific variation which caused overlap with species of differing dietary ecologies (raccoon; *Procyon lotor*), though this overlap could be explained by the inclusion of hard invertebrates in both species' diets. Degree of phylogenetic signal differed among DTA values and dental elements, with it being strongest in OPCR of m1 and weakest in OPCR of P4.

Variation in values observed when increasing sample size may reflect variation in diet and wear between individuals or variation in mesh properties between scans. Further research is necessary to clarify this trend. It is apparent that both dietary and phylogenetic signal in DTA values vary by dental elements and specific DTA variable.

Combining computational fluid dynamics and experimental data to understand fish schooling behavior

Yu Pan, George Lauder

Conservation of energy has long been thought to be a pivotal factor driving fish schooling behavior. However, understanding how fish within a school harness energy from interactions among members has proven to be difficult, mostly due to the complexities of measuring forces and flows generated by schooling individuals. Recently, methods in experimental fluid mechanics such as particle image velocimetry (PIV) have been successfully applied to explore the physical mechanisms and physiological functions of solitary fish swimming through flow visualization. Additionally, by combining motion tracking and analysis, computational fluid dynamic (CFD) techniques have been used to simulate fish locomotion, fostering a deeper understanding of fish swimming hydrodynamics. But the hydrodynamic environment of fish schools is

inherently three-dimensional and complicated, intensifying the difficulties associated with flow measurements. This necessitates a synergy of computational and experimental fluid techniques. CFD techniques can help obtain high-fidelity performance measurements and specific flow characteristics for deeper analysis. Meanwhile, experimental approaches can capture the precise locomotion of each schooling fish and provide extra flow information through direct measurements to enhance the accuracy and efficiency of CFD studies, while circumventing the limitations posed by high Reynolds number flow through data assimilation techniques. The combination of CFD models and experimental data allows us to attain new insights into the dynamics of fish schools, facilitating evaluation of functional significance and enabling comparative studies of schooling behaviors.

Dietary carbohydrates increase heat stress resistance of honey bees' foragers

Trisha Panganiban, Laura Haefner, Gabriela Robles-Pérez, Natalie Herbison

Nutrition is known to influence several aspects of organisms' life, from development to resistance to environmental stressors such as changes in temperature. Dietary carbohydrates are linked to higher heat and cold tolerances in many insect species. However, controlled laboratory studies with bumble bees and honey bees indicate that the upper thermal limit, a metric of heat tolerance that estimates the temperature at which individuals lose muscle control, is not influenced by access to food or short periods of starvation. Using a static protocol (constant temperature), we explored how short-term nutritional stress (limited access to nectar and access to nectar with low-sugar concentration) influences the heat stress resistance of honey bee foragers. In addition, we compared the heat stress resistance of bees fed with two commercially available honey bee candies used by local beekeepers, which differ in their sugar composition. We found that heat stress decreases in unfed bees and in bees provided with water and that sugar concentration increases bees' heat tolerance. Our findings suggest that while dietary carbohydrates might not directly impact bees' upper thermal limit, they do appear to influence their capacity to endure prolonged exposure to heat stress. Thus, carbohydrate availability in bees' diet might play a key role in enhancing their resilience during extended periods of elevated temperatures.

Sizzling Strategies: How High Temperatures Modify Anti-Predator Behaviors in Tree Swallows

Lillian Para, Maëlle Lefevre, Elizabeth Derryberry

With global temperatures rising, a pressing concern is understanding how exposure to high temperatures alters fitness-related behaviors in free-living animals. Like many other organisms, most songbird species are shifting their ranges north in response to climate change. However, with the notable exception of tree swallow (*Tachycineta bicolor*) ranges shifting south, we ask how temperature affects a key fitness-related behavior in these birds: nest defense from predators. We hypothesize that higher ambient temperatures will result in decreased defensive activity (including calls and behaviors) in response to a simulated nest predator due to heat stress. We used a repeated measures approach, running predator defense trials twice per nesting pair and opportunistically sampling on hotter and cooler days within the last week of incubation. Measures were taken both in the early and late breeding season, the latter having higher temperatures overall. We found that in the early season, as predicted some tree swallow defensive behaviors decreased with increased ambient temperature, however, this trend disappeared or reversed in the late season. These differences between early and late season trials suggest acclimatization to seasonal temperatures may affect nest defense. We anticipate this study provides a basis for future songbird defensive behavior studies in a rapidly warming world.

Assessing RNAi in *Lumbriculus variegatus* as a tool for reverse genetics

Iris Pardue, Kathy Gillen, Boyu Yang

Lumbriculus variegatus can regenerate over half of its body from cut segments, restoring body polarity and function in just a few days. The genes involved in this process are not currently known, so we plan to use RNAi to test gene function through reverse genetics. As this technique has not been used before in this organism, we are testing a variety of methods to introduce dsRNA into the worms. We have nanoinjected small amounts of dsRNA near cut sites, and are also exploring bacterial feeding techniques similar to the standard procedure in *C. elegans*. Our initial knockdown target is α -tubulin, as studies have shown that chemical inhibition of tubulin with colchicine inhibits regeneration. Ideally, a RNAi-based knockdown would show the same organism-wide phenotype of reduced regeneration as a chemical one. Preliminary testing in worms showed a decrease in regeneration that was inconsistent across organisms. However, without molecular testing, we do not know if α -tubulin levels were significantly affected or if we were observing natural variation in regeneration rates. To remedy that, we assessed Western blotting as a potential method to assay knockdown. Western blotting showed mixed results for detection of tubulin, but could be useful in the future if the technique is improved.

Investigation of Copulatory Structures in Spiny dogfish sharks using 3D Geometric Morphometrics

Catherine Paredes-Amaya, Rachel Keeffe, Brandon Hedrick, Patricia Brennan

Spiny Dogfish sharks (*Squalus acanthias*) belong to the ancient and diverse lineage of Chondrichthyes, a group that has undergone relatively little morphological change since originating over 400 MYA. Although chondrichthyans are among the few fish with intromittent organs, we know little about variation in their copulatory structures. The claspers of dogfish males are located in the pelvic fins. We investigated the morphological variation in these copulatory structures and compared them to variation in a non-copulatory structure, the pectoral fins. We dissected 23 pairs of pectoral fins, and 33 clasper pairs, and pelvic fins. We generated 3D models of these structures using a laser scanner. Claspers were scanned in open and closed positions, to capture clasper morphology during copulation and rest. We used AUTO3DGM to align 3D models with 3000 pseudo-landmarks. Landmark data were imported into R for analysis. We examined shape variation in pectoral and pelvic fins and their association with body size. We found no significant relationship between pectoral fin shape and body size, indicating that small and larger males have the same fin shape, however, as we expected, there are significant differences in the pelvic fin shape, including the closed claspers, between smaller and larger males. In further work, we will examine the open claspers, and asymmetry between right and left sides of the body.

Antimicrobial protection of immunologically underdeveloped marsupial neonates

Jongbeom Park, Aella Kaage, Mohamed Donia, Ricardo Mallarino

While prenatally protected in the sterile maternal womb, at birth, mammals are exposed to a plethora of microbes. Such a sudden shift in microbial environments is especially threatening to marsupial neonates as they are born immunologically underdeveloped. Unlike eutherians, marsupial newborns lack key lymphoid organs, such as the thymus and the lymph nodes, which are essential for functional adaptive immune responses. As the maternal pouch—where marsupial newborns spend several months—harbor microbes associated with diseases, marsupial newborns are under constant microbial threats. Here, we investigate how marsupial neonates protect themselves from pathogenic microbes using laboratory sugar gliders (*Petaurus breviceps*). Single-cell RNA sequencing of neonatal hematopoietic liver indicates that neutrophils are the most abundant immune cell type, comprising ~78.5% of leukocytes. Gene expression analysis shows that cathelicidin antimicrobial peptide genes are highly expressed in neutrophils. Detailed annotation of the cathelicidin gene family indicates that sugar gliders have 10 cathelicidin genes while humans and mice have only one. Multiple sugar glider cathelicidins

exhibit stronger antimicrobial activities than eutherian cathelicidins against mammalian pathogenic bacteria found in marsupial pouches. We also show that cathelicidins reduce proinflammatory responses by inhibiting bacterial lipopolysaccharide binding to immune cells. By combining transcriptomic profiling, genomics, and microbial and immunological assays, our findings suggest that highly duplicated antimicrobial peptide genes protect immunologically underdeveloped marsupial neonates.

Poké Pop-Up Museum: A Cosplay for Science Community Outreach Project Centered on Organismal Biology

Gregory Pask, Alexis Mychajliw, Gabriel-Phillip Santos

In higher education, community-engaged learning projects are effective methods for enriching curriculum, promoting creativity, and strengthening community connections. Within science, community outreach can break down traditional barriers to science and demystify the methods used to understand the natural world. Here we describe the Poké Pop-Up Museum project, a community outreach event developed by undergraduate students in two organismal biology courses, Entomology and Mammalogy, in a partnership with the educational initiative Cosplay for Science. Using the popularity of Pokémon, students designed and presented temporary exhibits highlighting how several Pokémon are inspired by real world insects and mammals, and then used this familiarity to discuss interesting questions in organismal biology research, all while cosplaying as a Pokémon researcher. The event was extremely successful, attracting over 200 visitors over a 3-hour period in a rural community. The successful cross-pollination between classes created a synergy around our departmental learning goals, and students had the opportunity to develop and practice their science communication skills in a realistic outreach environment (not simply an artificial outreach project only read by the professor). We offer this creative service learning model as an example of engaging and fun pedagogy in organismal biology that can serve both course learning goals and community needs.

Identification of a Sex Pheromone of the Winter Firefly, *Photinus corruscus*

Gregory Pask, Sarah Lower, Kyle Arriola, Sean Halloran, Hannah Holmes, Daphné Halley, Yiyu Zheng, Douglas Collins, Jocelyn Millar

Firefly flashes are well-known visual signals used by these insects to find, identify, and choose mates. However, many firefly species have lost the ability to produce light as adults. These “unlighted” species generally lack adult light organs and are diurnal. They are believed to use volatile pheromones acting over a distance to locate mates, but none have been previously identified in any firefly species. In this study, using coupled gas chromatography - electroantennographic detection, we detected a single female-emitted compound that elicited antennal responses from wild-caught male winter fireflies, *Photinus corruscus*. The compound was identified as (1S)-exo-3-hydroxycamphor (hydroxycamphor). In field trials at two sites across the species’ eastern North American range, large numbers of male *P. corruscus* were attracted to synthesized hydroxycamphor, verifying its function as a volatile sex attractant pheromone. Males spent more time in contact with lures treated with synthesized hydroxycamphor than those treated with solvent only in laboratory two-choice assays. Further, using single sensillum recordings, we characterized a pheromone-sensitive odorant receptor neuron in a specific olfactory sensillum on male *P. corruscus* antennae and demonstrated its sensitivity to hydroxycamphor. Thus, this study has identified the first volatile pheromone and its corresponding sensory neuron for any firefly species. It also provides a tool for monitoring *P. corruscus* populations for conservation and further inquiry into the chemical and cellular bases for sexual communication among fireflies.

Trophic ecology of *Chimaera*, *Hydrolagus coliei*, inferred from stable isotope analysis.

Buddhi Pathirana, Lauren Simonitis, Sora Kim

Spotted Ratfish, *Hydrolagus coliei* are chimaera, a cartilaginous fish distinct from sharks. This species is distributed from Alaska to Mexico and to depths

of 913 meters. Spotted Ratfish are known to feed via smell, but dietary details remain enigmatic. Here, we use stable isotope analysis, a biogeochemical technique used to track energy flow within ecosystems, to discern dietary patterns associated with ontogeny or sex. Ratfish were opportunistically collected near San Juan Island, USA in deep-water trawls. Muscle tissue was sampled from 38 formalin-preserved specimens, then extracted of lipid and urea with petroleum ether and deionized water, respectively, before isotopic analysis of carbon and nitrogen. The $\delta^{15}\text{N}$ values for this population ranged 14.5 to 18.2‰ and $\delta^{13}\text{C}$ values ranged -19.4 to -16.9‰; however, $\delta^{13}\text{C}$ values are likely affected by preservation. There was a weak correlation between total length and $\delta^{15}\text{N}$, but differences between females versus males. In general, females were larger and had higher $\delta^{15}\text{N}$ values than males, likely due to differences in diet or physiology. Dietary explanations include foraging at higher trophic level or in deeper waters while physiology differences could arise from pregnancy or growth. These isotopic results are some of the first for Spotted Ratfish and comparisons to other taxa within the habitats could provide important ecological context.

KEY WORDS: $\delta^{13}\text{C}$ & $\delta^{15}\text{N}$, Cartilaginous fish, Holocephali, stable isotope

Olfactogenetic approach to understanding odor detection in *Manduca sexta*

Anand Rao Patil, Joshua Swore, Melanie Anderson, Jeff Riffell

Insects sense a wide range of volatile chemical compounds present in the environment with high sensitivity and specificity. These compounds are detected by olfactory receptors: odorant receptors (ORs), gustatory receptors (GR), and ionotropic receptors (IRs). Insects also detect their hosts or mates through olfactory receptor-mediated olfaction. Olfaction plays an important role in insect behavior. Insect ORs are heteromers of transmembrane proteins (7 tm); odor specific odorant receptor and the odorant receptor co-receptor (Orco).

The tobacco hornworm *Manduca sexta* is a model lepidopteran insect organism widely used in the field of olfaction. Whole genome sequencing has identified the major chemosensory receptor families. Here, we are studying the role of *Manduca sexta* odorant receptors: MsexOR5 and MsexOR6, which are specifically expressed in female antennae and tuned to detect the plant odor linalool. We are developing an olfactogenetic approach to make transgenic *Manduca sexta* lines to ectopically express ORs in the *Manduca* antenna using CRISPR/Cas9-mediated HDR knock-in and piggyBac-transposon. We have generated and established an odorant receptor knockout line and found effects on the *Manduca* development. We performed EAGs and found that odor sensitivity was impaired in the knockout moth. In addition, we have isolated the MsexPUB gene promoter and found that the PUB promoter is active-functional in embryo and can be useful for *Manduca* transgenesis.

Our progress towards developing an olfactogenetic approach will help us study important chemosensory receptors involved in *Manduca* olfaction.

Ultrafast reversible self-assembly of living tangled matter

Vishal Patil, Harry Tuazon, Emily Kaufman, Tuhin Chakraborty, David Qin, Jörn Dunkel, Saad Bhamla

Tangled active filaments are ubiquitous in nature, from chromosomal DNA and cilia carpets to root networks and worm collectives. How activity and elasticity facilitate collective topological transformations in living tangled matter is not well understood. We studied California blackworms (*Lumbriculus variegatus*), which slowly form tangles in minutes but can untangle in milliseconds when stressed. Combining ultrasound imaging, theoretical analysis, and simulations, we developed and validated a mechanical model that explains how the dynamics of individual worms determines their collective topological state. The model reveals that blackworms use alternating helical gaits to enable both tangle formation and ultrafast untangling. The striking similarity between the worm gaits for tangling and untangling reflects the biological constraints on locomotion machinery. By identifying generic dynamical principles underlying the topological transformations of blackworms, our results can provide guidance for designing classes of topologically tunable active materials.

Brain transcriptomics vary with sex and breeding stage in a socially polyandrous shorebird

Tessa Patton, Sara Lipshutz, Kimberly Rosvall

Social polyandry, also called “sex role reversal,” is a unique mating system in which females compete for multiple mates and males provide the majority of parental care. Socially polyandrous species like northern jacanas (*Jacana spinosa*) can provide valuable insights into the molecular mechanisms of variation in reproductive behavior. We predicted that differences between sexes and breeding stages would be reflected in gene expression profiles. We conducted RNA-Seq with two brain regions of the social behavior network: the preoptic area of the hypothalamus and the nucleus taeniae. Differential expression analyses revealed that brain regions had distinct transcriptomic profiles. We found differences among males who were courting and parenting, including genes associated with the insulin-like growth factor pathway and hypothalamic-pituitary-adrenal axis. However, far more genes were differentially expressed between the sexes than breeding stages. Some of the top differentially expressed genes between sexes were involved in the hypothalamic-pituitary-gonadal axis. Taking a more global approach, weighted gene co-expression network analyses identified modules associated with sex differences in competitive traits, including body size, gonad mass, and weaponry. Some modules were enriched for hormonal signaling and neuron development processes. In other species, these candidate genes and pathways are associated with female care and male competitive traits, suggesting that some mechanisms may be universal, regardless of which sex performs specific behaviors.

Visual acuity differences in Southeast Asian treefrogs

Kyle Paul, Imerria Peoples, Matthew Fuxjager, Doris Preininger

Many animals rely on vision to navigate their physical and social world. The acuity of the visual system is thought to be extremely important because it determines the eyes' ability to discriminate static detail at a particular distance. However, visual acuity in many species remains a major mystery. We know little about the visual acuity of most anuran amphibians (frogs and toads). Out of over 7000 frog species, only 5 have been tested in this regard using an optomotor device, which is a drum of rotating black and white stripes. In this study, we exposed male and female individuals of three different Southeast Asian treefrog species to rotating stripes of different width (20 – 2.5mm) to test their optomotor response. If the tree frogs were able to perceive the moving stripes, they oriented in the direction of movement. We found that the spatial frequency eliciting tracking behavior differ between the treefrogs. Frequencies of 0.07 and 0.15 cycles per degree (cpd; minimum separable angle: 7.14° and 3.33°; stripe size 20mm and 10mm) are discriminated by the common tree frog. Fied-eared tree frogs are able to track 0.07 cpd while Wallace flying frogs showed no optomotor response. Motion and colorful ornaments that additionally stimulate the sensory system most likely enhance the precise perception of Southeast Asian Treefrog to detect prey and conspecifics.

Wedges have edges: Phylogenetic relationships in vertebral morphology of carcharhiniform sharks

Emma Pawlik, Monique Oliveira, Markens Alerte, Sonoma Arnaldy, Jamie Knaub, Michelle Passerotti, Lisa Natanson, Tricia Meredith, Marianne Porter

Sharks (subclass Elasmobranchii) are a diverse group of cartilaginous fishes. The largest order of sharks, Carcharhiniformes, is the most species-rich and its members occupy a wide range of habitats. Previous research has described carcharhiniform vertebral morphology; a double cone structure comprises four mineralized wedges (the intermedialia) that vary in form across genera. However, it is unclear if these changes reflect differences in ecology or phylogenetic relatedness. We investigated the variation in carcharhiniform vertebral morphology in a phylogenetic context. We hypothesized that phylogenetic relationships would impact vertebral morphology; Carcharhinus and Sphyrna would have different microarchitecture because they are the least related among genera investigated. We examined vertebrae from 5 genera (Carcharhinus, Negaprion, Prionace, Rhizoprionodon, and Sphyrna) representing 15 species of carcharhiniform sharks. We micro-CT scanned vertebrae

and measured dorsal, ventral, and lateral wedge angles, arch angles, and double cone angles. Our results showed that dorsal wedges were different in the least related genera and in the least related species within genus Carcharhinus. Within genus Sphyrna, we found that the bonnethead shark (*S. tiburo*) had significantly smaller double cone angles than other Sphyrnids, despite its close phylogenetic relationship to the scalloped hammerhead (*S. lewini*). Our findings support that vertebral shape may respond to a functional need; hammerhead sharks may have adapted a unique morphology to support the lateral movement of a large, laterally compressed head.

Hydrodynamic Performance of Various Swim Modes of the Class Chondrichthyes

Braedon Payne, Roi Gurka, Bryan Keller

There are four common swim modes exhibited throughout sharks: anguilliform, sub-carangiform, carangiform, and thunniform. The transition from anguilliform to thunniform represents a shift from oscillatory to undulatory locomotion. With this transition, there is a variation in how each shark generates forces and accommodates for resistant drag. These swim modes are not distinct; each shark's swim mode exists along a spectrum due to gradual modification through evolution. Therefore, there may potentially be a relationship between the hydrodynamics of sharks and their place in the phylogenetic tree. Using motion tracking software, we are able to track the position of the shark through time. This can be used to calculate the velocity and acceleration of the shark's center mass, and calculate total force output of the tail. The force output of each shark can be related to the shark's swim mode, which may be characterized by the head to tail amplitude ratio. This can be attributed to the phylogenetic tree based on the genetic similarities or the age of each shark species. We aim to describe the hydrodynamics between shark species, in order to shed light on this research effort which can continue for similar species in the future.

Using Single-Cell RNA Sequencing Methods to Investigate Genes Involved in Cnidarian-Algal Symbiosis

Stephanie Peak, Clara DiVincenzo, Tingting Xiang, Karl Castillo

Some cnidarians form symbioses with dinoflagellate algae of the family Symbiodiniaceae. Symbionts live in the host gastrodermal tissue, where photosynthates and inorganic nutrients are exchanged between partners. This association drives productivity and biodiversity in coral reef ecosystems; however, it is highly threatened by ocean warming. When this relationship breaks down from thermal stress and symbionts fail to repopulate coral tissue, the animal will die, resulting in loss of biodiversity and resources for humans. Despite its importance, the cellular and molecular mechanisms behind establishment, maintenance, and breakdown of the coral-algal symbiosis are relatively unknown. Bulk transcriptomic studies have provided some information about the cell biology of cnidarian-dinoflagellate associations; however, the resolution of whole-organism “-omics” is too broad to confidently predict host pathways required for symbiosis. We propose a method to achieve high-quality single-cell RNA sequencing data from cnidarians, and reveal potential genes involved in maintenance of the cnidarian-dinoflagellate symbiosis using the coral model organism *Exaiptasia diaphana* (*Aiptasia*). In this study, we compare cell type-specific gene expression between symbiotic and aposymbiotic (symbiont-free) phenotypes of *Aiptasia*, as well as between alga-containing and alga-free gastrodermal cells within the same organism. This research will enable targeted functional genomic studies to identify key genes responsible for maintaining the dynamic stability of cnidarian-dinoflagellate symbioses.

Drivers of oxygen storage and development in dependent Weddell seal (*Leptonychotes weddellii*) pups

Linnea Pearson, Emma Weitzner, Lars Tomanek, Heather Liwanag

The development of oxygen storage capabilities (TBO2) is a key ontogenetic milestone for diving mammals, as TBO2 directly impacts aerobic dive performance. In this study, we investigated the development of diving physiology and concurrent dive behavior of dependent Weddell seals. We hypothesized

TBO2 would be strongly correlated with dive experience rather than calendar age or body size. We longitudinally sampled pups ($n=8$) from 1 to 7 weeks of age at 4 age timepoints; summed blood, muscle, and lung oxygen stores (TBO2); and calculated the aerobic dive limit (cADL). Concurrent dive behavior data were recorded with flipper-mounted time-depth recorders. Our results show TBO2 development is correlated with age, but the underlying changes in mass drive oxygen storage capabilities in pups, and diving has little influence on TBO2. Weddell seals were able to maintain mass-specific TBO2 throughout early dependency, as increases in muscle O_2 stores made up for a decrease in blood volume. In weaned pups losing mass, TBO2 decreased from a peak before weaning. We observed a shift in oxygen storage from blood to muscle as pups aged, though blood remained the largest store. Additionally, pups dove well below their cADL, indicating pups had little hypoxia exposure while diving. Overall, the importance of mass and increased development of muscle O_2 stores provide insight into the factors affecting the successful transition to independence in this deep-diving predator.

Propagation and consequences of wing damage in the pollinator, *Megachile rotundata*

Koby Pearson-Bortle, Jacob Pithan, Kendra Greenlee, Joseph Rinehart

Organisms with wings often experience wing damage caused by collisions, predation, weather, and intraspecific interactions. This can decrease foraging and offspring provisioning, while increasing predation risk. However, little is known of the consequences of wing damage in solitary bees, such as the alfalfa leafcutting bee, *Megachile rotundata*. Due to their high level of flight activity and parental investment, we hypothesize that wing damage increases with age and that there are consequences for acquired wing damage. To characterize the propagation of wing damage, female bees were released and then collected at various ages. As predicted, wing damage was localized to regions of the wing near the edges and increased with age. To directly test for the effects of wing damage, wings of emerged females were clipped to mimic naturally occurring damage. Flight performance, behavior, and reproduction were compared between control and clipped individuals. Initially, wing damage had an effect on flight performance; however, the number of nests, number of brood cells, and provision size were no different between control, and clipped. Based on our results, the bees appeared to be able to compensate for the wing damage, because there was no difference in reproduction. This indicates that older females with wing damage are compensating either by changing their foraging behavior or flight mechanics.

Artificial grass as a potential stress reducer while introducing wild birds to captivity

Bradley Pedro, L. Michael Romero

Introduction of wild animals to captivity for research induces chronic stress leading to weight loss, increases in baseline corticosterone, and increased DNA damage. To mitigate these effects, providing enrichment to the captive environment has been proposed. Yet, studies investigating the physiological effects of captive environment enrichments are rare in wild birds. Here, we test the potential of a single enrichment factor by monitoring weight, baseline corticosterone, and DNA damage in two groups of house sparrows during introduction to captivity: (1) birds in standard laboratory cages with food dishes and (2) birds in cages where food is spread across artificial grass to simulate a more natural foraging environment. After 3 weeks, all birds switched environments for 3 additional weeks. Weight was monitored bi-weekly while baseline corticosterone and DNA damage were measured weekly. Initially, both groups lost significant weight and weight plateaued by about 2 weeks of captivity. However after switching treatment environments, only initially grass-caged birds continued to lose weight. After one week of captivity, grass-caged birds had lower DNA damage compared to standard-caged birds. Over time, standard-caged birds remained unchanged and initially grass-caged birds increased damage after switching treatment environments. There were no significant differences in baseline corticosterone across groups or over time. Results suggest limited support that artificial grass can be a substantial enrichment in mitigating the physiological consequences associated with introduction to captivity.

A Sugar-Proofed Lifestyle: Adaptations Underlying a Healthy REDOX State in Nectivorous Bats

Valentina Peña, Jasmin Camacho, Nicolas Rohner

For most mammals, regularly consuming large amounts of sugar induces a metabolic disease-state. Despite this, nectar bat species have evolved mechanisms that enable them to thrive on a high-sugar diet. Additionally, nectar bats have evolved specialized flight allowing them to hover over flowers for feeding on sugary nectar. The high-energy inputs required for hover-flight enables glucose oxidation to occur rapidly and directly in the flight muscles as evidenced by enhanced assimilation of carbohydrates, elevated GLUT4 expression in the pectoralis, lack of insulin response after feeding, and sustained hyperglycemia at rest. The excessive consumption and breakdown of dietary sugar may potentially expose nectar bats to greater levels oxidative damage in metabolically active tissues such as the liver, and skeletal muscle. With high metabolic rates and acute aerobic exercise there is a risk for oxidative tissue damage, however bats appear to be otherwise unaffected by this. To investigate this, wild bats were fasted and fed a glucose-rich diet with lethal sampling of liver and muscle tissue occurring at multiple time-points ($t=0, 10, 30, 60$ min). We integrate RNA-seq (liver) and targeted metabolite assays (liver, muscle) across nectivorous, frugivorous, and insectivorous bat species to investigate the adaptations underlying nectarivory in mammals.

Tendon mechanical structure varies across frog and toad species

Sara Penuela, Rebecca Wells, Crystal Reynaga

Tendons are composite structures made up of parallel collagen fibers. Tendons exhibit “spring-like” properties, capable of absorbing and transmitting forces to dampen muscle strain or amplify muscle power. Anurans (frogs and toads) are a well-studied model system, used to understand tendon mechanics, they move in diverse ways and often specialize in a single form of locomotion. However, little is known about how the morphological and mechanical properties may differ between locomotor types. We predict that species with similar modes of movement will have similar fiber diameter, cross-sectional area (CSA), density and spring properties. We used Transmission Electron Microscopy (TEM) to investigate 2D fiber structure. We used FIJI to analyze the images and quantify differences across an array of anuran species. TEM analysis suggests frog species specialized for jumping and swimming have smaller collagen fibril diameters and larger CSA. Species that primarily walk or run have larger collagen fiber diameters and smaller CSA, reflecting the lower strains the tendon experiences during walking. In the future, we plan to investigate how material strain properties of tendons correlate to fibril 3D organization using serial-block-face scanning electron microscopy. Investigating functional properties of biological springs, such as tendons, allows us to gain further insights to optimize design parameters for medical devices and other suspension-based engineered systems.

Group Exams: Helping students learn the material from the exam experience

Rachel Pepper

In many classes, exams are necessary as a way of assessing student learning, but are often thought of as summative assessment only. However, often, instructors want the exam to also be learning experience for their students. Here, I discuss the use of a group exam to achieve this goal in classes ranging from introductory to advanced level. My experience is in Physics and Biophysics courses, but a group exam could be implemented in many contexts. In my implementation, students take the exam individually and then repeat the same exam in groups of four. I discuss the details of my implementation, student response to the group portion of the exam, and learning outcomes.

Care-giver identity influences brood metabolism in bumble bees under starvation conditions.

Claudineia Pereira-Costa, Natalie Fischer, Sarah Woodard

One of the defining traits of eusociality is the presence of overlapping generations in the nest. The annually eusocial insects exhibit both maternal and alloparental (sibling) care, which is organized by a life history transition where first a parent rears offspring, then sibling care is expressed once workers emerge in the nest. Here, we explored social influences on larval development to identify how maternal and sibling brood care impact the developmental fate of workers in the bumble bee *Bombus impatiens*. We showed that queens influence larvae such that they ultimately develop into smaller-bodied workers that are also more resistant to starvation. We hypothesized that this phenomenon might be driven by differences in nutrient metabolism, enforced by care-giver identity during development. Thus, we next explored how care-giver identity impacts the nutritional status of adult offspring under starvation conditions. We found that care-giver identity influenced offspring hemolymph sugar levels under starvation conditions for a short period, whereas under longer durations (2 days), hemolymph sugar levels were depleted equally, irrespective of care-giver identity. These findings corroborate the hypothesis that bumble bee queens and workers uniquely influence the development of the first offspring in the nest. We propose that bumble bee brood care has been shaped by a suite of evolutionary and ecological factors, including a maternal influence on traits promoting incipient colonies survival.

Pooling together: pulling from the substrate governs prevailing convergent morphologies of reef fish

Tal Perevolotsky, Karin Olsson, Tamara Gurevich, Peter Wainwright, Roi Holzman

One of the iconic morphologies associated with reef fish is a deep, laterally compressed body, along with large protruding dorsal and anal fins. These convergent morphologies have so far been attributed to protection from predators, navigation of structurally complex habitats, and increased maneuverability, which has been considered the paradigmatic explanation, despite the fact it lacks both a mechanistic framework and empirical evidence.

Pulling from the substrate is a form of biting in which fish execute distinct head, body, and fin kinematics in order to exert a pull force on their substrate-attached prey. These kinematics have been directly linked to feeding performance, yet the relationship between fin and body morphologies and pulling remains unclear.

Using morphological measurements and classification of feeding mode (puller, non-pulling biter, suction) of species from common reef fish families, we tested the hypothesis that functional demands of pulling impose selective pressures that shape head, body, and fin morphologies. We fitted different models of morphological trait evolution to examine whether morphological traits evolve towards different adaptive optima according to their feeding modes. We found that biting pullers evolved towards different optima than non-pulling biters, which were characterized by a deep body, large anal and dorsal fins, and a tapering snout. Taken together, our results suggest pulling from the substrate as the mechanism underlying the prevailing converging morphologies displayed by benthic feeding reef fish. "

Cigarette tobacco reduces the survival of invasive parasites that affect Darwin's finches

Lorraine Perez, Jailene Contreras, Katia Goldberg, Gabriela Mena, Alexandria Soldo, Jaime Chaves, Sarah Knutie

Invasive parasites are a major threat to biodiversity worldwide so understanding the factors that control them is necessary to improve the health of affected host species. In the Galápagos Islands, the invasive nest ectoparasite, the avian vampire fly (*Philornis downsi*), is causing up to 100% mortality in nestling Darwin's finches. However, urban finch nests have fewer flies than non-urban finch nests. One explanation is that urban finches incorporate cigarette butts into their nests, which can decrease nest parasite abundance in other bird species. For our study, we exposed larval flies to cigarette tobacco-treated (concentrated or diluted) or untreated cotton, then we characterized pupation success, pupal deformities and success, and adult fly eclosure

success and size. The influence of moisture on the effect of tobacco treatment on fly health was also determined. Flies reared in tobacco as larvae had lower pupation success, larger pupal volume, and a higher prevalence of pupal deformities compared to control flies, regardless of moisture treatment. Furthermore, we found that tobacco-treated flies had lower eclosure success. In fact, very few tobacco-treated flies survived to adulthood. We also collected finch nests and quantified the prevalence and abundance of cigarette butts and abundance of flies in the nests. Although most urban finch nests contain cigarette butts (73%), the mass of cigarette butts was very low and did not correlate with fly abundance. Compared to past studies, finch nests require 10x as many cigarette butts to have enough chemicals to affect fly survival. Although tobacco can control vampire flies, finches do not incorporate enough cigarette butts to affect fly fitness.

Out of the shell: In silico deorphanization of MIH/CHH neuropeptide GPCR candidates

Jorge Perez-Moreno, Mihika Kozma, Neha Gandhi, Luisanna Hernandez-Jeppesen, David Durica, Tomer Ventura, Donald Mykles

Molt-inhibiting hormone (MIH), a neuropeptide member of the crustacean hyperglycemic hormone (CHH) neuropeptide superfamily, inhibits ecdysteroid molting hormone synthesis by a pair of molting glands or Y-organs (YOs). It is hypothesized that its receptor is a Class A G protein-coupled receptor (GPCR). Crustome, a transcriptome database assembled from 189 crustaceans (and 12 additional panarthropods), was used to deorphanize candidate CHH superfamily GPCRs, relying on sequence homology to three functionally characterized ion transport peptide GPCRs in silk moth (BNGR-A2, BNGR-A24, and BNGR-A34). Phylogenetic analyses revealed extensive expansion and diversification of crustacean A2, A24, and A34 receptors, designated CHH Family Receptor Candidates (CFRCs). Eleven of the 14 subclades were identified in decapod species. Modeling and sequence alignments of the ligand-binding region containing extracellular loop (ECL) 2 and ECL3 suggest that the CFRCs differ in neuropeptide binding affinity and specificity. In *Gecarcinus lateralis*, seven CFRCs, designated GI-CFRC-A2a1, -A24a, -A24b1, -A24b2, -A34a2, -A34b1, and -A34b2, were identified. Modeling showed that the ligand-binding ECL2 in all the CFRC sequences had a two-stranded β -sheet found in mammalian Class A GPCRs, whereas the ECL2 of decapod CFRC-A34b1/ β 2 had an additional two-stranded β -sheet. We hypothesize that this second β -sheet on ECL2 plays a role in MIH/CHH binding and activation, which will be investigated using functional assays. Supported by NSF IOS-1922701.

Host-pathogen transcriptomics in an avian host with varying degrees of prior pathogen exposure

Anna Perez-Umphrey, Edan Tulman, Jeremy Miller, James Adelman, Grace Ozyck, Steven Geary, Dana Hawley

Host and pathogen transcriptomics during *in vivo* infection can show signals of antagonistic coevolution and reveal key mechanisms of host-pathogen interactions that are likely important for driving phenotypic changes in pathogens during infection. Further, transcriptomics of host gene expression in hosts with and without immune memory can be particularly fruitful in revealing mechanisms of protective immune responses. We simultaneously examine gene expression in an avian host (*Haemorrhous mexicanus*) and in their endemic bacterial pathogen (*Mycoplasma gallisepticum*; MG). MG infections of house finches cause conjunctivitis of the eye, and the immune protection generated from infection is incomplete, allowing for reinfection. We experimentally inoculated house finches with one of three primary doses of MG (sham, low, or high dose), and after the infection cleared, birds received a secondary inoculation of either a sham or high dose. Conjunctival tissue was harvested three days post-secondary infection and poly-A-mRNA libraries were prepared for both house finches and MG present in the conjunctival tissue. By examining host gene expression in local tissues at the same timepoint as MG gene expression during *in vivo* infection, this will provide strong inference as to whether host immune gene expression is driving any detected phenotypic variation in pathogen gene expression. This also allows us to examine how prior exposure, and the degree of that exposure, mechanistically influences susceptibility to reinfection in the host.

Exploring the role of substrate curvature on fluid pumping with ctenophore-inspired artificial cilia

David Peterman, Margaret Byron

Ctenophores (comb jellies) were among the first swimming animals, and offer perspectives into the evolution of unique locomotion strategies. These animals bear the largest cilia of any organisms (~1 mm), which are bundled into paddle-like structures (ctenes) and arranged in rows around the body. These arrays of propulsors beat metachronally, enabling ctenophores to efficiently operate at intermediate Reynolds numbers (where viscous and inertial forces are both important). However, the degree to which the ctenes hydrodynamically interact with the body wall (substrate) is unclear, as is the role of substrate curvature and deformability. We explore pumping performance on flat and curved substrates with a ctenophore-inspired, magnetically actuated soft-robotic platform. In both the flat and curved cases, a jet of fluid hovers over the artificial cilia in intermediate Reynolds number settings ($1 < Re < 100$). Above this range, inertial forces begin to dominate, and a larger component of momentum flux is directed normal to the substrate. On flat substrates, this condition diminishes momentum flux in the swimming direction. However, on curved substrates, this normal component contributes locally to the momentum flux (and therefore thrust force) in the swimming direction. We use this platform to demonstrate distinct shifts in pumping performance across a biologically relevant range of phase lags, beat frequencies, and Reynolds numbers. Our results can help us understand the physical constraints imposed by fluid mechanics during the evolutionary history of ctenophores, spanning ~0.5 billion years.

Dietary isoleucine restriction increases catabolic flux of leucine and valine in lubber grasshoppers

Haley Peters, Kerri Conklin, Connor Clark, John Hatle

Nutrient (esp. amino acid) catabolism not only produces ATP but can also regulate metabolic health. The branched-chain amino acids (BCAAs; leucine, isoleucine, and valine) are essential for protein synthesis and stimulate cellular growth, however in excess they can be detrimental. Dietary modifications such as isoleucine restriction can delay age-related disease and are predicted to stimulate leucine and valine catabolism. Thereby, we quantified catabolism of each individual BCAA and measured organ sizes upon dietary isoleucine restriction. Lubber grasshoppers were fed high-quality artificial diets matched to the amino acid composition of vitellogenin (Vg; the precursor of egg yolk) with isoleucine included at 100%, 33%, or 0% of that in Vg. Carbon-13 labeled leucine, isoleucine, or valine was force-fed and tracked to breath CO_2 to quantify organismal catabolism. For only the 0% isoleucine group, leucine and valine cumulative catabolism each were significantly greater than the 100% isoleucine group, with respective 150% and 77% increases (ANOVA, $P < 0.05$). In contrast, for isoleucine cumulative catabolism, all artificial diets were similar. Isoleucine restriction at 0% severely decreased ovarian mass by 84% and fat body mass by 46%, relative to the 100% isoleucine group. Therefore, severe isoleucine restriction mimics a low-energy state catabolically, in which short-term application may alleviate metabolic defect via increased leucine and valine catabolism. We plan to further investigate the mechanism by which increased catabolism affects age-related disease.

The effect of school size and flow sensing on collective schooling behavior

Ashley Peterson, Nathan Swanson, Matt McHenry

Flow sensing is considered necessary to the formation and maintenance of fish schooling. We performed a series of behavioral experiments with rummy-nose tetras (*Hemigrammus rhodostomus*) to investigate the influence of flow sensing and its effects on schooling behavior. For this effort, we developed an arena for high-throughput kinematic measurements of schooling fish. We varied school size (from 15 to 60 fish) in fish with and without a lateral-line system (LLS; ablated by chemical treatment), and varied the proportion of fish without a LLS within a fixed-size school. Increasing school size had the greatest influence on average swimming speed, which decreased significantly. Schools without the LLS were slightly slower on average, regardless of school size. Similarly, the average distance between fish and the nearest-neighbor

distance (NND) both increased significantly with school size, while fish without a LLS were spaced slightly further apart within a school. These results suggest that the size of the school has a direct effect on the collective behavior of a school. An increase in the proportion of treated fish within a school resulted in an increase in the average distance and the NND. The average school speed decreased only slightly. Thus, fish without a functional LLS were capable of schooling with vision alone but flow sensing may aid in increasing school cohesion rather than acting as the primary controller for school formation.

Patterns of intergenerational DNA methylation inheritance in Acropora

Christopher Peterson, Carly Scott, Rashin Rashin-Ghaffari, Groves Dixon, Mikhail Matz

For sessile organisms at high risk from climate change, phenotypic plasticity can be critical to rapid acclimation. Epigenetic markers such as DNA methylation have been hypothesized as a mediator of plasticity. Methylation is associated with the regulation of gene expression, can change in response to ecological cues, and has been proposed as the basis for the inheritance of acquired traits. Within reef-building corals, gene body methylation has been shown to change in response to ecological stressors. If coral DNA methylation is transmissible across generations, this could potentially facilitate rapid acclimation to environmental change. We investigated methylation heritability in *Acropora*, a stony reef-building coral. Two *A. millepora* and two *A. selago* adults were crossed, producing eight offspring crosses (four hybrid, two of each species). For each sample, we used whole-genome bisulfite sequencing to identify methylated loci and allele-specific alignments to quantify inheritance at each locus. If methylation is heritable, differential methylation between the parents and between the two offspring alleles should be the same at a given locus. We found a mixture of heritable and non-heritable loci, with heritable portions ranging from 44% to 90% among crosses. Gene body methylation was more heritable than methylation in intergenic regions, and most loci had a consistent degree of heritability between crosses (i.e., the deviation between differential methylation in parents and offspring were of similar magnitude and direction). Our results provide evidence that coral methylation can be inherited but that heritability is heterogeneous throughout the genome. Future investigations into this heterogeneity and its phenotypic implications will be important to understanding the potential capability of intergenerational environmental acclimation in reef building corals.

Kinematics of Swimming Across Flow in a Rainbow Trout Model

Jack Peterson, Melina Hale

Research on sustained swimming in fish has focused on forward swimming in steady flow. However, in natural riverine environments fish must navigate a range of flow patterns and swim across flow. Understanding strategies for crossing flow will provide insight on swimming behavior and inform design of swimming robots. We studied young rainbow trout (*Oncorhynchus mykiss*) swimming across the flow in a flow tank (0.1 and 0.28 m/s speed) and analyzed the kinematics of seven individuals (TL: 7.6-8.8cm, 8.4cm average). Semicylinder volumes on both sidewalls provided refuge from flow and encouraged movement across the tank. Video analyses performed included measures of fin/tail beat frequency, velocity, and tail and fin movement patterns between cross-flow and forward steady swimming. The most common behavioral strategy (34 instances in 57 trials, used by all individuals) involved angling the body slightly paired with tail beating to move across flow. The other strategy observed (23 trials total, used by all individuals) was to drift downstream while crossing the tank followed by a redirection to return to forward-oriented swimming. Kinematic analysis of the most common strategy identified differences from steady swimming that includes asymmetry in tail and caudal fin bending and changes in pectoral fin movement. These behavioral approaches to crossing flow may optimize different aspects of performance, such as holding position in flow or increasing efficiency. Funded by The Office of Naval Research #N00014-22-1-2187

Varying Reproductive Rates in Queen Bumble Bees: A Reevaluation of Behavior in Early-Stage Nests

Blanca Peto, Sarah Woodard, Claudineia Pereira-Costa

Animals exhibit a variety of unique life history strategies that are linked with fecundity and fitness. For social animals that exhibit brood care, understanding how personal survival and reproduction are balanced with care for brood can provide key insights into social dynamics and evolution. Bumble bee queens initiate nests alone and carry out all brood care until their first workers emerge and take over these tasks. Early foundational work by bumble bee researchers described reproductive strategies employed by queens in early nesting stages and possible cues for transitions in reproduction. However, modern research has yet to fully characterize alternative reproductive strategies employed by bumble bee queens and examine their relationship to nest development and fitness. In this study, we examine the reproductive rate of *Bombus impatiens* queen nests, quantifying egg-laying, brood and nest development, and the timing of events within the nest. We determined that queens demonstrate a consistent pattern of overall colony development, but differ in their egg-laying rates and timing of subsequent events. Overall, this work helps to substantiate early descriptive work on bumble bees and provides a clearer understanding of the variety of life history strategies that bumble bees execute and their relationship to nest growth and survival.

Thermal acclimation studies in brook trout: Do native brook trout respond to a warming environment?

Evelyn Peyton, David Coughlin

As climate change alters the thermal environment of the planet, interest has grown in how animals may mitigate the impact of a changing environment on physiological function. We have examined how thermal acclimation alters swimming performance, muscle contraction kinetics and the gene expression and protein content of myotomal muscle in cold-water fishes. Thermal acclimation to a warm environment may blunt the impact of a warming environment on metabolism by allowing a fish to shift to slower isoforms of metabolically significant proteins such as myosin, creatine kinase, parvalbumin and heat shock proteins. This would result in lower energetic costs to offset the metabolic toll of rising water temperature. Our recent studies of hatchery brook trout (*Salvelinus fontinalis*) reveal a robust thermal acclimation response. These fish show significant shifts in swimming performance, muscle contractile properties and gene expression with changes in environmental temperature in the laboratory setting. But what is the response of native brook trout? Brook trout across their range are composed of many independent populations from different drainages with limited gene flow between the myriad streams and rivers of their fragmented distribution. These populations are highly variable in body form and size. Wild brook trout do acclimate to changes in water temperature throughout the year, with colder temperatures leading to faster swimming performance and faster muscle contraction kinetics. However, our results show that thermal acclimation shifts in gene expression are similar but not identical between native and hatchery fish. Variations in the acclimation response by native brook trout populations suggest differential survival rates as stream temperatures rise in the coming decades.

Simulated night shift work alters physiology and metabolism in a model diurnal songbird

Kevin Pham, Haruka Wada

Epidemiological studies show that rotating night shift work correlates to higher accumulation of subcutaneous fat and body mass, increasing risk of developing metabolic syndromes, such as insulin resistance and diabetes. This is thought to be caused by disruption of circadian rhythms, which alters the timing at which hormones, such as glucocorticoids, and downstream metabolites are mobilized. Although these indicators are elevated in rodent models subjected to simulated night shift work, empirical studies in diurnal species are lacking. Thus, the mechanism underlying disease outcomes induced by night shift work is not clear. To this end, we exposed diurnal zebra finches (*Taeniopygia castanotis*) to chronic, simulated night shift work, where the treatment group underwent a swap between a 12L:12D and 12D:12L cycle every 3 days and controls were maintained under a consistent 12L:12D cycle. We

measured negative feedback efficiency of glucocorticoids, glucose and β -hydroxybutyric acid levels, body mass, and furcular fat. We found that regardless of treatment, blood glucose levels significantly increased over time. Additionally, β -hydroxybutyric acid levels increased over time, and were significantly higher in the treatment group when compared to controls. Surprisingly, there were no changes in body mass or furcular fat across groups. Our results provide insight on how physiological and morphological indicators of metabolic syndromes differ in a diurnal bird model under night shift work and warrants further investigation.

The effect of living in flow on evolved strategies for gas exchange in South African tadpoles.

Jackson Phillips, Molly Womack, Shane Ngwenya, Gary Nicolau

Flow is a primary determinant of the abiotic factors that define different classes of waterbodies. One direct effect of flow is oxygenation. Flowing water is constantly disturbed, increasing dissolved oxygen, while still water is prone to low oxygen levels. There are two general types of respiratory strategies employed by aquatic organisms: water-breathing and air-breathing. Water-breathing is convenient and efficient in well-oxygenated water, while air-breathing requires coming to the surface, but allows gas exchange even in anoxic water. Anuran (frog) tadpoles (aquatic larvae) present an interesting case study in the effects of flow on physiological strategy, because tadpoles are found with and without functional lungs across different flow regimes. We find that stream tadpoles, regardless of lung status, are generally less tolerant of low oxygen levels, but that lungless stream tadpoles are by far the most sensitive to hypoxia. We find that lungless pond tadpoles are generally as tolerant to low oxygen levels as their lunged neighbors, but that one lungless pond species, *Schismaderma carens*, seems particularly hypoxia tolerant. In the course of the study, we also document several remarkable, apparently respiratory, behaviors by lungless tadpoles to take advantage of aerial respiration without the use of lungs, demonstrating the universal benefits of using air-born oxygen to deal with hypoxia.

Resident, but not migratory, songbird eye size varies with urban-associated light pollution levels

Jennifer Phillips, Alfredo Llamas, Todd Jones

Urbanization exposes large portions of the earth to sources of anthropogenic disturbance, driving rapid environmental change, producing novel environments and selective pressures on phenotypes. In this study, we examined whether morphological phenotypes in two residential species (Carolina Wren (*Thryothorus ludovicianus*) and Northern Cardinal (*Cardinalis cardinalis*)), and two migratory species (Painted Bunting (*Passerina ciris*), and White-eyed Vireo (*Vireo griseus*)), differed between urban core and edge habitats in San Antonio, Texas, USA. Specifically, we examined whether urbanization, associated sensory pollution (light and noise) and brightness (open, bright areas cause by anthropogenic land use) influenced measures of avian body size and lateral eye size. We found no differences in body size between urban core and edge habitats for all species except the Painted Bunting, in which core-urban individuals were smaller, younger males. Residential birds inhabiting urban-core areas had smaller eyes compared to their urban-edge counterparts, resulting from a negative association between eye size and light pollution and brightness across study sites; notably, we found no such association in the two migratory species. Our findings demonstrate how urbanization may indirectly influence phenotypes by altering population demographics and highlight the importance of accounting for age when assessing factors driving phenotypic change. Thus, we provide some of the first evidence that birds may adapt to urban environments through changes in their eye morphology.

Collective Orientation of Talitrid Amphipods

Magdalena Phillips, Jesse Granger, Sonke Johnsen

Collective navigation is the process whereby individuals take directional and positional cues from nearby animals. It has been theorized that the presence of other conspecifics may improve navigational accuracy; however, behavioral experiments on the effectiveness of collective navigation have been done almost exclusively with pigeons. The goal of this research is to assess how

collective navigation may assist in the orientation of *Megalorchestia* sp., a genus of talitrid amphipods native to the eastern North Pacific. Talitrid amphipods are ideal candidates for an invertebrate model system because they orient strongly towards the wrack line of coastal beaches. Their navigational abilities are based on a robust set of cue types, including visual, celestial, and magnetic. Additionally, talitrid amphipods are plentiful and easy to access in the intertidal zone. For this study, we conducted a behavioral orientation assay to test how well *Megalorchestia* orients towards the beach in three different group sizes: individually, in groups of five, and in groups of ten. Contrary to our expectations, we found that the largest group size ($n = 10$) was significantly slower, less directed towards the beach, and started moving earlier in the trial than the individual animals. The intermediate group size ($n = 5$) also started moving significantly earlier in the trial, but otherwise exhibited no differences in orientation from the individual animals. Our results provide the first evidence for beachward orientation in *Megalorchestia*. Overall, our results support the idea that collective navigation may not be beneficial for all organisms or in all situations.

Admixture mapping of call variation in the yellow-rumped warbler (*Setophaga coronata*) hybrid zone

Lan-Nhi Phung, Marcella Baiz, Daniel Pierce, Alan Brelsford, David Toews

In birds, vocalizations are presumed to be one of the important premating reproductive barriers that could affect the evolution of new species. Songs and calls are the two types of vocalization signals: songs are melodious vocalizations that play a role in territorial defense or mate attraction, while calls are short vocalizations that are used during flight and to alarm others. Specifically in oscines, songs have been shown to be learned, whereas the role of learning versus genetics of calls is less clear—some studies have suggested call variation has a stronger correlation with genetic differences among populations. To assess this relationship further, we focus on the well-characterized Rocky Mountains hybrid zone of two yellow-rumped warbler subspecies, the Myrtle warbler (*S. c. coronata*) and Audubon's warblers (*S. c. auduboni*), to perform an admixture mapping study for call variation in hybrids. This pair is known to extensively hybridize, and hybrids have highly admixed genomes—a spectrum between Myrtle and Audubon's ancestry—which is highly suitable for admixture mapping. First, we quantified acoustic attributes of calls to characterize individual call phenotype objectively ($n = 177$), then compressed the variation onto one axis using PCA. Second, we used genotype-by-sequencing to associate genotypes with call phenotypes using GEMMA, a program that controls for genome-wide ancestry estimates. During my presentation, I will discuss the associations we identified and more generally how they might inform the speciation processes.

Consecutive vs skip spawning in steelhead kelts: life history tradeoffs and physiological mechanisms

Andrew Pierce, Laura Jenkins, Lucius Caldwell, Lea Medeiros, Neil Graham, Douglas Hatch, James Nagler

Columbia River Basin steelhead trout are iteroparous. After spawning, many post-spawn adults (kelts) migrate downriver, but few repeat spawn. Conservation-focused kelt reconditioning programs capture, hold, and feed female kelts, then release rematuring fish to spawn naturally. We have investigated repeat spawning life histories and the physiology of spawning recovery and ovarian recrudescence using natural origin steelhead kelts, hatchery-origin kelts, and rainbow trout. The consecutive and skip repeat spawning life histories are found in steelhead kelts. Life history trajectory can be determined by plasma estradiol level at 20 weeks post-spawn. Size-standardized total egg mass at first spawning correlated positively with subsequent consecutive spawning, arguing against a current versus future reproductive investment tradeoff. The effect of post-spawn feed restriction on consecutive spawning depended on physiological status. In kelts fasted for 10 weeks after spawning, spawning plasma growth hormone levels were lower in consecutive versus skip spawners. In rainbow trout, increasing plasma insulin-like growth factor-1, decreasing estradiol, and decreasing liver *igfbp1b* mRNA level over the 8-12 weeks after spawning suggest that recovery from spawning and a transition to growth occurs during this period. Consecutive spawning kelts consistently showed elevated growth rate versus skip spawners over the first 10 weeks

after spawning. These results suggest that the reproductive decision takes place during the period after spawning and is influenced by both initial energetic status and post-spawn feeding.

Wavelength-frequency scaling for neuromechanical undulators in dissipative Environments

Christopher Pierce, Lucinda Peng, Xuefei Lu, Daniel Irvine, Hang Lu, Daniel Goldman

Nematodes and other undulatory locomotors modify their gait parameters (wavelength λ , frequency f , and amplitude, A) in response to changing conditions in the physical environment. Gaits are modulated through a combination of neural feedback, feedforward commands, and passive mechanical processes, allowing the organism to perform robust locomotion across diverse environments. We discovered that for a broad range of viscous and viscoelastic fluids as well as granular and solid gel environments, nematode gaits obey a consistent scaling relationship between wavelength and frequency where

$f \sim \lambda^2$, despite dramatic differences in the magnitude, spatial profile, and functional form of the environmental resistive forces. We explain this scaling relation using a biomechanical model of the nematode with few parameters; the organism is modeled as a viscoelastic beam immersed in a resistive environment and driven by a periodic muscle activation pattern. Our model explains both the origin of the observed scaling relation and why the scaling is independent of the type of environment surrounding the worm. We hypothesize that the scaling relation helps manage the tradeoff between energy dissipation in the body and the surrounding fluid as external resistive forces increase. The simplicity and generality of the model allow us to generate hypotheses about the gaits performed by other undulators operating in dissipative regimes, such as aquatic, swimming larvae, and terrestrial undulators, like snakes and sandfish lizards.

Wing bone evolution in manakins—mitigating putative costs of sexual selection for bizarre traits

Sofia Piggott, Ghislaine Cárdenas-Posada, Mallory Tucker, Elizabeth Brainerd, Sharon Swartz, Matthew Fuxjager

Vertebrate skeletal systems are thought to be highly conserved, evolving in response to strong natural selection for efficient and effective locomotion. One way we see these effects play out is through the design of largely similar bone morphologies across species, and especially within clades of closely related taxa. However, in some vertebrate groups, other forms of selection have had clear effects on the skeletal system by driving the evolution of highly unusual morphological traits. Here we use μ CT to explore these traits in a small family of birds called manakins (Family: Pipridae), where sexual selection has driven the evolution of diverse osteological modifications to support forms of acrobatic courtship behavior. Specifically, we test if there is morphological variation across their wing bones between species, and whether such modifications impact the biomechanical properties of the bones under selection. Our results show that while there is variation between species in their morphological traits, the biomechanical properties of these bones remain largely conserved. We suspect that this conservation is due to the emergence of so-called “cost ameliorating” traits, which in this instance are likely osteological traits that function to preserve the biomechanical integrity of the bone itself.

Does heterozygote advantage maintain variation of a gene of major effect for muscle mass?

Lucas Pineiro, Theodore Garland

The mechanisms that can maintain genetic variation within populations under directional selection have been an important topic in evolutionary genetics for more than a century and are also of practical importance for agriculture. During replicated selective breeding for high voluntary wheel-running behavior in laboratory house mice, a gene of major effect was discovered, which, when homozygous, causes the so-called “mini-muscle” phenotype, characterized primarily by a 50% reduction in hind limb muscle mass, and numerous pleiotropic effects. The genetic variant causing this phenotype is a

single C-to-T base pair change (Myh4Minimisc) between exon 11 and 12 of the Myh4 skeletal muscle gene. The mini-muscle phenotype has been observed in two of four High Runner (HR) lines (lab designation HR3 and HR6) and in one of four non-selected control lines (C5). Within these lines, the phenotype was lost in C5, reached fixation in HR3, and has remained polymorphic within HR6 after 100 generations. Population genetic modeling at earlier generations indicated the Myh4Minimisc allele must have been under positive selection in the HR lines and either neutral or disfavored in the C lines. We tested the hypothesis that heterozygotes run more on wheels than does either the wildtype or Myh4Minimisc homozygote. Using individual mice for which wheel-running data were available, tissue samples from multiple generations were collected and genotyped by sequencing.

CRISPR-based Natural Transformation Tools for *Vibrio fischeri*

Brian Pipes, Michele Nishiguchi

Natural transformation (NT) is a type of horizontal gene transfer, where bacteria can actively take up and integrate exogenous DNA. Symbiotic bacteria that are transmitted environmentally are likely to use this mechanism of DNA transfer, yet no system has observed this in the wild. We used the *Euprymna scolopes*-*Vibrio fischeri* symbiosis model to develop novel methodologies to induce NT in *V. fischeri*. *V. fischeri* has the ability to become competent and uptake exogenous DNA under specific conditions, such as the presence of chitin oligosaccharides or during experimental overexpression of the chitin induced competence regulatory factor TfoX. We designed a novel NT inducing TfoX expression vector that incorporates a CRISPRi cassette targeting the *Dns* gene for repression, enhancing NT rates compared to that of standard TfoX expressing vectors. Additionally, we developed a TfoX expression vector containing a CRISPR-Cas9 counterselection cassette that enables scarless and markerless genetic modifications via induced NT. Characteristics such as biofilm formation, host colonization, nutrient uptake, and host recognition that are important mechanisms for symbiotic competency are then used to test the efficiency of NT and subsequent colonization in situ. By advancing the use of NT for genetic manipulation of *V. fischeri*, these enhanced CRISPR-based NT induction vectors expand the utility of *V. fischeri* as a genetically tractable model bacterium for symbiosis research.

Fluctuating thermal regime preserves longevity and quality in the pollinator, *Megachile rotundata*

Jacob Pithan, Joseph Rinehart, Kendra Greenlee

Insect cold storage is commonly used to extend dormancy and improve synchronization of development. Cold storage is generally implemented using a static thermal regime (STR) at a constant low temperature because of its simplicity; however, this has been shown to be detrimental to pollinator performance and longevity. Using a fluctuating thermal regime (FTR) during storage reduces cold-induced mortality and improves longevity in insects. Although FTR seems inherently better than STR, there are possible fitness and energy costs. We predict that FTR treated bees will experience improved longevity and survival compared to STR, but will decline in performance and reproduction with time. To explore potential tradeoffs, we conducted a study where post-diapause quiescent alfalfa leafcutting bees were stored under STR at a constant 6°C and compared with bees given a daily 1-hour pulse of 20°C (FTR). Over several months, emergence, longevity, locomotion, and reproduction were measured. Initially, emergence between STR and FTR was similar, but over time bees stored in FTR experienced faster development rates and reduced cold-induced mortality with no effect on individual mass. Similarly, longevity diverged over time with FTR bees living longer than STR bees. Flight performance and walking performances did not differ with treatment or time. Contrary to our prediction, these results suggest that FTR can improve survivorship and longevity without physiological conditions suffering in bees.

Testing the “Adaptive Sterilization Hypothesis” in mice inoculated with *Chlamydia muridarum*

Ned Place, David Peck

The “adaptive sterilization hypothesis” argues that the tendency of sexually transmitted infections (STIs) to cause infertility likely reflects an evolutionary adaptation of pathogens. To test this hypothesis experimentally, female mice were inoculated with *Chlamydia muridarum* to determine whether tubal occlusion modulates mating behavior in a manner that might increase transmissibility of *Chlamydia*. Increased mating frequency and promiscuity are factors associated with greater risks of acquiring STIs. Similar to *C. trachomatis* infections in human females, *C. muridarum* can ascend the reproductive tract of mice, damage and occlude the oviducts, and cause infertility. However, ovarian function and mating activity are maintained following tubal occlusion. Twenty C57Bl/6 mice with regular estrous cycles were given intra-vaginal inocula of *C. muridarum* and 32 days later paired with a male for 90 days. Ten saline-treated females served as controls. Three *Chlamydia*-inoculated females were infertile due to bilateral oviductal damage and mated 8 (± 0.0) times. Control females mated on average 4.6 (± 0.3) times, and 17 *Chlamydia*-inoculated fertile females with unilateral oviductal damage mated on average 4.7 (± 0.2) times. Female mice with *Chlamydia*-induced tubal infertility mated more frequently (approximately every 11 d) than did fertile females (approximately every 20 d), which is consistent with the adaptive sterilization hypothesis. To determine whether *Chlamydia*-induced sterilization is truly adaptive, future studies will need to demonstrate increased promiscuity and sexual transmissibility within populations of freely breeding mice.

Directional control of phototactic behavior in sea stars

Theo Po, Matt McHenry

Sea stars control their direction through hundreds of tube feet without a brain. The tube feet continue to move after being excised from the body. Thus, the direction of locomotion may emerge through the collective action of the local control of the feet, or through descending commands from the animal's central nervous system. We investigated this hierarchy of control through experimental manipulation of *Protoreaster nodosus*. We found that sea stars often change their direction, but that they follow a straighter path when exposed to a light source. When inverted and touching the water's surface, the sea stars exhibited fictive locomotion without mechanical coupling to a substrate. In this inverted preparation, the direction among the tube feet could be either highly variable (low-polarization) or consistent (high-polarization). For either of these behavioral states, the tube feet became more polarized when we placed a glass slide atop the feet. However, the motion of the glass was less directed in stars with a low-polarized state than those with the high-polarized state. This result suggests that the direction of locomotion may be similarly dictated by the behavioral state of the animal when the body is mechanically coupled to the substrate. These observations support a model of directional control where the tube feet operate under local control, but a central controller can alter the behavioral state for more directed motion.

Effects of elevated CO₂ exposure on post-molt recovery of pinching force in the ornate blue crab

Robert Podolsky, Zoe Munson

The crustacean musculoskeletal system relies on hardening of an external cuticle following molt. This process involves incorporation of mineral salts, including calcium carbonate, to increase hardness and durability. Deposition of calcium carbonate can be inhibited by reduced pH conditions, putting calcifying species at increasing potential risk as the world's oceans absorb atmospheric CO₂, which lowers seawater pH. We studied how cuticle of the ornate blue crab, *Callinectes ornatus*, recovers after molt under different CO₂ conditions. Following molt, juvenile crabs were placed in atmospheric flow-through containers that matched current (400 ppm) or expected future (1000 ppm) pCO₂ conditions. During recovery, crabs were measured daily for two features that reflect post-molt skeletal recovery—pinching force and flexural stiffness of the chelae. For a small sample of crabs, pinching force tended to recover more quickly for animals held at higher CO₂ (lower pH) conditions. This unexpected result agreed with other work on *Callinectes* crabs

showing that calcification was unexpectedly greater under low pH conditions, apparently as physiological compensation for the lowered saturation state of CaCO_3 . The pattern of change in flexural stiffness was inconclusive. These results warrant further study of recovery over longer intermolt periods to see whether pinching forces in the two conditions ultimately reach similar levels, or if post-molt performance is a persistent correlate of greater calcification under low pH.

The Role of Vitamin D Provisioning in Regulation of Diapause in Annual Killifish

Jason Podrabsky, Amie Romney

Differential packaging of proteins, metabolites, or hormones and other small molecules into oocytes can affect developmental outcomes such as survival and developmental rate. In some species, differential packaging can program an embryo for alternate developmental trajectories in response to environmental and ecological cues received by the female. In the annual killifish *Austrofundulus limnaeus*, females can produce two different developmental phenotypes, embryos that enter diapause II and those that “escape” dormancy and development continuously. Recently, regulation of vitamin D signaling in embryos has been shown to determine if embryos enter diapause or develop continuously in this species. We hypothesize that females may differentially package embryos with vitamin D to determine their developmental trajectory. Mass spectrometry was used to measure vitamin D levels in freshly fertilized embryos produced by females that were monitored and found to produce diapausing or escape embryos. We found that females that produce a high proportion of escape embryos have higher levels of vitamin D in their ovaries, and provision significantly higher levels of vitamin D into their embryos. These data are the first evidence of differential maternal packaging that can regulate embryonic diapause in a vertebrate species. In the future, we plan to explore the environmental or ecological cues that result in increased vitamin D packaging in embryos to identify the cues that regulate production of diapausing embryos in annual killifish.

A simple model to explore effects of age on reaching performance

Delyle Polet, Christopher Richards

Ageing can have profound effects on muscle and on arm reaching performance- that is, moving the hand from one position to another in space in a controlled manner. However, exactly how muscle changes can affect reaching performance remains elusive. A key challenge is the complexity of the motor control system, leading to an extremely large parameter space to explore. Instead, we explore reaching performance using a simplified computational model, consisting of one planar joint connected to two antagonistic muscles. The muscles are hill-type with parallel passive elements. Using optimal control with CasADi, we explore the effects of muscle and activation parameters on various aspects of reaching performance- time to target, effort, work, and robustness to perturbations. Despite the model's simplicity, several common features of reaching emerge from optimal control, including triphasic activation and bell-shaped velocity profiles.

Optimal gearing of dynamic musculoskeletal systems: maintaining physiological similarity

Delyle Polet, David Labonte

Terrestrial animals of different sizes often exhibit different gear ratios in their musculoskeletal systems. It is thought that this is some adaptation of the musculoskeletal system to overground locomotion. The most common explanation focuses on a static mechanical analysis and relates to the differential scaling of muscle and weight forces. However, little work has assessed how the gear ratio influences dynamics, and might be tuned to the size of the organism and the physiology of the driving muscle. We explore this problem through the lens of physiological similarity (or “effective inertia”), i.e. the degree to which maximum muscle strain rate or strain capacity constrains the work that can be done as a function of the payload. Gearing modulates the physiological similarity index, and we investigate whether an “optimal” gearing exists, such

that maximum work can be supplied by a muscle for a given payload and physiology. We show that such an optimum exists for a number of simplified limb arrangements and mass scaling relationships.

Poison dart frogs use feature analyzers to detect prey items

Jasausha Pope, Nigel Anderson, Doris Preninger, Matthew Fuxjager

Finding food and avoiding predators is critical to all animals, and natural selection therefore favors the evolution of diverse mechanisms to accomplish these tasks. In anurans (frogs and toads), prey recognition is thought to occur via neural feature analyzer systems, where individuals classify and detect prey by appraising its shape and the direction the shape moves. If, for example, individuals see a rectangle moving in a direction that is parallel to the rectangle's long axis, then the anuran classifies the object as a prey item. By contrast, if individuals see the same object move perpendicular to the object's long axis, then they see the object as an unfamiliar item. However, while this feature analyzer system is thought to be conserved across most amphibians, few studies have tested this possibility by looking for evidence of its existence in diverse anuran taxa. Accordingly, we present a study in which we test for the feature analyzer in a poison dart frog (*Epipedobates anthonyi*). We find that individuals positively orient to rectangles moving parallel to the rectangles' long axis, while a few individuals even tried to eat this stimulus. Yet, these same frogs did not respond to rectangles moving perpendicular to the rectangles' long axis. Our findings are therefore consistent with the idea that feature analyzers are a conserved mechanism for prey recognition in frogs and toads.

Ant search: coordinated, efficient, and not just random!

Stefan Popp, Anna Dornhaus

How do animals search for resources of unknown locations? We know relatively little about it, especially in collective systems like social insect colonies, where the individuals should coordinate to maximize group success. In my PhD I investigated the search behavior of *Temnothorax rugatulus* ants in a large lab arena.

I show that 1) ants do not walk purely randomly, as was previously assumed, but meander regularly in smooth left-and right turns. This systematic element decreases the number of path self-crosses while not increasing the distance ants disperse from the nest, making their search patterns more space-filling.

2) Colonies in this genus often relocates their nest, requiring the foragers to become familiar with their new environment for effective navigation and search. In our experiments, over 3 days after being placed into a new arena, colony-level exploration decreased, but ant dispersivity, speed, and path straightness all increased over time, possibly reflecting a shift from familiarization or marking to searching.

3) Previous work showed that ants walk straighter on surfaces which are marked with chemical footprints of nestmates. We find the opposite effect, which we explain by 2 reasons: footprints make ants walk straighter, and interindividual variation combined with environmental heterogeneity mean causation in the reverse direction. The latter, together with extreme sensitivity to environmental cues, are probably more ecologically relevant than the reaction to footprints.

Wnt-Dependent and -Independent Repertoire of Dkk3 in Tunicate Neurodevelopment and Neurodegeneration

Sydney Popsuj, Alberto Stolfi

The existence of a basic molecular toolkit underlying body plan development and evolution means that certain signaling pathway components may have different functions in a variety of processes. As such, understanding how one molecule can be implicated in diverse and often contradictory developmental processes is of the utmost importance. A clear example of this is Dkk3, a member of the Dickkopf family of Wnt inhibitors. Dkk3 has roles in neuro-protective, neurodegenerative, and neural remodeling events in humans and other animals, which have puzzled researchers studying the molecular mechanisms underlying these processes. The model organism *Ciona* offers a unique opportunity to study these functions outside the realm of disease or neurological injury, because of its biphasic life cycle. We have identified Dkk3 as a

molecular marker of many differentiated neuron types including different brain neurons, motor neurons, and sensory relay neurons, suggesting that Dkk3 could play a role in the differentiation of the larval nervous system, as well as the programmed cell death of larval neurons during metamorphosis. Although we present evidence that Ciona Dkk3 is capable of inhibiting Wnt signaling in vivo, we hypothesize that Dkk3 might act through both Wnt-dependent and Wnt-independent mechanisms. Using cell-specific CRISPR-Cas9-mediated knockouts and ectopic overexpression of Dkk3, we plan to further investigate the functional repertoire of this gene in the Ciona nervous system.

Why are birds dark in cold, wet places?

Cody Porter, Faye Romero, Dean Adams, Rauri Bowie, Eric Riddell

Convergent evolution is widely regarded as a signature of adaptation. But testing the adaptive consequences of convergent phenotypes is challenging, making it difficult to exclude non-adaptive explanations for convergence. Here, we combined feather reflectance spectra and phenotypic trajectory analyses with visual and thermoregulatory modeling to test the adaptive significance of dark plumage in songbirds of the California Channel Islands. By evolving dark dorsal plumage, island birds are generally less conspicuous to visual-hunting raptors in the island environment than mainland birds. Dark dorsal plumage also reduces the energetic demands associated with maintaining homeothermy in the cool island climate. We also found an unexpected pattern of convergence, wherein the most divergent island populations evolved greater reflectance of near-infrared radiation. However, our heat flux models indicate that elevated near-infrared reflectance is not adaptive. Analysis of feather microstructure suggests that island-mainland differences are related to coloration of feather barbs and barbules rather than their structure. Our results indicate that adaptive and non-adaptive mechanisms interact to drive plumage evolution in this system. This study sheds light on the mechanisms driving the association between dark color and wet, cold environments across the tree of life, especially in island birds.

Compounding complexity: Investigations of expressed opsin diversity across stomatopod species

Megan Porter, Sitara Palecanda, Mireille Steck

Stomatopod crustaceans have the largest spectral diversity of photoreceptors known from any animal. Past studies of the eyes of larvae and adults of the stomatopod *Neogonodactylus oerstedii* demonstrated that expressed opsin diversity exceeded predictions based on photoreceptor diversity alone, with opsins in the adult retina displaying complex expression patterns across the eye. In this study we expand on the *N. oerstedii* visual opsin diversity using transcriptomics to characterize opsin expression in the adult eyes of eleven additional species of stomatopod representing ecological, visual system, and taxonomic diversity across the order. Additionally, larval eyes from four species were sequenced to look at patterns of opsin expression across development. The number of visual opsins expressed in stomatopod adult eyes ranged from six in *Psuedosquilla marmorata* to 36 in *Pseudosquilla ciliata*. In larval eyes, expressed opsins ranged from 5 in *Alima pacifica* to 24 in *Pullosquilla thomassini*. Squilloid species in which adult retinas have lost photoreceptor complexity have correspondingly lost expression of at least six spectral clades of opsins. In *Alima pacifica*, despite similar sets of opsins expressed in both larval and adult retinas, expression levels reveal a set of predominately larval vs predominately adult opsins associated with differences in the habitat of each stage. This preliminary look at opsin diversity across stomatopod species suggests complex relationships between visual opsin diversity and ecology.

Lake Malawi cichlid fishes as model for the evolution of facial development

Kara Powder

Cichlids of the East African rift lakes are one of the most species-rich vertebrate lineages and a textbook example of an adaptive radiation. Within bony fishes, cichlids are notable for their sheer number of species, and their morphological diversity. For instance, specialization of the facial skeleton in cichlids has produced an unparalleled range of craniofacial morphologies that are critical for the evolution of these species. Notably, the evolutionary history

and genetic architecture of cichlid (e.g. >99.75% nucleotide similarity between pairs of species in Lake Malawi) make them an ideal model to investigate genotype-phenotype relationships, identify novel regulators of facial development, and explore how evolution of developmental programs generates organismal adaptations. Our lab employs techniques including Next-Generation sequencing, genetic mapping, geometric morphometric shape analyses, and experimental embryology to examine the molecular and developmental origins of variation in facial shape across Lake Malawi cichlids. Ongoing projects have identified species-specific responses to altered epigenetic modifications, used genomic approaches to identify the DNA changes that underlie adaptations across Lake Malawi, and examined how the genetic background of each species interacts with sex to generate distinct patterns of morphological variation. In all, this work leverages comparative approaches in genetics and developmental biology to understand the evolution of organismal form. This work is supported by NSF-IOS #1942178, NIH P20GM121342, NIH R15DE029945, and the Clemson Creative Inquiry program for undergraduate research.

Symbionts to the rescue: Symbiodiniaceae facilitate coral survival in extreme bay environments

Maya Powell, Verena Schoepf, Sarah Solomon, Karl Castillo

Coral reefs are crucial to global biodiversity and local economies, yet highly vulnerable to climate change, making them a research priority. Semi-enclosed bays in Curaçao are uniquely extreme and variable, and semi-analogous to future ocean conditions as warming and acidification continue. Studying corals in these multi-stressor environments and comparing them to corals on fringing reefs will help us uncover mechanisms for coral resilience. This study aims to investigate coral-associated microbial and algal symbiont (Symbiodiniaceae) interactions and their role in coral acclimatization and stress tolerance. *Siderastrea siderea*, *Siderastrea radians*, and *Porites porites* samples were collected from bays and reefs in Curaçao. We show that *S. siderea* and *P. porites* host higher proportions of stress resistant Symbiodiniaceae in bays compared to reefs. We also found that microbial community composition is distinctly different between sites for *S. siderea*. Microbiome communities for all coral species have high proportions of bacteria associated with diverse metabolisms, such as sulfur-cycling in inland bays. Thus, stress-resistant Symbiodiniaceae potentially facilitate the survival of their coral hosts in these extreme inland bay environments. Our study suggests that microbial composition is influenced by environmental conditions, and microbial diversity and metabolic flexibility is likely critical for success across environments. These results show how Caribbean coral holobionts are affected by multi-stressor environments, and may respond as anthropogenic climate change continues to intensify these stressors.

Bat Reproductive Processes and Pathogen Response

Lisa Powers

Roughly three-quarters of emerging infectious diseases come from animals (zoonotics). This motivates researchers to identify the animal species that are hosts to pathogens that may become the next emerging infectious disease, and to understand the biology that allows host species to be a reservoir. Many animal physiology studies neglect sex, either by omitting females or by not including sex as a factor in analyses, in the interest of simplifying the design. Here I present the rationale for deliberate inclusion of sex and female reproductive characteristics in the study of potential reservoir hosts, using bats as an example. Bats merit consideration on this topic due to the relatively large percentage of species that are associated with zoonotics, and also the breadth of diversity of reproductive traits among species. I will present examples that demonstrate how differences in female reproductive status and life history characteristics can predict pathogen prevalence in bat populations, and discuss how bats' unique reproductive ecology has the potential to play a role in their response to other pathogen infections. Pathogen studies that consider the effects of reproductive processes help to identify common characteristics of host species. These characteristics can: uncover patterns that underlie wide intraspecific variation in immune response; inform decisions about when, where, and which species to sample in the field; and improve models of spillover risk.

A Framework for Fast, Large-scale, Semi-Automatic Inference of Animal Behavior from Monocular Videos

Eric Price, Pranav Khandelwal, Daniel Rubenstein, Aamir Ahmad

Video recording devices are becoming a staple for collecting animal behavior data in the wild. They allow capturing diverse behaviors of animals in a non-invasive manner. Converting the captured videos into behavior data involves manually annotating frames to classify behaviors or using machine learning (ML) to automate the process. ML is an attractive proposition but relies on custom-built algorithms and large, manually annotated, ground-truth datasets to train those algorithms – a labor-intensive feat that is required for each study system. We present an open-source interactive tool called Smarter-labelme that can rapidly generate large amounts of reliable training data, train an animal detector and behavior classifier, and use the trained network to semi-automatically generate more training data from unseen videos. Our bootstrapped workflow allows the rapid evolution of the detector and classifier networks to become more robust and accurate with each training event. The tool generates a bounding box, ID, and behavior for each animal, which can be used to train other ML models or directly for behavioral analysis. We demonstrate the effectiveness of our tool on aerial videos of zebras recorded in Kenya. After training with ~4000 annotations, the time spent per new annotation was reduced by ~90%. We also demonstrate the tool's utility in performing behavior analysis by quantifying the activity budget of zebra groups over large spatiotemporal scales in their natural habitat.

Microbial predictors of bleaching in Great Barrier Reef *Acropora millepora*

Karim Primov, Mikhail Matz, Mark Kirkpatrick

Identifying the underlying basis for a complex trait such as bleaching resistance is crucial for identifying coral populations warranting high conservation status. *Acropora millepora*, a main Indo-Pacific reef-building coral, exhibits differential bleaching response throughout the GBR despite high levels of gene flow (Fuller et al., 2020). Corals which had higher bleaching scores, however, possessed higher proportions of thermotolerant *Durussdinium* symbionts. No information on microbial community composition among bleaching susceptible and resistant *A. millepora* exists, however, which could provide insight into the driver of differential bleaching response and symbiont community variation between GBR *Acropora millepora* populations. In the current study, microbial community profiles will be characterized to identify whether differential bleaching response and symbiont community variation correlate with microbial community variation. In addition, specific microbial taxa may be useful indicators of bleaching resistant or susceptible coral genotypes. Results from this study will inform conservation management on whether microbial communities, in addition to environmental differences, provide an underlying basis for differential bleaching response among populations of ecologically-significant corals threatened by climate change.

Sea turtle detection of ammonia odors: Implications for navigation to nesting beaches

Lillian Prince, Kayla Goforth, Jadya Sethna, Kenneth Lohmann, Catherine Lohmann

Loggerhead sea turtles (*Caretta caretta*) return to their birth regions to reproduce as adults, a behavior known as natal homing. Previous studies suggest turtles use Earth's magnetic field to relocate their natal regions, but it is likely sea turtles use additional cues to localize specific beaches. Migratory sea birds can detect and follow odor plumes of airborne ammonia, produced from seabird fecal matter and bacteria in marsh habitats, to relocate nesting islands. Given that sea turtles detect air and water based odors it is possible that turtles use ammonia as an additional navigational cue. To determine if turtles are able to detect and respond to airborne ammonia odors, turtles were exposed to two concentrations of airborne ammonia (10 μ M and 100 nM), along with food and water odors. Turtles spent significantly more time with their nares out of water and took more breaths when odors of ammonia were present compared to odors of sea water; behaviors performed by turtles when they detect an odor of interest. Responses to ammonia and food odors were similar thus it appears that sea turtles can detect airborne ammonia at

biologically relevant concentrations. Results indicate turtles may use ammonia odors to assist in navigation to near-shore coastal habitats and reproductive areas. Experiments are currently underway to determine the minimum concentrations at which turtles will respond to airborne ammonia.

Sensorimotor apparatus underlying head movements in hawkmoths

Agnish Prusty, Payel Chatterjee, Sanjay Sane

Walking and flying insects stabilize their gaze primarily through compensatory head movements. Such head stabilization behavior ensures a stable visual field on the retina during locomotion, despite minimal movement of their compound eyes relative to the head. To achieve head stabilization, the neck sensorimotor apparatus integrates sensory feedback from the visual and vestibular systems. This multimodal feedback drives the activity in multiple neck muscles spanning the head-prothoracic segment. In Diptera, vestibular feedback is obtained from halteres, which sense body rotations during complex aerial maneuvers. In addition, the mechanosensory prosternal organ situated in the ventral neck region provides rapid feedback about head movements. However, we know very little about compensatory head movements in non-Dipteran insects, which lack halteres. Specifically, for nocturnal moths operating under dark conditions, visual feedback is much slower and less reliable. Previous studies showed that moths require antennal mechanosensory feedback for head stabilization. The prosternal organ was also not reported in moths. We conducted detailed investigations of the neck sensorimotor apparatus in the Oleander hawkmoth *Daphnis nerii*. Using X-ray microtomography, we identified neck mechanosensory bristle fields resembling the prosternal organ similar to flies. Sensory afferents of this organ project into and likely stimulate neck motor neurons with soma located in the prothoracic ganglion and sub-esophageal zone. X-ray microtomographs revealed 11 bilaterally symmetric pairs of neck muscles, their geometry, orientation and attachment points. Our electromyography studies demonstrate that neck muscles are activated by antennal mechanosensory feedback. Together, these data provide insights into the mechanisms underlying head stabilization in moths.

Twist of Nature: The mechanical design of hawkmoth antennae helps to control flight

Adam Puchalski, Kostya Kornev

The beauty of nature has always sparked innovation, giving rise to novel materials with unique qualities. In particular, the mechanical intricacies of insect antennae remain a treasure trove of untapped potential. These antennae possess remarkable bending abilities, enabling insects to perform a variety of tasks, from navigation to finding mates. This study embarks on a comparative journey, examining the mechanical properties of antennae from three hovering hawkmoths: *Manduca sexta*, *Manduca quinquemaculata*, and *Xylophanes tersa* and one non-hovering hawkmoth *Ceratomia catalpa*. Through a series of bending experiments, we discover and quantitatively characterize the fascinating features of directional dependence of bending rigidity of antennae. The resistance of the antennae to bend toward the dorsal and ventral sides is drastically different and is distinguished from their resistance to bend sideways. These intriguing characteristics prompt questions about antennal performance during flight. We experimented with antennae in the wind tunnel to show unique antenna bending and twisting features. These new discoveries suggest that the mechanical design of the antennae of flying insects may contribute in flight control. By harnessing the mechanical aptitude displayed by insect antennae, our goal is to craft materials that seamlessly combine functionality with ecological sustainability.

Biomechanics of leaf fracture in herbivores: a scaling study on leaf-cutter ants

Frederik Puffel, Olivia Walthaus, Victor Kang, David Labonte

Herbivores large and small need to mechanically process plant materials. The ability to do so is determined by the ratio of two forces: the maximum force they can generate, and the minimum force required for material fracture. We measured both forces for *Atta vollenweideri* leaf-cutter ants which vary

by more than one order of magnitude in body mass. Maximum bite forces increased with strong positive allometry, such that large workers generate about 16 times higher peak forces than small workers, twice as much as predicted from differences in body size alone. In stark contrast, the forces required to cut standardised polymer sheets were independent of size, indicating that larger workers can cut a broader range of materials, and cut the same leaf with less mechanical effort than smaller workers. Although cutting forces were size-independent, they differed by a factor of two between pristine and worn mandibles. Pristine mandibles cut with forces close to a theoretical minimum defined by the toughness and thickness of the cut material, suggesting they are ideally sharp; only with increasing mandibular wear do cutting forces begin to depend on mandible geometry. The increase in cutting force due to mandibular wear may be particularly problematic for small ants, which require a larger fraction of their maximum bite force to cut the same plant.

Contextual Factors Affecting Human Perception of Emotion in Cats from Photos

Amanda Puitiza, Monique Udell

Cats are currently one of the most common companion animals in the United States. Beliefs about cats can affect general management, care, and welfare practices. However, little is known about how humans perceive the emotional state of cats and to what extent contextual factors such as environment, coat color, and one's own emotional state may affect human perception of cat emotional states. Using an online survey, we asked participants to rate the emotional state of cats from photos with AI-generated backgrounds. Each cat was shown twice in a randomized order: once with an indoor and once with an outdoor background. Participants also rated their own feelings towards viewing each image. An initial analysis suggested that average human emotional response to the cat images and the average emotional rating attributed to the cat were positively correlated, $r(338) = 0.55$, $p < 0.001$. Differences between how humans rated the emotional state of the cats based on the type of background, exper-rated valence scores, and coat color will also be analyzed.

Investigating giant retinal ganglion cell function in the bottlenose dolphin (*Tursiops truncatus*)

Rejana Pullarkat, Makayla DePinto, Lorian Schweikert

Retinal ganglion cells (RGC) bridge the gap between photoreceptors and the brain to provide neural information that is critical to physiology and behavior. Atypically large RGCs (i.e., giant RGC) have been observed in cetaceans and other mammalian groups, prompting discussion about whether giant RGCs are a product of allometry or evolved to serve a particular function. To address this, we investigated the form and function of giant RGCs in the common bottlenose dolphin (*Tursiops truncatus*) by 1) evaluating the retinal topography of giant RGCs and 2) testing whether there is a subpopulation that express melanopsin (OPN4), a photopigment previously implicated in circadian rhythm entrainment and pupillary response. Histological staining and anti-melanopsin immunohistochemistry were employed separately on the whole-mounted retinas taken from a single subadult dolphin. Examinations of the retinas allowed us to produce separate topographical maps of all giant RGCs and those expressing melanopsin (cells/m²). We found that both typically-sized RGCs and giant RGCs express melanopsin, but only within a subpopulation of cells distributed non-uniformly over the topography of the retina. These results provide new insight into the function of giant RGCs and their potential role in both image-forming and non-image-forming vision.

Look Mom, No Hands: Effects of perch diameter on vertical gap crossing in arboreal snakes

Joshua Pulliam, Sydney Blacksten, Mason Dooley, Kamau Braxton-Hall, Julia Alexander, Scarlett Ewing, Jeffery Anderson, Jake Socha

Arboreal animals locomote through environments characterized by branches that differ in diameter, texture, and compliance. Climbing upward requires that the animal balance or grip the origin while reaching for the target. For snakes,

reaching is done with the body; the greater the reach, the more balance or grip required by the remaining body. Here, we investigated how perch diameter affects vertical gap crossing ability in arboreal snakes. We hypothesized that larger perches provide a larger balancing surface area, enabling the snakes to cross greater distances. We recorded three species of snake, *Chrysopelea paradisi*, *Dendrelaphis pictus*, and *Ahaetulla prasina*, crossing vertical gaps between two horizontal perches. A motion capture system (Vicon) was used to track points at 10% (snout-vent length) increments along the snake's spine. The origin perches were three PVC pipes (d, 21.5–48.4 mm). The target was a 1.3 cm diameter pipe wrapped in artificial foliage to incentivize the snake to cross. During the cross, the target was raised until the snake achieved its maximum height. The tracked points were used to calculate maximum height, instantaneous velocity, and ratio of traveled distance versus maximum height (sinuosity). Preliminary results suggest greater variability in climbing velocity as diameter decreases, decreases in average velocity as diameters increase, and sinuosity increases along the body of the snake during the cross. Supported by NSF 2027523 and 1922516.

Molecular and functional organization of the octopus visual system

Judit Pungor, Jeremea Songco-Casey, Angelique Allen, Cristopher Niell

Cephalopods have a sophisticated visual system that guides a wide array of visually guided behaviors: from hunting prey, avoiding predators, and finding mates, to underlying their impressive camouflage abilities. Although superficially similar, the camera-type eyes of cephalopods and vertebrates emerged independently, resulting in one of the most stunning examples of convergent evolution. However, because the cephalopod brain evolved independently from that of other highly visual species, the neural organization of their visual system is dramatically different. But compared to other model organisms, relatively little is known about its molecular and functional organization. Our group uses multiple approaches to explore this enticing visual system in *Octopus bimaculoides*. We conducted single cell RNA sequencing and in situ hybridization of their optic lobes to identify and localize molecularly distinct cell types within the system. We also developed a two-photon calcium imaging protocol for the cephalopod central brain to identify basic functional response properties in the optic lobe, and are using this to further explore responses to more complex visual features, such as the polarization of light.

Interplay of acclimatization and adaptation modulates resilience to climate change in marine invertebrates

Hollie Putnam, Kevin Wong, Ariana Huffmyer, Hendrikje Jorissen, Eva Majerova, Antoine Puisay, Yann Lacube, Michael Henley, Claire Lager, Carmela Nuñez-Lendo, Caroline Dubé, Laetitia Hedouin

Parental provisioning and transgenerational epigenetic inheritance are mechanisms by which information from parental environments can be transmitted to offspring. This is particularly critical information for sessile, reef building corals, as environmental-performance mismatch can be detrimental. Here using a transplant experiment, we examined the impact of sites with differing thermal variance on the reproductive capacity (fecundity, egg size, eggs per bundle, and sperm motility) of adult *Acropora hyacinthus* colonies in Moorea French Polynesia, as well as mechanisms whereby environmental information can be transmitted across a generation (maternal egg mRNA and sperm DNA methylation). *A. hyacinthus* from the lower thermal variance foreereef site had consistently higher fecundity, larger egg size, more eggs per bundle, and greater sperm motility compared to those transplanted to the backreef. Differentially expressed genes demonstrated that environment during gametogenesis influenced maternal mRNA provisioning. Genes with greater expression levels in the high thermal variance backreef location included, for example, those associated with thermotolerance (e.g., caseinolytic peptidase B protein and saccin) and a histone variant H2B L4, whereas histone H2A had higher expression in the foreereef samples. Methyl binding domain enrichment bisulfite sequencing (MBD-BS) of sperm DNA showed differential methylation of translation and sperm capacitation genes. This study of reproductive performance, maternally-provisioned gene expression, and paternally-inherited methylation patterns, identifies that offspring performance is shaped across a generation by prior environmental history and epigenetic mechanisms.

Neural Effects of Motion Parallax in the Tobacco Hornworm Hawk Moth

Ruchao Qian, Jamie Theobald

Visual detection of wide-field motion is an intensely studied area because of its utility and relevance to animals as they move through their environments. However, laboratory stimuli often omit a feature that is nearly ubiquitous during natural movements, differential image motion due to distance, or parallax. This cue complicates visual processing, but offers important cues about object distance that animals might otherwise be unable to infer. To examine the effects of parallax on neural processing, we used extracellular neuronal recordings in the ventral nerve cord in hawk moths. The Tobacco hornworm hawk moth (*Manduca sexta*), a swift and agile insect frequently confused with hummingbirds, exhibits not only complex flight abilities, but, by insect standards, superior vision. We exposed moths to a virtual environment simulating motion parallax through a moving flower and dot fields, aimed to influence the moth perception of the flower distance based on the speed differential between the flower and the dot fields. Preliminary findings suggest that distinct spike patterns emerge in the ventral nerve cord based on the visual stimulus. This research underscores the complexity and efficiency of the visual transduction pathways in *M. sexta*, offering insights into the integration of vision and motor responses in this fascinating pollinator.

Anthropogenic Noise and *Opsanus tau* Advertisement Calls in Various New York City Soundscapes

Thomas Quigley, Paul Forlano, Sydney Gdanski, Xylo Lazrinth, Rachel Rodriguez

Anthropogenic noise in the marine environment is considered pollution. Recent studies have examined how noise pollution impacts animal behavior, fitness and even community structure. New York City is a highly modified archipelago with many sources of anthropogenic noise. As a result, it likely has many different soundscapes. New York City is also home to *Opsanus tau*, the oyster toadfish, which depends on advertisement calls for reproductive success, and which might be impacted by noise. Indeed, recent research found that oyster toadfish at Pier 40 (Hudson River, Manhattan) call most when vessel traffic abates at night.

The goal of this study was to sample several urban marine soundscapes that also provide habitat for oyster toadfish, characterize those soundscapes and examine bioacoustic patterns in advertisement calls. Hydrophones were deployed at four locations in New York City from April to July, 2023. Random samples of all sound (excluding oyster toadfish) indicate that Pier 40 had the loudest mean SPL ($M = 115.8$ dB re $1 \mu\text{Pa}$, $SD = 11.3$ dB) while Sheepshead Bay (southern Brooklyn) was quietest ($M = 103.5$ dB re $1 \mu\text{Pa}$, $SD = 6.4$ dB), a difference of 12.3 dB. Advertisement calls and anthropogenic noise will be further examined using R and Amazon Web Services. We predict that *O. tau* calling behavior will vary based on the characteristics of anthropogenic noise at each location.

Morphological specialization to nectarivory in *Phyllostomus discolor*

Laura L. Quinche, Sharlene Santana, Alejandro Rico-Guevara

This study delves into the morphological adaptations associated with nectarivory in *Phyllostomus discolor*, contributing to our understanding of specialized feeding behaviors in bats. Nectarivory requires unique cranial and soft-tissue modifications to extract nectar efficiently. While it is well-documented in certain phyllostomid subfamilies, this paper shifts the focus to nectarivorous traits in the *Phyllostomus* genus within Phyllostominae. Through dissections and scanning electron microscopy, the researchers examined the tongue and palate morphology of *P. discolor*. Hair-like papillae on *P. discolor*'s tongue, similar to those found in Glossophaginae and nectarivorous Pteropodids, suggest convergent adaptations for enhanced nectar consumption across different lineages. Furthermore, a skull morphometric analysis was performed, comparing *P. discolor* with other nectarivorous, omnivorous, and carnivorous bat species. The analysis shows that *P. discolor* lacks common cranial traits of nectar-feeding bats, possibly due to its omnivorous diet and larger size relative to specialized nectarivores. The study's findings propose a distinctive

evolutionary solution for *P. discolor*: an elongated, highly mobile tongue with papillae that facilitates effective nectar extraction while preserving its dietary versatility. The study contributes to our understanding of adaptations in non-specialized nectarivorous bats and provides a basis for future research on the functional roles and ecological implications of these adaptations.

Stingray Wing Dynamics

John Michael Racy, Adam Summers, Ed Habtour, Carmen Escobedo, Bart Boom

The majority of nature-inspired stingray robot developments have been focused on engineering efficient soft actuators. The architecture of the skeletal system (pectoral fins) of stingrays are rarely examined as an essential subsystem of their propulsive action. In this study, the role of the dynamic behaviors of stingray pectoral fins in their propulsive dynamics are examined computationally and experimentally. The complex actuation system of the pectoral muscles is removed to determine, in isolation, the mechanistic connections between the morphology of the pectoral fins and propulsive performance. Simple 3D-printed samples of fin skeleton were fabricated to emulate the fin rays, made up of mineralized cartilage radials, that run from pelvic girdle to wing tip. The study identifies two important architectural patterns: 1) offset - the angle between the ends of radials of successive fin rays; and 2) trajectory - the splay of the fin rays as they span out from the pelvic girdle. The models with offset radials generate traveling waves, while those without do not, when actuated vertically in still water. Models with trajectories remain stable at increased frequency while those without become out of sync. Finite element analysis shows the mode shapes dynamics as a function of offset and trajectory patterns. Comparison of mode intervals reveals how skeletal arrangement can directionally change fin stiffness, and correlates with the behaviors of physical models.

Diet shifts alter the activity and distribution of digestive enzymes in an herbivorous fish

Kirsten Clerre Rafanan, Michelle Herrera, Caitlyn Catabay, Donovan German

Digestion is a chemical process performed by digestive enzymes. Thus, we examined the activity levels of seven digestive enzymes along the digestive tract of the marine herbivorous fish *Cebidichthys violaceus*. This fish species is considered an obligate herbivore. We reared *C. violaceus* in the laboratory on carnivore, omnivore, and herbivore diets for six months and compared the digestive enzyme activities among the fish on the different diets, and with wild-caught fish consuming their natural foods. Generally, enzymatic activities were lower in the laboratory than in wild-caught fish. For amylase, a strong anterior-to-posterior activity gradient along the gut seen in wild-caught fish largely disappeared in the lab-fed fish. Interestingly, N-acetyl- β -D-glucosaminidase (NAGase) activity (digests chitin breakdown products) showed a strong spike in the distal intestines of the lab-fed fish, but not the wild fish. This increase in NAGase hindgut activity matches with *Paracoccus* sp. (known NAGase producers) being a dominant microbe in the distal intestines of the lab-fed fish, although the role of this enzyme in the digestive process remains unknown since the lab diets contained no chitin. Overall, our results show that *C. violaceus* can tolerate a wide range of protein and carbohydrate levels. Given that this fish species is being explored for aquaculture, the totality of our data (including growth rates) suggests that live algal diets may be best for this herbivorous fish.

High temperature induces nitrative stress and DNA methylation in gonads of Atlantic sea urchin

MD Rahman, Esmirna Cantu

Global warming is one of the greatest threats to living organisms. Among them, sea urchins are severely impacted on reproductive fitness by rising seawater temperatures due to climate change. In this study, we used highly sensitive radioimmunoassay and advanced biochemical and molecular techniques to investigate the effects of heat stress on global DNA methylation, cellular apoptosis, and nitrative stress in gonads of the Atlantic sea urchin. Young adult sea urchins were exposed to 24, 28, and 32 °C for one week

under controlled laboratory conditions. Higher temperatures (28 and 32 °C) significantly increased global DNA methylation in gonads (~3.4- to ~6.2-fold in testes and ~2.7- to ~5.1-fold in ovaries) compared to controls. The number of apoptotic nuclei in gonads was much higher in high temperature groups. Caspase-3/7 activity also increased in testes and ovaries in high temperature groups. Nitrate/nitrites, an indicator of reactive nitrogen species, levels were increased around 2.6- to ~5.2-fold in testes and ~1.9- to ~3.8-fold in ovaries in high temperature groups. Collectively, our results suggest that elevated temperatures drastically induce oxidative stress and cellular apoptosis associated with global DNA methylation in gonads which may cause the trans-generational epigenetic inheritance in sea urchin populations.

Global Urban Bird Survey: An Innovative Community Science Approach

Islamiat Raji, Paul Preston, Alex Mutati, Dan Mennill, Lynn Martin, Massamba Thiam, Paul Robert Martin, Fran Bonier

Urbanization is one of the most extreme forms of landscape transformation, generally reducing biodiversity. However, species vary in their ability to persist and breed in urban environments. This variation in urban tolerance can be estimated using standardized surveys in cities across the globe. We are conducting a standardised urban bird survey using innovative community-based methods. By partnering with local experts and engaging community members, we have established a robust framework for data collection across a range of cities, including Dakar (Senegal), Nairobi (Kenya), Paris (France), and Toronto (Canada). We employed a stratified, randomised sampling approach to survey the core of each city. Using audio recordings, we capture bird vocalizations during the dawn chorus for 15 minutes, visiting each survey point four times across the breeding season. The results of this research provide crucial insights into the distribution of birds within cities globally, overcoming the limitations of previous studies that were biased toward economically developed regions and green spaces. Using randomized sampling and acoustic surveys, our study broadens its scope to encompass rapidly expanding, biodiverse, and heavily urbanized areas. By conducting cross-city comparisons, we can identify avian diversity hotspots, contributing valuable insights for sustainable urban development and the coexistence of healthy avian populations.

Seafaring with Added Cargo: Simulations of Swimming Blue Blubber Jellyfish with Prominent Oral Arms

Cittali Ramirez, Laura Miller, Matea Santiago, Alexander Hoover

The blue blubber jellyfish, *Catostylus mosaicus*, is characterized by eight prominent oral arms extending from its central bell and a distinctive staccato-like rhythmic pulsing. This species displays relatively high swimming efficiency compared to other medusa due to its oblate bell shape, allowing for passive energy recapture. Additionally, this jellyfish is important ecologically and economically as its massive population blooms can disrupt ecosystems and potentially serve as a sustainable human food source.

With the aim of creating a three-dimensional biological simulation of blue blubber swimming, we used the immersed boundary method to numerically simulate the contractions of the elastic bell as the jellyfish propels itself through seawater. Notably, the motion of the bell is not prescribed, and the model kinematics are tuned to biological data specific to the blue blubber jellyfish. Furthermore, periodic active tension is used to model muscle contractions. The resulting emergent behaviors, the swimming speed and vortex structures, are compared with experimental data to validate the model. Of particular significance, this model takes into account the prominent oral arms unique to the blue blubber jellyfish while most other mathematical models of oblate jellyfish omit the oral arms. Consequently, investigating how these oral arms impact swimming kinematics and efficiency becomes a focal point of interest.

Seeing glia: Molecular and EM descriptions of glia in the gastropod mollusc *Berghia stephanieae*

Desmond Ramirez, Harshada Sant, Alexzander Cook, Brandon Drescher, Yuelong Wu, Richard Schalek, Jeff Lichtman, Paul Katz

Glia play important, and still under-appreciated, roles in the structure and function of nervous systems (NS). Despite detailed studies of individually identifiable neurons in gastropods, glia in these animals are poorly described. No histological techniques uniquely targeted them and no molecular markers were known until recently. Here we describe glia types within the NS of the nudibranch *Berghia stephanieae*. We identified marker genes for glial subtypes using single cell transcriptomics (scRNA-seq), and visualized their expression using in-situ hybridization chain reaction (HCR). Apolipoprotein, a known marker for *Drosophila* astrocytes, was differentially expressed in scRNA-seq by glia in *Berghia*. Apolipoprotein HCR revealed abundant glia in the NS, suggesting it may be a pan-glial marker. Small glia outlined neuropil regions and were embedded within the ganglionic sheath. A few giant glia were found per ganglion, encasing numerous neuronal soma. These appear analogous to cortex glia in *Drosophila*. *Berghia* glia were also identified in a volume EM dataset of a rhinophore ganglion. Glia had distinct ultrastructural features and diverse morphologies. Some partitioned axon bundles or synaptic areas of neuropil. Others bordered the sheath and vasculature. Membrane-to-membrane junctions between glia suggest a potential glial signaling network. Modern molecular and imaging techniques provide unprecedented access to glia in a gastropod NS. Future research on molluscan glia will offer insights into the function and evolution of glia across animals.

Distribution of Prey and Feeding Behaviors in the Anole Lizards of Cayman Brac

Akshaya Ranjit, Grace Anderson, Taylor Black, Jeremy Blackburn, Mia Kholy, Katherine Starr, Michele Johnson, Thomas Sanger

Cayman Brac, the smallest of the Cayman Islands, is home to two species of insectivorous, arboreal anole lizards: the short-snouted brown anole, *Anolis sagrei*, and the long-snouted green anole, *Anolis maynardi*. The different head shapes of these species may facilitate their consumption of different prey, but this has not yet been tested. In this study, we measured the height and diameter of the perches where each species of lizards was found, and where they captured prey. We also used transects to quantify arthropod diversity, abundance and biomass, recording the height and diameter of each potential prey item. We found that brown anoles tend to perch at the bases of tree trunks, whereas green anoles generally perch in the leafy canopy. Abundance of potential prey was greatest on the ground, while the greatest prey biomass was available among insects flying at heights of greater than 1m. Despite these differences in arthropod communities, both species of anole were most often observed opportunistically feeding in the same microhabitat where they typically perched. Our next steps involve measures of anole bite force, gape size, and stomach contents to address how head shape may be directly associated with prey consumption in this relatively simple animal assemblage.

Identifying genes mediating local adaptation using targeted versus whole-genome approaches.

Nathan Rank, Elliott Smeds, Abigail Keller, Caroline Williams, Elizabeth Dahlhoff

Populations persist because individuals possess genetic variation that allows them to adapt to their environment. Rapid changes in environmental conditions make it challenging for populations to adapt and leave them vulnerable to extirpation. Prior investigations of variation among metabolic enzyme variants have identified candidate genes contributing to local adaptation, yet modern genomics tools provide opportunities to identify candidates for selection outside the canon of central metabolism. We applied a combination of targeted and 'de novo' analyses to explore the genomic basis of local adaptation in populations of the willow beetle *Chrysomela aeneicollis*. Whole-genome sequences were obtained for beetles used in performance experiments and across different populations in California. We assessed genomic differentiation among populations, tested for relationships of two candidate genes [phosphoglucose isomerase (Pgi) and succinate dehydrogenase (Sdh-b)] to recovery

from heat exposure, and identified novel genes associated with larval performance. Genome-wide association analyses identified novel genes associated with recovery from heat exposure. Climate-associated environmental variation and geographic distance contributed to genomic variation among populations and pointed to non-synonymous variation in loci associated with cytoskeletal function, ion transport and cold tolerance. Demonstrating relationships between novel regions of the genome and candidate genes contributes to understanding the genetic basis of local temperature adaptation in a well-studied organism and expands our knowledge of how organisms respond to rapid environmental change.

Adult male house sparrows outperform females and juveniles in vertical tagged flights

Olivia Rataezyk, Elizabeth Cramer, Natalie Wright

Some, but not all, species of birds exhibit sexual dimorphism in flight muscle size. Our previous work has found that male House Sparrows (*Passer domesticus*) have significantly greater flight muscle masses than do females. To investigate how sexual dimorphism in flight muscle size might affect flight performance, we flew 136 wild-caught House Sparrows in a vertical flight cage with and without model tags (1.4g, approximately 5% of adult body mass). This flight cage aimed to ensure that birds used maximal effort during their flight, as vertical flight requires more power than horizontal flight. All birds were flown twice, and half of all birds were fitted with a model tag in their second flight to gauge the impacts of added weight on vertical flight ability. All birds were given one hour to acclimate before being flown again. In their first flight, adult birds were significantly faster than juvenile birds, and there was no difference between males and females. This remained true in control second flights. However, in tagged second flights, males were significantly faster than females and age had no significant impact on overall flight velocities. In addition, tagged males significantly increased their wingbeat frequencies. Other groups had consistent wingbeat frequencies across all trials.

Three-dimensional myological signals of dietary specialization in strepsirrhine primates

Aleksandra Ratkiewicz, Edwin Dickinson, Cassidy Davis, Shruti Koli, Ashley Deutsch, Michael Granatosky, Adam Hartstone-Rose

Diffusible iodine-based contrast-enhanced computed tomography has emerged as a valuable tool to support detailed studies into muscle anatomy. Compared to traditional gross dissection techniques, the resulting “digital dissections” allow muscle fibers to be studied in situ by preserving three-dimensional spatial relationships for analysis. This makes it possible to measure new variables (e.g., fascicle orientation and tortuosity) that were previously unavailable to researchers. In this study, we use this technique to explore the relationship between dietary ecology and masticatory muscle anatomy across 8 dietarily diverse strepsirrhine species in digital space. We observed that muscle volumes and fascicle lengths derived from these digital analyses generally agree with those measured from gross dissection. As found with traditional techniques, physiological cross-sectional area (a proxy for muscle force production) was greatest in species with mechanically challenging diets, and fascicle lengths (a proxy for muscle excursion potential) were longest in frugivorous and wood-gouging species, and shortest in folivorous taxa. Fascicle orientation also seems to have a clear dietary association: most folivorous taxa have masseter and temporalis muscle vectors that intersect acutely in the parasagittal plane, while these vectors intersect obliquely in more frugivorous species. Finally, we observed notably greater magnitudes and interspecific variation in tortuosity within the adductors than the abductors. These data highlight the heretofore unrecognized ways that three-dimensional analyses can inform our understanding of masticatory muscle adaptation.

Identifying Resilience in *Zostera marina* Meadows in the San Juan Islands, Washington

Baylen Ratliff, Tina Whitman, Bart Christiaen, Olivia Graham, Audrey Vinton, Catherine Harvell, Brendan Rappazzo, Carla Gomes

Eelgrass (*Zostera marina*) is a vital ecosystem engineer in the Salish Sea and is an indicator for ecological health in Puget Sound. Eelgrass meadows around the San Juan Islands have declined significantly in recent years. Eelgrass declines limit the many ecosystem services eelgrass meadows provide, and can disrupt entire food webs. Seagrass wasting disease (SWD), caused by the protist *Labyrinthula zosterae*, is among the modern stressors facing these meadows. Cooler, deeper waters can moderate disease levels. This survey aims to identify resilience (biotic or abiotic conditions that reduce SWD susceptibility) in eelgrass meadows. Here, we surveyed 11 subtidal eelgrass meadows around the San Juan Islands in summer 2023 for biometrics and disease analyses. Sites significantly differed in canopy height, normalized sheath length, leaf count, and second youngest leaf area. Disease prevalence (proportion of infected leaves) was significantly different between survey sites, though disease severity (proportion of infected leaf area) was not. Sites had variable environmental conditions—such as depth, turbidity, and water flow—that may have an influence on SWD susceptibility. In initial assessments, disease prevalence was lower at deeper sites, suggesting that deeper meadows could be potential refugia from disease and climate warming. In the face of global and local climate change stressors, this study reinforces the importance of holistic analyses of disease and environmental conditions to better understand eelgrass resiliency.

Behavioral characterization of auditory escape responses in the mosquito *Aedes aegypti*

Michael Rauscher, Apple Patel, Gabriella Wolff

The significant public health challenges posed by mosquitoes prompts study of all aspects of their life history and sensory biology. Mosquitoes have sensitive hearing, which has been studied for the role it plays in courtship and mating, during which males are attracted to females in large part based upon conspecific wingbeat sounds. Prior work has shown that the mosquito *Aedes diaantaeus* may use its auditory system to mediate a predator escape response as well. By attracting free flying mosquitoes with a conspecific auditory signal, then playing putatively aversive auditory stimuli, Lapshin and Vorontsov (Entomological Review, 2018) identified a negative phonotaxis in response to sounds in the frequency range of 140–200Hz. This range encompasses the wingbeat frequencies (and low-order harmonics) of aerial predators of mosquitoes such as dragonflies and robberflies. Here, we use a tethered flight behavioral paradigm to study the kinematics of the auditory behavioral response in more detail and show that the related *Aedes aegypti* responds similarly to stimuli within the same frequency range. High-speed videography shows that wingstroke amplitude and frequency markedly increase over and above that observed for auditory stimuli outside of this range at the same points following stimulus presentation. As with *Aedes diaantaeus*, no clear steering component was observed in response to stimuli presented from different azimuths, suggesting a role for multimodal integration of auditory and visual escape responses.

Influence of waterfall-climbing style and ontogeny on fast-start performance in gobiid fishes

Shrika Ravichandran, Kelly Diamond, Amanda Palecek-McClung, Heiko Schoenfuss, Richard Blob

Many species of gobiid fishes climb waterfalls using one of two distinct mechanisms. “Powerburst” climbers use pectoral fin adduction followed by rapid axial undulation, whereas “inching” climbers show limited lateral undulation, and instead ascend rockfaces via alternating oral and pelvic sucker attachment. Climbing is most common among juveniles entering freshwater from the ocean. Migrating juveniles use fast-starts to escape predators but, because many predators do not climb, adult gobies living above waterfalls may face less pressure on escape performance. In this context, we used high-speed video to compare fast-start performance between juveniles and adults from powerburst (*Sicydium punctatum*) and inching (*Sicyopterus stimpsoni*) climbers. We

predicted adults of both species would show lower escape performance due to reduced exposure to predators; moreover, inching climbers might perform poorly compared with powerburst climbers as the former climbing style limits lateral undulations, whereas the latter relies on rapid axial movements. We found that, compared at the same age, both species had similar lateral bending during fast-starts, but bending was less in juveniles than adults. Distance traveled during escapes showed similar patterns. Adults of powerburst climbing *S. punctatum* showed faster peak escape velocities than all other groups, but accelerations of adult *S. punctatum* were matched by juveniles of inching *S. stimpsoni*. These results show that factors beyond climbing style and predator exposure likely contribute to variation in goby fast-start performance.

Using a Vertically Integrated Project to characterize ovarian follicles in a sexually plastic fish

Heather Ray, MadeLynn Anderson, Mahrissa Clark, Ian Curnutt, Zach Hawkins, Kai Park, Devaleena Pradhan

We designed and implemented a Vertically Integrated Project (VIP) approach to involve a group of undergraduate students in a collective research project to characterize distinct stages indicating oocyte development and maturation. We studied the bluebanded goby, *Lythrypnus dalli*, a bidirectionally hermaphroditic fish that can be used to study mechanisms pertaining to oogenesis in adult females and during the process of adult sex change. As a first step, we characterized the ovarian cycle through gross morphology and histological examination of thin sections of ovaries from naturally cycling wild-collected adults. We separated the ovary into 4 phases over the reproductive cycle and characterized five distinct follicle stages. The early phases (I-2) contained mostly early-stage follicles (I, II) while later phases (3,4) were dominated by late-stage follicles (IV-V). The numbers of stage IV follicles were most prevalent in phases 3 and 4 while stage V follicles were only found in phase 4 ovaries. Additionally, there were notable differences in interstitial space between phases 2 and 3. These data will identify if *L. dalli* have similar patterns of oocyte development as seen in other teleosts. Future studies will investigate molecular and cellular mechanisms regulating oogenesis within a VIP framework. More senior undergraduate students and graduate students will participate in long-term research through increased project ownership and mentorship of incoming students.

Using linguistic analysis to assess anti-predator combinatorial calls in red-winged blackbirds

Katelyn Ray, Katharine Maurer, Mark Hauber, Sharon Gill

Diverse taxa use alarm calls to communicate threats to reproductive success. In many species, individuals produce the same note repeatedly in the presence of predators, with variation in rate or structure communicating risk. However, some combinatorial calls, in which individuals combine multiple calls with specific syntax, may communicate more complex information, but are poorly understood in part due to analytical challenges of identifying predictable sequences. We tested the hypothesis that red-winged blackbirds produce combinatorial alarm calls in response to nest threats, including avian predators and brood parasites. Red-winged blackbirds recognize these threats and produce multiple alarm call types during nest defense, but whether they combine calls in predictable sequences is unknown. We recorded blackbird alarm calls to taxidermic models of nest threats and used the linguistic method of multiple distinctive collocation analysis (MDCA) to test if particular call sequences were more common than chance. MDCA revealed a significant attraction between two call types (check-see2; attraction = 1.43, $P = 0.05$). These results suggest the presence of an order-specific combination used during high threat scenarios. Combinatorial calls and the use of syntax among birds remain poorly understood, and our work contributes to a growing body of evidence that this complex communication system evolved in diverse lineages of avian and mammalian species.

Polar Pelts: Morphology and Thermal Function of the Pelts of Weddell Seals

Stella Raymond, Dana Twisk, Allyson Taylor, Heather Liwanag

Weddell seals (*Leptonychotes weddellii*) are the southernmost breeding mammal on Earth and therefore face extreme conditions from birth. Initially, these Antarctic pups possess no substantial blubber layer. Unlike many phocid (true seal) pups, Weddell seals begin swimming in polar waters at around two weeks of age, relying primarily on their lanugo (neonatal) fur for thermoregulation. Because seawater is 25x more conductive than air, this is thermally challenging for young pups. We hypothesized that lanugo pelts would be morphologically distinct from adult pelts, and that these differences would make lanugo pelts superior insulators both in air and in water, as compared to adults. To test this, we compared the morphological characteristics (hair length, circularity, and density) and thermal function (thermal resistance) of Weddell seal lanugo ($n=6$) and adult pelts ($n=5$) in air and water. We found that lanugo hairs are longer and more circular compared to adult hairs. Lanugo pelts also had a higher fur density than adult pelts. As expected, both pelt types had reduced thermal resistance in water compared to air. Additionally, neonate pelts had higher thermal resistance than adult pelts in both conditions. These results demonstrate the importance of the lanugo coat for thermoregulation in Weddell seal pups, while they develop their blubber layer. This has implications for how climatic changes that accelerate melting of the fast ice could impact pup survival.

Leopard Shark Teeth Series Reveal Life History Patterns with Stable Isotopes

Angelique Rea, Sora Kim, Jonathon Kuntz

The Leopard Shark (*Triakis semifasciata*) is experiencing population declines in Northern California, highlighting the need for conservation initiatives; however, the life history of highly mobile organisms is difficult to elucidate. Stable isotope analysis (SIA) has been used to understand shark ecology across various timescales depending on the tissue analyzed. Horizontal rows of Leopard Shark teeth are lost every 40 - 55 days, with six to seven teeth representing a year-long time series. The isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of dentine in teeth reflects the dietary sources of an individual, which vary between estuarine and marine habitats. Thus, for the Leopard Shark, teeth series can represent seasonal movement ecology. To understand connectivity between Northern California Leopard Shark subpopulations, we collected individuals from four regions in close proximity: Lower South San Francisco Bay ($n = 9$); Drake's Estero State Marine Conservation Area ($n = 10$); Tomales Bay ($n = 5$); Bodega Bay ($n = 5$). After dissection, teeth were demineralized, and homogenized before being analyzed for $\delta^{13}C$ and $\delta^{15}N$ on an Elemental-Analyzer coupled to an Isotope Ratio Mass Spectrometer. While we found some regional overlap in isotopic niche, divergence of isotopic niche between brackish and marine dominated regions is evident. In addition, isotopic teeth time series can help us understand how long individuals were spending in each region, aiding in future conservation and management decisions.

Nutrient-sensing genes mediate resource allocation in Japanese quails

Gebrehaweria Reda, Ádám Lendvai, Levente Czeglédi, Sawadi Ndunguru, Brigitta Csernus

Resource allocation in organisms is monitored by nutrient-sensing pathways that can trigger physiological changes or alter gene expression. The mechanistic target of rapamycin (mTOR) pathway has been suggested to play a role in adaptive plasticity, enabling individuals to re-allocate resources when faced with a limited energy budget. However, how resource allocation is regulated through the expression of genes governing this pathway and their effects on fitness remains understudied, particularly in birds. We used dietary restriction (DR) to simulate resource depletion and examined its effects on body mass, reproduction and gene expression in Japanese quails. Quails were subjected to ad libitum (ADL) feeding or 20%, 30%, and 40% restriction levels for two weeks. All restricted groups exhibited reduced body mass, whereas reductions in the number and mass of eggs were observed only under more

severe restrictions. DR led to decreased expression of mTOR and insulin-like growth factor 1 (IGF1), whereas the ribosomal protein S6 kinase 1 (RPS6K1) and autophagy-related genes (ATG9A and ATG5) were upregulated. Regardless of the treatment, proportionally higher reproductive investment was associated with individual variation in mTOR expression. These findings reveal the connection between resource allocation and the expression of mTOR and related genes in this pathway.

Elastic swing initiation during locomotion

Brandon Reder, Nicolai Konow, Andrew Biewener

Tendons transmit muscle force to bones, store energy to quickly release it via recoil and augment joint rotational velocity. Tendon length in Tibialis Anterior (TA), a foot dorsiflexor, has been estimated during human locomotion but the time-varying forces transmitted to the ankle remain unknown. Using tendon force and ankle kinematics from treadmill-locomoting Guinea fowl (*Numida meleagris*), we tested the hypothesis that TA tendon recoil assists swing initiation. We predicted TA force rise in late stance followed by rapid ankle dorsiflexion and simultaneous TA force decay. We also predicted TA force decay and foot dorsiflexion velocity to be greatest for incline running, intermediate for incline walking and level running, and lowest for level walking. Tendon force peaked in late stance and decayed during foot dorsiflexion in early swing. As hypothesized, ankle dorsiflexion velocity decreased from incline running ($1047 \pm 269^\circ/\text{s}$; mean $\pm$ S.D.) to level walking ($455 \pm 174^\circ/\text{s}$), with statistically significant differences between all conditions except for level and incline walking (Tukey's test, $\alpha=0.095$, $p=0.072$). Contrary to our hypothesis, TA force decay for level walking (12.19 ± 2.70 N) exceeded level running (8.66 ± 5.94 N), incline running (6.91 ± 4.04 N), and incline walking (5.54 ± 1.86 N). Our findings add to the evidence of tendon elastic recoil augmenting joint rotational velocity, in this case aiding safe foot clearance during fast and incline locomotion.

Investigating high morphological variation in the dermal skeleton of *Lepidochelys kempii*.

Amanda Redman, Kathryn Kavanagh

The Kemp's ridley (*Lepidochelys kempii*) is the smallest and most endangered sea turtle. The species suffered >90% population decline between 1950-1975 but has slowly rebounded with increased protection. We examined skeletal and genetic variation in an opportunistic sample of ~300 cold-stunned individuals from Cape Cod, Massachusetts. We found >40% of the samples had unusual scute and scale variations, a large deviation from the reported species phenotype. Skeletal variations, often asymmetric, involve one or more regions on an individual. Alternate hypotheses to explain the variation include disrupted genetics such as hybridization, developmental genetic patterning anomalies, or physical pressure during critical periods of embryogenesis. Anecdotal reports suggested a high rate of hybridization, but results of RAD-seq analysis on 95 morphologically variable samples found no evidence of hybridization; the genetic outliers were not concordant with morphological outliers. Based on known network dynamics during the embryonic patterning of the carapace, we expected that developmental patterning anomalies would result in predictable suites of variation. The randomness of the expressed variations and their asymmetry suggests a simple developmental regulatory change is not sufficient to explain our results. Environmental causes of sea turtle skeletal variation are not well understood but developmental models introducing perturbations may prove to be a useful tool. Understanding the underlying mechanisms driving scutation variation has broader ecological implications for this recovering species and sea turtle biology in general.

Microplastic Analysis in the Appalachian Wilderness

Cierra Reed, Jason Davis

One of the most pressing concerns facing our environment is plastic pollution. Though efforts have been made to reduce the amount of plastic in our ecosystem, this type of pollution still presents a tremendous, almost omnipresent, threat. Microplastics are one of the most dangerous forms of plastic pollution as their small size allows them both to be broadly distributed across a range of habitats and to readily penetrate tissues resulting in digestive

complications, chemical poisoning, and cell degradation. Though these pollutants are a topic of much research, there remain areas and aspects in which they are relatively understudied. One such area includes the diverse waterways of Appalachia, and far southwestern Virginia in particular, a vast and ecologically diverse region home to some of the world's most unique plant and animal species. This study seeks to provide insight into how microplastic distribution and type differ across varying levels of disturbance relative to the features of particular water sources including speed, turbidity, pH, and temperature. Samples were collected from a range of waterways in the Appalachian region and then analyzed through a combination of spectroscopy, microscopy and chemical analysis, with a focus on cataloguing the various types and sizes of plastics in these water sources and relating those to the macrofeatures of those waterways. We hypothesize that waters with increased turbidity and heat might experience lower levels of plastic pollution.

Future of Fin Filmography: A novel system to record and analyze fin movements.

Makenzie Reed, Deeksha Seth, Devaleena Pradhan

Parental care quality and quantity are important for reproductive success. The bluebanded goby, *Lythrypnus dalli*, is a harem marine fish in which the male performs parental care. This species has two prominent parental care behaviors, fanning using fins, and rubbing by moving the body in multiple orientations. While this behavior has been described and documented previously, the precise mechanisms of fin movement and body orientation have not been described biomechanically. Our goal is to model and describe these behaviors in 2D and 3D. Preliminary work identified that caudal and pectoral fins move utilizing different fin shapes and speeds lasting 1-3 secs. We then developed a novel imaging apparatus in collaboration with an engineering design team. This apparatus allows two high-speed cameras to be mounted in orthogonal positions for 3D viewing. Additionally, the apparatus is adjustable to record different locations within and between tanks, while calibrations on the apparatus allow for repeatable and reliable measurements. Finally, the apparatus includes a new nest tube design which is clear on one side to allow for side view recording. By understanding the baseline mechanisms of animal movement, we are able to understand how changes in behavioral regulation alter behavior performances important for reproductive success. In future, we will perform in vivo pharmacological manipulations to alter endocrine pathways to better understand the mechanisms of precise fin kinematics to help or hinder reproductive success.

Seasonal variation in thermal tolerance and hypoxia tolerance of a threatened minnow and a congener

Jessica Reemeyer, Lauren Chapman

Freshwater organisms face multiple threats to their ecosystems, including warming associated with climate change and low dissolved oxygen (environmental hypoxia), which increasingly co-occur and likely interact because both affect aerobic metabolism. Understanding tolerance thresholds for these environmental stressors as well as the plasticity of responses is key for informing conservation of imperiled species. In this study, we measured physiological performance of two species: one federally listed as Threatened in Canada (Pugnose Shiner, *Miniellus anogenus*) and a non-imperiled congener (Blackchin Shiner, *Miniellus heterolepis*). Routine metabolic rate, hypoxia tolerance, and upper thermal tolerance were measured streamside over a period of five months to capture seasonal acclimatization (plasticity) and to quantify safety margins (difference between the stressor threshold and the temperature or dissolved oxygen in the environment). These nonlethal experiments were performed in the field on fish directly after collection, which allowed fish to recover and be returned to the site of capture. and will therefore inform on the environmental tolerances of wild fish as well as the seasonal plasticity in these traits, and how vulnerable these fish are to climate warming and increasing hypoxia. The comparative framework (imperiled versus non-imperiled congeners) provides insight into drivers of imperilment and the value of surrogate species as a conservation tool. We found that the Threatened Pugnose Shiner exhibited a higher level of thermal vulnerability than the

non-imperiled Blackchin Shiner. We conclude that researchers should be cautious when using surrogate species to inform tolerance limits of imperiled species, as they may represent overestimates of true tolerance.

Methods of estimating maximum oxygen uptake by fish—different results, biological bases, and uses

Bernard Rees, Jessica Reemeyer, Samantha Brieske, Sandra Binning, Timothy Clark, Jeremy De-Bonville, Rachel Eisenberg, Graham Raby, Jodie Rummer, Yangfan Zhang

The maximum rate at which animals can take up oxygen from their environment is an important feature of their physiology and ecology. In fishes, maximum oxygen uptake rate ($\dot{M}O_2$ -max) is commonly quantified by measuring $\dot{M}O_2$ either during swimming at increasing speeds or immediately after an exhaustive chase. Recent studies that apply both techniques to the same individuals show that these two methods typically yield different estimates of $\dot{M}O_2$ -max. Furthermore, in many cases, the order of individual $\dot{M}O_2$ -max within a group is not repeatable when measured by these two methods, that is a fish that has a high $\dot{M}O_2$ during swimming does not necessarily have a high $\dot{M}O_2$ after chasing. This collective data analysis explores methodological and biological reasons for these differences, as well as recent advances that could enhance the repeatability of $\dot{M}O_2$ -max. One explanation for the lack of repeatability is that the traditional methods measure different traits, each with their own behavioral, biomechanical, and physiological determinants. Therefore, each method is suitable to address different sets of questions in fish biology and they are not necessarily interchangeable with one another. Recommendations are to match the method used to determine $\dot{M}O_2$ -max to the biological contexts, and, when possible, compare methods for the species of interest in order more fully understand of the causes and consequences of variation in oxygen uptake among and within fishes.

An investigation into the physiology and ecology of the slug millipede, *Petaserpes cryptocephalus*

Corey Reese, Matthew Huang, Sunghwan Jung, Brian Lovett, Mary Salcedo

Arthropods can harbor a great deal of chemical complexity to deal with a diversity of behaviors such as predation, mate attraction, or defense mechanisms. Millipedes specifically, generate unique classes of repellants that can range from benzoquinones to entirely novel chemicals. The millipede *Petaserpes cryptocephalus*, couched in the family Polyzoniidae, uses repellent chemicals to deter predaceous ants and other insects using a uniquely viscous secretion, which also acts as a glue, filled with novel, repellent chemicals. Slow-moving, this species is a fungivore and lives in small groups under decomposing logs in forests of the Eastern United States. Poorly documented, little is known of the life history of this millipede and its chemical defenses. As an important arthropod in breaking down detritus, we asked “Can we qualitatively analyze *P. cryptocephalus* and its secretion to create a similar bioinspired insect repellent?” Therefore, in this premiere study, we measured *P. cryptocephalus* through morphological description, locomotion, chemical characterization using FT-IR spectroscopy, rheology, histology, and ecology to gain a better understanding of its biology. We were able to tie these aspects together for a comprehensive understanding of its ecology and physiology. Not only do these results provide a further understanding of how millipedes behave in the environment, they point toward how natural bio-glues and chemical defenses can provide stepping stones to novel insect management, for new ideas in our modern world.

Iron wars over ligand soup: Exploring chemical crosstalk in Symbiodiniaceae - bacteria interactions

Hannah Reich, MacNeill Matthews, Nicole Cunningham, Corinne Richard, Cassidy Stadtfeld, Hayden Wink, The Students of Siderophore Superlab (Haverford-College-Bio300), The TAs of Siderophore Superlab (Haverford-College-Bio300), Kristen Whalen, Elizabeth Harvey

Nutritional exchanges among cnidarians, endosymbiotic dinoflagellates (Family: Symbiodiniaceae), and bacteria enable the ecological dominance

reef-building corals in oligotrophic oceans. In some instances, bacteria can acquire forms of trace elements otherwise inaccessible to their eukaryotic partners. For example, bacterial siderophore (Greek: iron carrier) production of small, low-molecular weight ligands with high Fe^{3+} affinity can facilitate the uptake of insoluble iron. To ascertain the presence of siderophore production in Symbiodiniaceae-associated bacteria, we isolated ~150 strains of bacteria (spanning 25+ genera) from key Symbiodiniaceae cultures and observed widespread siderophore production via a colorimetric Chrome Azurol S (CAS) assay. To evaluate the sensitivity of bacterial growth to host iron stress, isolates of two ‘core’ bacterial genera (*Marinobacter*, *Labrenzia*) were grown on ‘spent’ media (algal filtrate) from algal hosts with symbiotic (*Symbiodinium microadriaticum*) and free-living ecologies (*S. natans*) reared in either iron deplete or replete conditions. Bacteria were grown on the spent *Symbiodinium* media for 12 days and assayed for siderophore production every 4 days. These experiments elucidate a biotic route of iron exchange as well as the abiotic triggers for its activation. Attaining a multidisciplinary understanding of iron exchanges within the coral holobiont is a critical for identifying biogeochemical scenarios that exacerbate coral bleaching responses.

Individual-group feedbacks and the role of perception in a simulated communication network

Michael Reichert, Barney Luttbeg, Elizabeth Hobson

Communication takes place within a network of multiple signalers and receivers. Social network analyses (SNA) provide tools to understand how an individual's social positioning affects group dynamics, and the subsequent biological consequences. However, SNA has rarely been applied to communication, in part due to the logistical difficulties of monitoring communication networks in the field. We therefore generated a simulated communication network to obtain insights into how variation in individual communication behaviors generates social structure, and how social structure feeds back onto individual communication interactions. We simulated competitive acoustic signaling interactions among chorusing individuals and varied the chorus density, individual hearing sensitivity, attention and other variables to examine effects on calling output and connections among competitors. Our findings indicate an important role of perception and cognition on the structure of communication networks. When hearing was more sensitive, communication networks were more saturated. Individuals' signaling behavior also affected their position within communication networks, but the strength of these effects decreased with increasing chorus size. Physical proximity to competitors played an important role, but a distinctive communication network structure emerged when signal active space was limited. In sum, our model generates many novel predictions about communication networks that can be tested experimentally, and we identify important assumptions about information processing in complex communication scenarios that remain to be investigated.

Syntax in the vocal communication of a wild songbird

Emma Reinhardt, Keith Sockman

A vocalization has syntax if the order of unbroken sound-traces, called syllables, alters receiver behavior. However, it is unclear the extent to which syntax occurs outside of communication in humans, for whom the order of words, such as those in this abstract, affects the signal. The individual songs of Lincoln's sparrows (*Melospiza lincolnii*) contain several syllable types produced in a predictable order. Each syllable type may be reproduced in rapid succession to form a trill. Trills can vary in their performance, a measure of a relationship between frequency bandwidth and syllable-production rate that is purportedly constrained by a biomechanical trade-off. To examine whether syllable order affects receiver behavior, we exposed wild male Lincoln's sparrows to songs of two digitally manipulated syllable type orders: natural and randomized. Order did not appear to affect subjects' non-vocal agonistic displays. However, compared to natural order, randomized order reduced the post-playback latency and frequency bandwidth (and therefore performance) of subjects' trills. These results show syntax in the vocal communication of a wild songbird, in that the order of vocalized syllables affected receiver behavior. However they do not yet reveal a clear adaptive basis for

syntax. Future studies are required to elucidate whether syntax affects, for example, the salience, valence, origin, or association of the signal and how receiver responses may affect the signaler.

Male competition signaling in Japanese rhinoceros beetles

Zoe Reinhold, Brook Swanson

Japanese rhinoceros beetles *Tropoxylum dicotomus* are distinguished by large, pronged horns extending from the heads of the males. Male beetles use these horns to battle over females and sap feeding territories on trees. These exaggerated structures likely evolved as weapons or as a signal to other beetles of the resource holding potential of an individual. To understand how these structures are used in resolving competition, we staged a series of interactions between males. Half of the beetles were calorie restricted to manipulate condition and trials were conducted with beetles both size matched, and with pairings made by random. Winners and losers were tracked for each fight and behavioral sequences were documented and analyzed. Most interactions did not end with physical fights between the beetles, instead there were contacts and what appeared to be assessment, then one of the beetles retreated and the other claimed the territory. However, in some cases, the horns were used to throw the other beetle from the territory. Both horn size and body size, but not male condition were found to be significant factors predicting fight outcome.

Competition has greatest effect on behavioral thermoregulation in high quality thermal environments

Julie Rej, Alex Gunderson, Eric Riddell

Temperatures are rising at an alarming rate, forcing species to behaviorally thermoregulate by seeking cooler microclimates. Two factors that dictate the costs and benefits of behavioral thermoregulation are the spatial structure of available microclimates and the presence of competitors. The effects of these factors on thermoregulation have been explored extensively in isolation, but we know little about how they interact. To address this gap in our knowledge, we used an individual-based model to determine how competition for space hinders thermoregulation, and if the effects of competition depend on the spatial arrangement of the thermal environment. We found that an even distribution of shade had the greatest impact on thermoregulatory ability when a competitor was present. An even distribution of shade increases the quality of a thermal environment which leads to an improved thermoregulation ability, but our results show that the presence of a competitor can negate these effects. Additionally, there is an increased energetic cost to maintaining preferred temperatures in the presence of a competitor. Our results demonstrate the importance of considering species interactions in evaluating thermoregulatory potential and costs in current and future environments.

Growth plate specific genes found using the unusual ossification of the metatarsal and pisiform

Philip Reno, Sungdae Park, Kelsey Kjosness, Sherrie Wallace, Sarah Doelp, Maria Biancaniello, Douglas Menke

We utilize the substantial variation in the location of growth plates within the mammalian skeleton to identify genes specific to growth plate patterning. Third metatarsals (MT3) form only a single growth plate located at the distal end, and the pisiform (in non-human mammals) forms an active growth plate unlike the other carpals. Comparison between the growth plates of mouse distal MT3 and pisiform to generalized endochondral ossification in proximal MT3 and other carpals controls for the effects of age, systemic growth factors, and biomechanical environment. We identify numerous differentially expressed genes (DEGs) using RNA-seq in 4- and 9-day old mice. DEGs from the MT3 are disproportionately represented in gene ontology (GO) categories including limb development and growth and factors associated with the *Fzd8-Ror2-Wnt5a* pathway. The Pisiform-Carpal comparison includes GO terms associated with skeletal and cartilage development and abnormalities of the carpal bones. A limited set of DEGs shared by both the MT3 and Pisiform-Carpal datasets includes *Wnt5a*. *Wnt5a* is expressed in the perichondrium in both ends of the MT3 and the pisiform, but expression at the columnar/hypertrophic cell boundary is stronger in the distal MT3 and pisiform growth plates.

The related *Wnt10a* gene is solely expressed in the bone collar of the distal MT3 and pisiform. This confirms previously identified roles for Wnts in regulating osteogenesis and cell polarity within the growth plate.

Thermoregulatory and Immune Responses to Ebola-like Particles in Thirteen-Lined Ground Squirrels

Matthew Repke, Lisa Powers, Luis Viquez-R, Briston Bayle, Sara Talmage, Isabel Steinberg, DeeAnn Reeder, Ken Field

Hibernation induces a significant physiological change through decreased metabolism, respiration, heart rate, and immune responses. In this study, we sought to understand how antiviral immune responses were affected by hibernation, using the thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*) as a model organism. Ebola virus-like particles (eVLPs, which present Ebola antigens but are not infectious) were used to trigger an antiviral response. Twenty-four squirrels were housed in a hibernation chamber at 4°C, while eighteen were maintained at room temperature (21°C). The squirrels were given adjuvants, which are biological molecules that help mount a more robust immune response and probe different immune pathways. Blood was drawn before immunization and 35 days later to measure the anti-Ebolavirus antibodies. Additionally, subdermal temperature loggers recorded body temperature every ten minutes throughout the study. Analysis of the thermoregulatory response of both hibernating and non-hibernating squirrels to eVLPs is ongoing. Additional immune response measures, such as eVLP-specific T cell proliferation assays, are being used to assess the type of cell-mediated immune response. It is predicted that hibernating squirrels will have a reduced humoral immune response and therefore have lower antibody titers than non-hibernating squirrels due to the likely contraction of immune responses during hibernation. By comparing the thermoregulatory responses to the magnitude and type of immune response in each squirrel, we will determine how different behavioral responses affect antiviral immunity.

Abundant phytoplankton may mitigate impact of high temperature on growth of sea star larvae

Alexander Reyna, Sophie George

Increase in ocean temperatures is impacting marine invertebrates worldwide. However, little is known about the effects of high temperatures on the larval stages of most species. The purpose of this study was to determine the effects of rising temperatures on growth and development of *Pisaster ochraceus* larvae. Larvae were kept at 22°C for 13 days then transferred to temperatures of between 15°C to 18°C for a further 15 days. Controls consisted of larvae kept at temperatures between 9°C to 11°C throughout the experiment. For both temperature treatments, samples were collected for 23, 31, and 37-day-old larvae and ImageJ used to measure total larval length, total larval width, stomach width and length, and ciliated band length. At high temperatures, 23- and 31-day-old larvae were significantly larger, with larger stomachs and longer ciliated bands. Most of these differences disappeared among 37-day old larvae from both temperature treatments but remained significant for total larval width and ciliated band length. Longer ciliated bands may have allowed for increased feeding, faster growth and development. These results suggest that if phytoplankton is abundant in the water column, *P. ochraceus* larvae may be able to survive marine heat waves.

Surveys that prioritize many sites have better outcomes: a case on finding the Big Black Rocksnail

Calvin Rezac, Robert Ellwanger, Samantha Donohoo, Paul Harfield, Ashely Ruppel, Matthew Wagner, Nathan Whelan

Freshwater gastropods are among the most imperiled organisms on the planet. Research guidelines and policy recommendations are needed so surveys are designed to provide the best information possible for conservation. To develop guidelines and recommendations, we examined the case of *Lithasia hubrichti*, a freshwater gastropod endemic to the Big Black River in Mississippi, USA which was last seen in 1965. In 2022, mollusk surveys resulted in finding putative *L. hubrichti* alive. Conchological and radular morphological comparisons among 2022 and historical samples confirmed

species identification. Subsequently, genomic analyses indicated that the species has persisted with a large population size for many years, and that the rediscovered population has an effective population size of approximately 8,000,000. Although this value is high, quantitative sampling at the time of rediscovery indicates that the value is plausible. Thus, *L. hubrichti* was locally abundant at the site of rediscovery, but unsampled and overlooked, which led to a premature extinction declaration. Given our findings, and other recent freshwater mollusk rediscoveries, we conclude that freshwater gastropod surveys should emphasize sampling as many sites as possible when targeting rare species, rather than expending high sampling effort at a small number of sites. We also advocate for policies that encourage partnerships with private landowners and surveys that include as many taxa as possible, both of which were required to rediscover *L. hubrichti*.

Mitochondrial capacity in two Mimidae species: a migratory and non-migratory comparison

Emma Rhodes, Kang Nian Yap, Geoffrey Hill, Wendy Hood

Although mitochondria produce 90% of the energy that fuels migration, their role in the evolution of life histories that include migration is essentially unstudied. We collected 11 Gray Catbirds (*Dumetella carolinensis*, GRCA), which are migratory, and 10 Northern Mockingbirds (*Mimus polyglottos*, NOMO), which are non-migratory, in Coastal Alabama during fall migration. Our goal was to investigate mitochondrial capacity and physiology of both a migratory and non-migratory species from the same avian family. Because they are migratory, we predicted that GRCA would have higher maximum mitochondrial respiration (state 3), basal respiration (state 4), respiratory control ratio (RCR) (state 3/state 4), and mitochondrial density compared to NOMO. We found no significant differences in state 3, state 4, or RCR data between the two groups. Additionally, while not significant, the NOMO respiration data trended higher overall than GRCA. GRCA had higher mitochondrial density than NOMO although this was not significant ($p = 0.07$) until fat score was included in the linear model ($p = 0.05$). Our study demonstrates that variable life history traits may determine mitochondrial capacity other than migration. While NOMO are non-migratory, they are exceptionally active birds, engaging in flapping flight throughout the day.

Cognitive variation and its genetic basis in hybridizing chickadees

Amber Rice, Georgy Semenov, Joan Marie Spinelli, Austin Russell, Noel Martinez, Haley Kenyon, Alex Huynh, Robin Johnsson, Timothy Roth, Scott Taylor

When hybridization occurs, selection against hybrids reduces gene flow and maintains species barriers. Although learning and memory are known to play important roles in preventing hybridization, whether they contribute to selection against hybrids is less understood. Further, although hybridization is widespread and cognition is linked to fitness in many taxa, whether and how hybridization affects cognition remains unclear. Black-capped (*Poecile atricapillus*) and Carolina chickadees (*P. carolinensis*) naturally hybridize, and also rely on learning and memory to cache and retrieve food as an adaptation for overwinter survival. Previous research on wild-caught adult chickadees indicated that hybrid chickadees show reduced performance in multiple cognitive tests. Here, we asked whether hybrid chickadees still differ from their parental species in cognitive ability when reared under common conditions. We therefore reared hybrid and parental species chickadees under standardized environmental conditions and tested their performance on a series of cognitive tests, including associative spatial learning, reversal learning, problem solving, and response to novelty. Additionally, we sequenced the genomes of the captive-reared chickadees, so that we could test for associations between SNPs and cognitive performance. Our results provide insight into how hybridization may influence cognition, and how genomic variation may be associated with cognitive performance.

Dolphin Reproductive Evolution Explored Through In Vitro Semen Analysis

Jacqueline Rich, Jonathan Cowart, Dara Orbach

The biotic and abiotic features of the vaginal environment are important in regulating sperm cell function and selection. Adapting to mating in an aquatic environment has imposed unique constraints on cetacean (whales, dolphins, and porpoises) vaginal features compared to their terrestrial counterparts. Cetaceans possess vaginal folds, protrusions of the vaginal wall into the lumen, which have been hypothesized to promote sperm viability by preventing salt-water from reaching the cervix and uterus. The abiotic vaginal environment, such as pH, may also affect sperm viability and behavior in cetaceans. We explored the effects of biotic and abiotic vaginal features of common bottlenose dolphins (*Tursiops truncatus*) on sperm function. Two semen samples were collected voluntarily from each of five adult male dolphins housed at facilities in Florida. Each sample was exposed to biologically relevant solutions of varying pH and salinity immediately after collection, and a computer-assisted sperm analysis (CASA) software was used to objectively analyze sperm motility and kinematic parameters. Semen samples were formalin-fixed to measure sperm integrity and viability post-hoc. The data support the saltwater exclusion hypothesis for the function of cetacean vaginal folds and indicate variable intra- and inter-male effects of pH, suggesting that vaginal pH may be a mechanism to modulate sperm competition in cetaceans. In vitro semen analysis within varying chemical environments has begun to enhance our understanding of reproductive evolution in aquatic systems.

Rhythmic chew cycles with distinct fast and slow phases are ancestral to gnathostomes

Brian Richard, Meghan Spence, Mateo Rull-Garza, Yonas Roba, Daniel Schwarz, Jason Ramsay, J.D. Laurence-Chasen, Callum Ross, Nicolai Konow

The task of processing food before swallowing is critical to the survival of most gnathostomes. Tetrapods have chew cycles containing four distinct phases (fast close, slow close, slow open, and fast open) with slow close being the power-stroke where food is mechanically processed. Maintaining chew phases should facilitate efficiency by introducing rhythmicity, whilst aiding in more controlled and thus safer occlusion, and has been hypothesized to be unique to mammals. Basal aquatic-feeding vertebrates also process food intraorally, but whether these taxa partition each chew cycle into distinct phases remains unknown. Here, we show that chew cycles in anamniotes are also partitioned into at least three, sometimes four, distinct phases that are as rhythmic as those of mammals, with a lack of distinct phases sometimes occurring during gape opening. Basal fishes and aquatic feeding amphibians partition variability into the initial fast close phase, better resembling mammals than basal amniotes (lepidosaurs). Surprisingly, terrestrial-feeding salamanders demonstrate a third pattern with the second closing phase, near-contact, being faster than the first. Our results suggest that rhythmic chewing with cycles containing distinct fast and slow phases is ancestral to gnathostomes. There appears to have been a complex evolutionary history involving changes in cycle phase duration and jaw velocity through gnathostome phylogeny. This history leaves unclear the mechanical and sensorimotor underpinnings of injury-preventing occlusal precision and energetic efficiency during food processing.

Modelling the effects of intrinsic muscle properties on the coordination of human reaching

Christopher Richards, Tiina Murtola

Goal-directed reaching is an intricate task involving coordination of several muscles enabling us to perform crucial daily activities. We aim to investigate how the neuromuscular system achieves accurate and robust reaching. Specifically, we ask how muscle intrinsic force, length, velocity and activation properties impact reaching performance. We developed a conceptual planar (2D) reaching model with shoulder, elbow and wrist joints, each driven by a pair of Hill-type muscle models. To coordinate muscle excitations, a simple PD controller was used to grossly mimic sensorimotor control. Our model broadly replicated natural reaching behaviour. For "slow" reaches (2s time limit), we found reaching to be a minimally demanding task in terms of muscle

mechanics; it only required 10 – 20% fmax. Additionally, we tested the model in several configurations where we altered the complexity of activation dynamics and the inclusion/exclusion of force-length-velocity properties. We found that force-velocity properties are necessary for stable reaching. Moreover, unlike simplistic 1st order activation dynamics, more realistic 3rd order activation required a “co-contraction” coordination strategy to compensate for neuromechanical delays not present in the 1st order model. We will also explore muscle dynamics during more demanding reaching tasks such as reaching at high speed or in the presence of perturbations. Thus far, our modelling provides evidence that intrinsic muscle properties impact coordination strategy and reaching performance.

Striking gold: successes in rare species detection using eDNA, acoustics, and visual surveys

Corinne Richards-Zawacki, Allie Byrne, Caren Goldberg, Justin Kitzes, Sam Lapp, Jamie Voyles

Biodiversity loss is one of the most serious environmental crises of our time. As species become rare, distinguishing continued declines and extirpations from nascent expansions and recoveries becomes increasingly painstaking – how do we best find the needles in the haystack across space and time to monitor population health or the success of conservation interventions? For many taxa, traditional methods of species detection are inefficient at best and destructive at worst. Here we demonstrate the success of two non-invasive methods for species detection, environmental DNA (eDNA) and acoustics, as a supplement to traditional visual encounters surveys in amphibians, the most threatened vertebrate group. The spread of the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) resulted in dramatic declines and changes to Panama’s amphibian communities in the early 2000s. The two critically endangered Panamanian golden frogs (*Atelopus varius* and *A. zeteki*) were among the hardest hit. For years these iconic frogs were thought to be extinct but eventually a handful of individuals were spotted in a few of their historic localities. Here we report on successes in detecting these rare frogs using eDNA, acoustics, and visual surveys and discuss how these complementary methods can help us understand how some individuals and populations persist in the face of the now endemic pathogen while others have not.

Comparing fecal and circulating hormone levels in an endangered snake across its active season

Melanie Richter, Beth Roberts, Mark Sandfoss, Steve Reichling

There are clear differences in the seasonal hormone patterns exhibited by animals utilizing different reproduction strategies. However, reptiles are sorely underrepresented in endocrinology studies, particularly oviparous snakes. The endangered Louisiana pinesnake (*Pituophis ruthveni*) is a large, egg-laying colubrid found in the southeastern United States. The Memphis Zoo is part of a multi-zoo captive breeding/release program attempting to save this enigmatic species. The zoo has a large breeding population of adults that are housed under conditions designed to mimic the natural environment. We utilized this population to collect fecal and blood samples from adult male and female snakes (20.23) throughout the calendar year to outline the natural hormonal variation in this species. After validating extraction methods and assays, we measured four hormones (corticosterone, testosterone, estradiol, and progesterone) in samples collected (200+ fecal and 600+ blood samples) over two years. We found clear differences in patterns exhibited by males and females in both sample types. Comparing differences and similarities between the two sample types we can determine how different sample time scales can affect observed hormone levels. We are also able to use reproductive physiological markers to determine how intra-individual variation in hormone levels are reflected in reproductive status. This study will help us better understand the seasonal hormonal patterns in egg-laying snakes, and to aid in the recovery of this endangered species.

How temperature decouples a universal trade-offs between gas exchange and water loss in amphibians

Eric Riddell

Trade-offs between linked traits are a fundamental aspect of biology and influence complex phenotypes, such as life history, behavior, and physiology. Comparative studies across diverse taxa have indicated that the relationship between gas exchange and water loss rate is a universal trade-off among terrestrial organisms. This physiological trade-off occurs due to the reliance on moist respiratory surfaces for gas exchange; thereby necessitating water loss to fuel the energetic requirements for work. However, the role of temperature in shaping the trade-off between these two linked traits remains underexplored. Here, we use a dataset spanning several species of salamander from different experiments in our lab to understand the effect of temperature on the universal trade-off between gas exchange and water loss. Our analyses indicated that gas exchange and water loss are tightly coupled in these amphibians; however, the residual variation in the relationship is strongly driven by the experimental temperature. We found that individuals lose less water than expected (i.e., positive residuals) at warm temperatures and more water than expected (i.e., negative residuals) at cool temperatures. We then discuss the proximate and ultimate mechanisms that may be driving the variance in the trade-off and extend our findings to the selective factors that many terrestrial organisms face on the planet.

Digital Footprints: What Rapid Evolution of a Genetic Algorithm Reveals About Selectors

Annie Riffée, Birch Ambrose, Jason Davis

The usage of computer algorithms to simulate evolution allows us to rapidly explore complex interactions between a range of genetic and environmental factors as well as the functions and activity of different selective agents. For example, just as the coloring of a butterfly can tell us things about the color vision of a bird, the adaptation of an evolutionary computer model can tell us things about the brains and behaviors of the selectors that are acting upon it—in this case, the humans that we’ve solicited to play a computer game. For this project, we’ve created a system that uses human participants as the selectors on a population of simple digital organisms; over time, the organisms that they are selecting for evolve around the player’s search pattern, leaving us with an end population that gives insight into the participant’s strategies, whether intentional or unintentional. By measuring the emergent patterns of phenotypic and genetic change in populations of these digital organisms (such as mimicry, aposematism, evolution of modulatory genes, etc.) we can make inferences about how individual selectors perceive and process presented information. In this presentation we will discuss our preliminary findings, with a focus on how demographic features and behaviors of human selectors may correlate to patterns of selection and evolutionary output.

Climbing without Feet: Forces and Stability in Vertical Snake Locomotion

Calvin Riiska, Michelle Lee, Joseph Mendelson, Jennifer Rieser

Movements through arboreal habitats are challenging, particularly for limbless animals like snakes that lack appendages that can be crucial for gripping, propulsion, and stabilization. Here we created a simplified model surface to mimic large-diameter trees: a climbing wall consisting of a flat, smooth, vertical surface with force-sensing protrusions that serve as “footholds.” The combination of temporally resolved force data with synchronized 3D kinematic data from marker-based tracking allows us to analyze the distribution and variation of forces applied by the snakes’ bodies. Studying corn snakes (*Pantherophis guttatus*), we find that during ascents, forces over the anterior third of the body are directed strongly downward, with a vertical component often up to 10 times greater than the horizontal. The middle third produces strong vertical forces, but also high lateral forces with magnitudes nearly half the body weight. During descents, the middle and posterior parts of the body apply stronger vertical forces than when ascending. Our results suggest the importance for the anterior part of the body to apply strong vertical forces while the midsection and posterior apply vertical forces and stabilizing lateral forces during ascents while in descents the midsection and tail support more

weight relative to their horizontal output. Further work will investigate how species specialization and surface textures affect physical interactions and overall success of locomotion.

Effects of cavity diameter on nesting preference in *Megachile Rotundata*

Joshua Rinehart, Courtney Grula, Joseph Rinehart, Julia Bowsheer

The alfalfa leafcutting bee (*Megachile Rotundata*) is a solitary cavity nesting bee. In this species the size of the nesting cavity influences body size, sex, survival, number of offspring, and diapause status. While previous studies have examined the effects of cavity size on various metrics in *M. rotundata*, these studies utilized bees that emerged from the commercial standard 7mm diameter cavities. This study is the first to examine how cavity diameter affects nesting preference when bees emerged from different cavity diameters. Our goal was to examine the effects that nest diameter has on *M. rotundata* by manipulating the size of cavities that are available for nesting by bees reared in 5, 7, and 9mm cavity diameters. We provided the bees with nesting cavities that vary in size from three to nine millimeters in one millimeter increments. We measured the following: number of offspring created in each cavity diameter, number of cells created by the position of the cavity, time required for nest completion, and diapause status of offspring. We found that cavity size affected nesting preference and offspring diapause status, but not time to complete the nest or number of cells created by the position of the cavity.

Ontogenetic changes in southern sea otter fur morphology

Kate Riordan, Heather Liwanag, Nicole Thometz, Francesca Batac

Sea otters have the densest fur of any animal, and their fur exhibits morphological adaptations to trap a layer of air for insulation. Newborn sea otters have a natal pelage that they will eventually molt and replace with a pelt resembling the adult pelage. Past studies have investigated the morphology and hair density of adult otter fur, but these characteristics have not been measured for other age classes, including for the natal pelage. This study quantified ontogenetic changes in hair morphology of southern sea otter pelts. We measured guard hair length and circularity, shape of cuticular scales on guard hairs and underhairs, and overall hair density for otter pelts across six age classes: neonate, small pup, large pup, juvenile, subadult, and adult. Neonate and small pup pelts had significantly longer guard hairs than older age classes. Natal pelage guard hairs were similarly shaped but smaller in diameter than adult guard hairs. Hairs of the natal pelage had similar cuticular scale patterns as adult hairs, indicating the importance of this structure for the function of the fur. Natal pelage had a lower hair density than the pelage of older age classes, with the adult pelage exhibiting the highest hair density. Overall, the morphological differences between natal and adult pelage in sea otters suggest functional differences that may make sea otter pups more vulnerable to heat loss.

Effects of ontogeny and oiling on the thermal function of southern sea otter fur

Kate Riordan, Nicole Thometz, Francesca Batac, Heather Liwanag

For most marine mammals, the use of fur as an insulator has been replaced with more streamlined blubber. Yet the sea otter relies on a dense pelage with an air layer for insulation. Adult sea otters have a different type of pelage than younger sea otters that possess natal pelage, and these pelage types differ in morphology. In this study, we investigated the ontogenetic changes in thermal function of southern sea otter (*Enhydra lutris nereis*) pelts in air, in water, and when oiled with crude oil. Pelt thermal conductivity, pelt thickness, and thermal resistance were measured for six age classes: neonate, small pup, large pup, juvenile, subadult, adult. Pelt conductivity was significantly higher for pelts in air than in water, with oiled pelts having the highest conductivities. Oiled pelts had the lowest thermal resistance, which suggests that regardless of age, all sea otters are vulnerable to the effects of oiling. To scale up our laboratory findings, we used a volume-specific geometric model of conductive heat transfer for a simplified sea otter body across age classes and treatments. Neonates, small pups, and large pups are more vulnerable to the effects of

oiling compared to older age classes, due to a higher surface area to volume ratio. Overall, body size and age play a more important role in the thermal abilities of sea otters than previously thought.

Do Rho A pathways play a role in closure of the avian ductus arteriosus (*Gallus gallus*)?

Jessica Rippamonti, Edward Dzialowski

The ductus arteriosus (DA) is an oxygen-sensitive embryonic blood vessel present in all developing amniotes that connects the pulmonary artery to the aorta, shunting blood away from the nonfunctioning lungs and toward the fetal respiratory organ. Permanent ductus closure upon birth or hatch establishes proper separation of pulmonary and systemic circuits in the neonate. Increased arterial PO₂ at birth or hatch stimulates DA closure via smooth muscle contraction. Smooth muscle contraction is governed by myosin light chain kinase (MLCK) stimulating increased cross-bridge cycling and through increases in actin polymerization. The Ras homologous protein family (Rho) of GTPases are involved in calcium sensitization and contraction of vascular smooth muscle. We examined the role of the Rho GTPase pathways through the MLCK pathway and actin polymerization in the closure of the avian DA in late-term chicken (*Gallus gallus*) embryos. Using a Danish Myo Technology wire myograph, DA physiology was examined when exposed to activators and inhibitors of the Rho GTPase pathways. Activation of Rho A pathways with CN01 (0.2 units/ml) produces contraction of the DA. Blocking the Rho pathway with Y-27632 (10 mM) removes the O₂-sensitivity of the vessel, while the vessel is still able to respond to PE (100 mM). This suggests the Rho pathway is involved in maintaining baseline tension and contractile response to O₂. This research was funded by Grant R15HL14887 from NIH-NHLBI.

Opportunities and limitations of eDNA metabarcoding for detecting aquatic and terrestrial organisms

Sakib Tahmid Rishan, Richard Kline, MD Rahman

The earth is struggling to address a terrible biodiversity loss that threatens food supply, health, and safety as well as the decline of precious species and precious genetic diversity. Identifying cryptic species or juveniles using traditional monitoring methods like visual surveys and estimating individuals might be challenging. Environmental DNA (eDNA) is a relatively new technology that has the potential to be a faster, non-invasive, and cost-effective tool for monitoring biodiversity, conservation, and management practices. eDNA has been extracted from materials that are both ancient and present, and its applications range from the identification of individual species to the study of entire ecosystems. In recent years, eDNA has been used more in ecological preservation and conservation studies. However, several technological problems still need to be solved. To get rid of false positives and negatives from current eDNA technologies, the validation and calibration processes must be enhanced at every level. There is a significant need for greater information about the physical and ecological constraints on eDNA use, as well as its synthesis, current state, expected lifespan, and potential modes of movement. Due to the widespread use of eDNA research, it is also essential to assess the extent and breadth of these studies. In this study, we discussed the prospects, challenges, and limitations that are caused by improper usage of eDNA metabarcoding in aquatic and terrestrial species.

How flower longevity and environmental conditions affect epiphytic bacterial abundance

Afagwu Rita-Nkem, Ciara Stewart, Avery Russell

Floral longevity determines when pollinators can visit and thus plays a vital role in plant reproductive ecology. Because floral tissue is metabolically expensive, flower longevity tends to be highly influenced by environmental factors including temperature, humidity, and precipitation. Flowers are often colonized by abundant and diverse epiphytic microbial communities that may also influence metabolic costs of flower maintenance. Yet how environment and flower age interact to influence microbial abundance is unknown, despite the potential importance of these processes for understanding flower microbial community assembly and function. We investigated (1) if floral bacteria

changed in abundance as flowers aged, and (2) whether shorter or longer-lived flowers accumulated bacteria more quickly, (3) and how environmental conditions shaped these patterns. We tagged flower buds of eight plant species over two years to determine flower age from anthesis to senescence. Each day after anthesis, flowers were removed and washed to examine changes in epiphytic bacterial abundance (via plating) and we collected environmental data from a nearby weather station. All species accumulated bacteria as flowers aged, but flowers that senesced quickest accumulated bacteria fastest. Furthermore, higher temperatures negatively affected both floral longevity and microbial growth. Analysis of other environmental effects are pending. Our results suggest that shorter lived flowers may tolerate more bacteria and/or longer-lived flowers may have mechanisms to reduce bacterial colonization and growth.

Heat and repeat: Do wild birds habituate to consecutive thermal challenges?

Atalanta Ritter, Emily Levy, Kimberly Rosvall

As heat waves increase in frequency and intensity, there is an urgent need to understand how organisms respond and potentially adapt to successive thermal challenges. Heat exposure can either habituate or sensitize an animal to future heat. But these hypotheses remain underexplored, especially in temperate bird species, many of which are experiencing population declines. To address this gap, we conducted a pilot experiment heating nestboxes of tree swallows (*Tachycineta bicolor*) for 4 hours when chicks were twelve days old, and again when the same chicks were thirteen days old. To achieve sublethal temperatures, we used air-activated Uniheat packs in experimental nests and contrasted them with control nests that received inert heat packs. After each experimental period, we took blood samples from 3-5 chicks per nest. We measured gene expression of HSP90AA1, a heat shock protein that repairs cellular damage incurred by heat and other stressors. If HSP90AA1 gene expression decreases on the second day of heat, this suggests a habituation response. However, if gene expression increases on the second day, this suggests a sensitization response. Our results will broaden our understanding of avian responses to heat waves, with potential to inform conservation strategies and predictive climate change models.

Swimming with fossils: student research of ammonite locomotion using computation, robotics, and play

Kathleen Ritterbush, Nicholas Hebdon, David Peterman, YunJi Choi, Mikelia Heberer, Garrett Butler, Jay Merrill

Ammonites are squid-like animals that went extinct alongside large dinosaurs but left a spectacular fossil record of seashells on every continent. We study form-function relationships of ammonite conch seashells: how would water flow around the conch allow the animals to travel with speed, agility, or efficiency? Our specimens include fossil replicas and idealized shapes rendered in 3D for computer simulations, and built into robots or low-tech "pool toys" for real-world experimentation and play, respectively. Here we synthesize results from the past five years of work, and frame questions for new student projects. First, "streamlining" is relative; the locomotion challenges of conch shape are size-dependent. We explain the trade-off between lateral compression and viscous drag. Second, flow around conchs is speed-dependent. We show the trade-off between top speed and efficiency. Third, external shape features (ridges and nobs) fine-tune flow challenges of gross conch shape. We highlight eddy and vortex formations. Moving forward, we need to model greater nuance for jet propulsion and animal locomotion to learn how animals might take advantage of their complex conch shapes. Student research opportunities include synthesis of fossil trends in locomotion-constraining shape features; advanced computational fluid dynamics simulation design; boosted reproducibility of experimental and robot models; and savvy trajectory analysis for a three-dimensional seascape.

Deducing the Evolution of Allorecognition and Primordial Immunity in Cnidarians

Alberto Rivera, Andy Baxeavanis

The allorecognition gene complex (ARC) in cnidarians provides a model for understanding the evolution of the primordial immune system in bilaterians. The allorecognition system of the hydroid *Hydractinia symbiolongicarpus* plays an essential role in mediating self vs. non-self recognition in cnidarian colonies. This ability to recognize genetic identity is analogous to the immune system of bilaterian animals. Similarities have been noted between the allorecognition complex in *Hydractinia* and the major histocompatibility complex (MHC) in higher vertebrates, including humans. At the genomic level, we find that both complexes have contiguous stretches of similar, highly duplicated genes, strongly suggesting a homologous relationship between the ARC and MHC. Protein structural prediction methods, such as AlphaFold2, also indicate structural similarity between the proteins encoded within the *Hydractinia* ARC and the MHC. We are currently elucidating the structure of the ARC in a related hydrozoan species (*Podocoryna carnea*) to determine the degree of conservation of the allorecognition complex between *Hydractinia* and *Podocoryna*. Through the use of genome annotation software (BRAKER) and protein structural prediction (AlphaFold2), we identified a number of candidate *Podocoryna* allorecognition proteins. Phylogenetic approaches suggest strong homology between cnidarian allorecognition genes, as well as the conservation of synteny in this gene complex across cnidarians. Future comparative genomics studies will use these findings as a foundation for determining whether there is macrosynteny between the ARC and the bilaterian MHC.

Femoral Gland Gene Expression in Parthenogenetic Lizards of the Genus *Aspidoscelis*

Joshua Rivera, Joseph Rangel, André Luiz Carvalho, Adam Leaché, Matthew Fujita

Chemical communication, facilitated by the secretion and recognition of pheromones, plays a vital role in the behavior of squamate reptiles. These chemical signals are linked to various intraspecific and interspecific interactions, such as kin recognition, mate choice, and aggression. In some cases, pheromone composition may even be linked to speciation. For example, some morphologically conservative lizard radiations exhibit divergence in pheromone composition. Whiptail lizards of the genus *Aspidoscelis* possess femoral glands, which likely play a key role in their chemical communication. However, the composition of the pheromones and the genetic mechanisms regulating expression of the glandular content are not well understood. In this study, we compared the expression of pheromone-related genes between parthenogenetic and sexually reproducing whiptail lizards, anticipating minimal differences due to the presence of pseudocopulatory behavior in all-female parthenogens. We collected lizards in the field, extracted gland chemicals, and excised femoral gland and forelimb tissues for transcriptomic analysis. We used gene ontology and combined pathway analyses to investigate the diversity of proteins associated with glandular tissue and infer potential biochemical pathways. Finally, we performed differential gene expression analyses to compare glandular gene expression between male and female *A. gularis*. Our findings shed light of the genetic machinery that maintains the evolutionarily ubiquitous femoral glands and contributes to our understanding of chemical communication in reptiles, paving the way for future research in this field.

Boat sound's effect on embryonic/juvenile Little Skates' (*Leucoraja erinacea*) auditory sensitivity

Micaela Rivera, Rosland Putland, Lilly Hall, Allen Mensinger

Anthropogenic sound is an increasing problem in marine environments that can impact aquatic animals. While most studies have investigated anthropogenic sounds on adult animals, its impact on embryonic or juvenile animals remains understudied. Sessile organisms are unable to escape sound exposure making them particularly vulnerable to anthropogenic sound. The little skate (*Leucoraja erinacea*) attaches their egg cases to plants or other stationary objects, where embryonic skates develop for up to 9 months prior to hatching. Thus, their fixed position in shallow waters can expose them to

sounds from vessels and/or coastal engineering during their critical developmental window. The impact of boat noise on the auditory sensitivity of little skates were examined prior to hatching and one and four weeks post hatch. Auditory evoked potentials (AEPs) were recorded before and after 15 minutes of boat noise. Preliminary results show that embryonic skates had a threshold shift across all frequencies (100-800 Hz), while juvenile skates had threshold shifts predominantly at lower frequencies (100-300 Hz). These results suggest that the auditory sensitivity of embryonic skates may be more impacted by anthropogenic sound than hatched skates. This study provides an understanding of the auditory sensitivity of developing little skates before and after sound exposure and provides a guide for researchers to further investigate the auditory sensitivity of animals at different developmental stages.

A century long perspective on grey wolf ecology: comparing within and between individual diet.

HyeJoo Ro, Robin Traylor, Daniela Kalthoff, Anmol Sanghu, Sora Kim

Carnivores can shape the ecological dynamics of their communities through foraging patterns, which vary based on resource use and availability. Grey wolves (*Canis lupus*) are a large, social carnivore that inhabit a wide range of environments worldwide. In this study, we explore grey wolf dietary ecology in Sweden over the past c. 100 years, with a focus on individual dietary variation. We used carbon ($\delta^{13}\text{C}$) and nitrogen stable isotope ($\delta^{15}\text{N}$) compositions to investigate diet and trophic dynamics. We collected stable isotope data from grey wolves, and their potential prey including herbivores (cervids, lagomorphs), omnivores (foxes, badgers), and marine mammals (seals). The isotope values ranged widely for both grey wolves and prey species, which suggests that grey wolves targeted a diverse set of prey species, and there were environmental (i.e., baseline) differences. For finer scale resolution on individual diet, we serially sampled dental collagen from each individual and found that each wolf varied among individuals suggesting that individualistic prey choice is important when assessing population diet. Taken together, the variations in isotope compositions among and within individuals support grey wolves as generalist predators that make a variety of prey choices. Other variables we considered that influence diet include sex and ontogeny. Understanding trends in the variation of apex predator diets can give insight to complex food webs and how they could be impacted by environmental change.

Multiple lizard phenotypes respond to the latitudinal thermal gradient via countergradient variation

Travis Robbins, Tiffany Hegdahl, Brandon Wolfsohn, Benjamin Haussmann, Stephanie Cromwell, Paul Ayayee

Temperature influences mechanisms associated with both biotic and abiotic factors across all organismal and ecological organizational levels. Examining these mechanisms at the organismal level, where natural selection occurs, is critical to understanding evolution and how organisms adapt to changing environments. At the organismal level, the thermal environment influences biochemical processes that determine physiology, morphology, behavior, reproduction, performance and ultimately fitness. Although we have some knowledge about how individual traits respond to changes in the thermal environment, we do not yet understand how multiple traits respond in concert to result in the phenotypic suites associated with distinct populations. We studied phenotypic evolution in lizard populations across a latitudinal thermal gradient to examine coadaptation associated with phenotypic suites. Despite ectotherm physiology slowing down in cooler temperatures, this species of spiny lizard (*Sceloporus consobrinus*) grows larger in the cooler, higher latitude populations, a response consistent with countergradient variation. The life history traits we examined (body size, growth rate, and reproductive effort) all positively covaried with latitude and temperature. Unless individuals from cooler environments are universally superior, other traits should inversely covary along the thermal gradient, and/or cold-adapted populations should assimilate more energy or utilize energy more efficiently. To this end, we assessed energy use across the gradient by examining metabolism of digestion, digestive efficiency, gut microbiomes, and locomotor performance.

Ectoparasite load generates tradeoffs in male color badge intensity and badge size in male lizards

Katherine Roberts, Eric McElroy, Lance McBrayer

Male condition-dependent signals convey information on health, dominance, and/or immunocompetence. Yet, the severity of parasitic infections mediates the differential expression of signals among populations, thereby creating variation in signal intensity. Here, male signal expression (brightness, patch size) was quantified in two Florida scrub lizard (*Sceloporus woodi*) populations that vary in ectoparasite loads. Males without mites were caught only in the early breeding season. The largest males had the darkest blue throat badges and its border. In contrast, males with ectoparasites (range = 1-368) were captured throughout the breeding season and showed a habitat dependent pattern of how ectoparasite load, body size, and body condition correlated with badge characteristics. In SPS, large lizards in good condition that are heavily parasitized had the largest badges. Neither body size, nor ectoparasite load was associated with badge darkness in SPS. Lizards from longleaf pine (LLP) share a singular pattern with SPS: larger males have larger badges. Body condition and ectoparasite load are unrelated to badge sizes in the LLP. Also, the most heavily parasitized males in LLP had the brightest, not darkest, badges. Moderate and low parasite loads had the darkest abdomens and low parasite loads have the darkest throats. Thus, body size appears to have similar effects on badge area across habitats, yet parasite load and body condition have habitat-dependent effects on badge characteristics.

Multiple Displacement Amplification Facilitates HiFi Genome Sequencing of Microscopic Animals

Nickellaus Roberts, Michael Gilmore, Torsten Struck, Kevin Kocot

Obtaining adequate input material for long-read genome sequencing remains a roadblock in obtaining high-quality genomes from small-bodied (e.g., < 1 mm) organisms. Multiple displacement amplification (MDA) is an in vitro technique that makes use of phi29 DNA polymerase to amplify minute amounts of DNA yielding micrograms of DNA suitable for long-read sequencing. Here, we explored the utility of MDA for generating template DNA for PacBio HiFi sequencing and demonstrate the efficacy of this approach comparing a genome produced for the model nematode *Caenorhabditis elegans* generated from MDA DNA from just one-half specimen (102 Mbp assembly; 336 contigs; N50 = 0.9 Mbp bp; L50 = 39; BUSCO_nematoda C:94.9%, S:92.2%, D:2.7%) with a genome obtained via sequencing a pool of worms. We demonstrate comparable coverage across regions of varying GC richness and repeat content and a low incidence of chimeras. Using this technique, we also sequenced the genome of the meiofaunal gastrotrich *Lepidodermella squamata* (122 Mbp assembly; 157 contigs; N50 = 3.9 Mbp; L50 = 13; BUSCO_metazoa: C: 80.8%, S:78.0%, D:2.8%). This simple and straightforward methodology has the potential to help generate contiguous genomes of other microscopic taxa whose body size would otherwise preclude long-read sequencing.

The modified pharyngeal jaw constrains the diversification of fish feeding kinematics

Alexus Roberts-Hughes, Christopher Martinez, Katherine Corn, Peter Wainwright

Modifications to the pharyngeal jaws – a prey processing system located posterior to the mouth cavity – are widely considered a key innovation that enhanced diversification within the feeding apparatus of several prominent fish clades. Seen in cichlids, damselfishes, wrasses, and a few other groups, these musculoskeletal alterations are believed to increase the evolutionary independence of the oral and pharyngeal jaws, thereby facilitating their diversification. To test this classic hypothesis, we conducted comparative phylogenetic analyses to assess the effects of this novelty on the diversification of feeding kinematics and morphology across a phylogenetically diverse sample of spiny-rayed fishes. We quantified interspecific head shape as well as jaw and craniofacial motions during suction feeding strikes using 689 high speed videos collected from 228 species with and without modified pharyngeal jaws. Contradicting long-held predictions, we found greater disparity in all traits and faster rates of oral jaw kinematic evolution in fishes without the

specialized prey processing system. In light of these and other recent findings, a reinterpretation of the macroevolutionary consequences of pharyngognathy is needed. The modified pharyngeal jaw is undoubtedly a functional innovation as it enhances the strength of the prey processing system, facilitating exceptional transition rates to feeding on hard and tough prey. However, it also restricts the diversification of the feeding system, revealing that the impact of pharyngognathy is more nuanced than previously thought.

Investigating toxicokinetics of newt (*Taricha*) tetrodotoxin (TTX) in garter snakes (*Thamnophis*)

Kelly Robinson, Haley Moniz, Chris Feldman, Amber Stokes

Animals that consume toxic diets provide excellent models for understanding molecular and physiological adaptations to ecological challenges. Garter snakes from several populations in western North America prey on Pacific newts, which employ tetrodotoxin (TTX) as a powerful antipredator defense. These snakes possess mutations in sodium ion channels (Nav), the molecular targets of TTX, that decrease the ability of prey toxins to bind to these proteins, and permit normal muscle and nerve function. Although we know the molecular mechanism underlying TTX resistance, we still lack an understanding of other mechanisms including the physiological processing of TTX. We explored toxin metabolism in garter snakes to determine if TTX-resistant snakes either rapidly eliminate TTX or, actively sequester the poison. We examined differences in rates of TTX elimination between TTX-resistant and TTX-sensitive snake species. We quantified TTX concentrations and calculated half-life of TTX in four tissues across four time points following exposure to TTX. We found that TTX half-life varied considerably across snake treatment groups, species, and resistance. Interestingly, TTX-resistant populations of *T. couchii* and *T. sirtalis* eliminated TTX quicker than their TTX-sensitive counterparts. These results align with a recent hypothesis on the evolution of toxin resistance; the ability to rapidly metabolize toxins allows for increased toxin intake, leading to greater accumulation of toxins, which might then promote the evolution of other resistance mechanisms and chemical defenses.

Do bees' heat tolerances improve following an acute heat exposure?

Gabriela Robles-Pérez, Natalie Herbison, Trisha Panganiban, Laura Haefner, Victor Gonzalez, Thomas Tscheulin, Theodora Petanidou, John Hranitz

Bees are the most efficient pollinators, making them a key species in ecosystems and in human welfare and sustainability. Climate change, one of the causes of bee decline, is affecting worldwide temperatures, possibly altering the bees' foraging activities and thereby, pollination. To cope with rises in temperatures, some organisms have been able to adjust their heat tolerance following acute heat exposure, thus enhancing their capacity to withstand or recover from elevated temperatures. Herein, we assessed whether bees' heat tolerance increases following acute heat exposure. We conducted experiments on the Greek island of Lesbos and used forager bees from four social species. We placed bees in an incubator at 38 °C in the dark for a 4h period (heat shock treatment). Then, we measured bees' critical thermal maximum (CTMax) following the 4h heat shock treatment and another group with a post-heat shock recovery after 24h at 27 °C. Bees incubated at 27 °C (average ambient temperature at the study site) for 4h and 24h served as control groups. We found that bees' average CTMax did not improve following acute heat stress, except for that of the sweat bee *H. scabiosae* which increased from 1.7 to 2.1 °C. Our results indicate that bees might have limited capacity to enhance their CTMax following acute heat exposure, with some species being more sensitive than others to rapid temperature changes. Bees are the most efficient pollinators, making them a key species in ecosystems and in human welfare and sustainability. Climate change, one of the causes of bee decline, is affecting worldwide temperatures, possibly altering the bees' foraging activities and thereby, pollination. To cope with rises in temperatures, some organisms have been able to adjust their heat

Isolating the Effects of Individual Nest Characteristics on Offspring Phenotypes in the brown anole

John Rodgers, Mike Norris, Daniel Warner

The influence of the external environment on offspring phenotypes and survival is well described for a variety of oviparous (i.e., egg-laying) species. Much of this previous work has focused on replicating natural incubation environments in the laboratory, based on the characteristics of maternally chosen nest sites in the wild. Although several environmental factors affect offspring phenotypes (e.g., substrate type, soil moisture, temperature), most studies do not assess the relative contribution of each factor to variation in offspring phenotype. To understand how multiple nest characteristics interact to affect offspring phenotypic variation, we studied the brown anole lizard (*Anolis sagrei*) and evaluated three major factors that typically vary among their nest sites: substrate type, incubation temperature, and soil moisture. Preliminary analysis suggests relatively moist soil increases egg mass during development at a greater rate than other treatments. Egg mass has a direct effect on hatchling body size, which is a primary driver of fitness variation in juvenile lizards. Further analyses will be conducted to isolate the effect of each environmental parameter on offspring phenotypes, and to determine which factor- substrate type, incubation temperature, or soil moisture- has the largest impact on hatchling body size and other morphological traits.

Emergence, assembly and evolution of an ascidian histocompatibility locus

Henry Rodriguez-Valbuena, Tony De-Tomaso

Histocompatibility is the ability to discriminate between self and non-self tissues, and has been described in species throughout the metazoa, from the first multicellular organisms to mammals. Despite its universal presence, histocompatibility genes utilized by different phyla are unique- for example, the MHC genes are found in all jawed vertebrates, but no ancestral locus exists in any invertebrate species. Thus, the origins of these sophisticated recognition systems are not understood. A well-studied histocompatibility system exists in the botryllid ascidians, members of the chordate subphylum, Tunicata, and provides an opportunity to do so. Histocompatibility in the botryllids is controlled by a single, highly polymorphic locus called the fuhc. In contrast to the vertebrate MHC, fuhc-based histocompatibility is not conserved within the tunicates, allowing studies of the origins and evolution of this system. Here we find that candidate histocompatibility genes in the fuhc locus each appear at different points in tunicate evolution, suggesting they were ultimately co-opted to participate in the allorecognition system of botryllids. In non-styelidae species, these genes are neither linked, nor polymorphic, and found in scattered, syntenic regions, including housekeeping genes. These regions assembled into a single locus that encodes ligands, receptors and other candidate proteins in the histocompatibility reaction, which subsequently became polymorphic. Once established, this region has mostly maintained its structure within the botryllid lineage. These characteristics are similar to the vertebrate MHC and other invertebrate histocompatibility loci, suggesting that- despite the absence of phylogenetic relationships, evolution of histocompatibility systems may proceed through or require a common genomic organization.

Exploring *Culex territans* mosquitoes role in trypanosome transmission to frogs

Isabella Roeske, Joanna Reinhold, Chloe Lahondere

Amphibian populations are in decline worldwide due to a variety of factors, including numerous pathogens that affect them. Anuran trypanosomes are parasitic protozoa present in frog populations globally. *Culex territans* is a mosquito species that feeds on amphibian hosts, primarily frogs, and is a known vector of hepatozoon parasites. We hypothesized that *Cx. territans* transmit trypanosomes to their amphibian hosts via saliva during blood-feeding. To test this hypothesis, we first tested field-caught mosquitoes and frogs for these pathogens at Mountain Lake Biological Station (Pembroke, VA). We focused on collecting *Cx. territans* mosquitoes as well as blood from two of their hosts, the green frog, *Lithobates clamitans*, and the bullfrog, *Lithobates catesbeianus*. Blood-fed mosquitoes and frog blood were screened for trypanosomes using DNA extraction and PCR followed by Sanger sequencing for species

identification. *Trypanosoma ranarum* were found in several samples, indicating Cx. territans can uptake trypanosomes and potentially transmit the parasites to frogs. We then allowed starved Cx. territans to feed on a trypanosome positive frog to test for vector competence. At 14 and 21 days post blood-meal, we dissected the mosquitoes and collected saliva. PCR of the 21 day old samples showed presence of the parasite in the saliva and salivary glands, which leads us to believe Cx. territans is likely a competent vector of trypanosomes. This research adds another critical piece to the puzzle of the epidemiology of anuran pathogens.

Using $\Delta 17\text{O}$ to estimate metabolic and exogenous body water inputs in captive house sparrows

Elizabeth Rogers, John Whiteman, Seth Newsome, Zachary Steele, Karen Caceres, Alexander Gerson

Terrestrial [BR1] animals gain water from two main sources: ingestion of food/drinking water, which is derived from precipitation, and endogenous synthesis of metabolic water. Metabolic water and precipitation have distinct values of $\Delta 17\text{O}$, a measure of $\delta 17\text{O}$ ($17\text{O}/16\text{O}$) relative to $\delta 18\text{O}$ ($18\text{O}/16\text{O}$), providing a way to estimate relative inputs of endogenous and exogenous water into the body water pool from a single sample. We aimed to develop a model to predict fractional body water inputs from the $\Delta 17\text{O}$ of water distilled from avian blood samples. To do this, we housed captive house sparrows (*Passer domesticus*) in metabolic phenotyping chambers custom built with low-cost, 3D printed components to continually measure animal body mass, food consumption, and water intake using Arduino microcontrollers. We used open-flow respirometry with rapid multiplexing to measure O_2 consumption. Birds lived in the metabolic phenotyping chambers for 35 days; we exposed birds to two temperature treatments (25°C and 5°C) and two diets (high salt and low salt) which we predicted would change the fractional inputs of metabolic water and food/drinking water, respectively. We rotated treatments and collected blood samples every 7 days, allowing for full turnover of the body water pool, such that each bird experienced each combination of conditions. The results of this project will enable the use of the $\Delta 17\text{O}$ technique for novel studies on avian ecophysiology.

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Neural mechanisms of sound source localization in a vocal fish

Loranzie Rogers, Jonathan Perelmuter, Nicholas Lozier, Brooke Vetter, Thomas Quigley, Julian Davis, Andrew Brown, Paul Forlano, Joseph Sisneros

Sound source localization requires animals to bilaterally compare directional auditory cues while attending to sound sources. For terrestrial vertebrates, this is accomplished by comparing interaural differences (i.e., time and level). In contrast, aquatic vertebrates, such as teleost fishes, cannot rely upon the same cues due to the inner ears' minimal interaural distance and the higher sound speed underwater. Instead, fishes are posited to utilize their differentially oriented and directionally sensitive inner ear otolithic end organs (sacculi, utricle, and lagena) to localize underwater sound sources. However, the degree to which each inner ear end organ contributes to underwater sound source localization is unclear. Here, we describe how differential binaural inputs contribute to sound source localization behavior in the plainfin midshipman fish (*Porichthys notatus*), a vocal teleost fish that extensively utilizes sound to facilitate reproductive interactions. Using behavioral experiments, we show that midshipman fish can successfully localize sound sources following the bilateral or unilateral removal of various combinations of the inner ear. Using neuroanatomical data, we identify the central auditory pathway of the inner ear otolithic end organs, distinguish sites of auditory convergence, and model the directional sensitivity of the inner ear otolithic hair cells in 3D space. Taken together, our results suggest that teleost fishes require the integration of complex directional auditory information from the array of inner ear end organs to facilitate sound source localization.

Spilling your guts: Eelgrass isopod gut contents and harmful algal blooms

Rylie Rogers, Dawson Johnson, Christine Weilhoefer, Ryan Kenton, Rosa León-Zayas, Cecilia Brothers

Harmful algal blooms (HABs) are becoming more frequent and widespread due to anthropogenic influences and climate change. As primary consumers graze on harmful algae, biomagnification of toxins, such as domoic acid, can cause potentially dramatic effects on trophic interactions. One such primary consumer is the eelgrass isopod, *Pentidotea resicata*, which serves as a food source for many fish species. Isopods, epiphytic algae on the eelgrass *Zostera marina*, and seawater samples were collected during July and August in the Salish Sea, Washington to determine whether isopods consume HAB species and accumulate toxins. Microscopic analysis of the potential food source (epiphytic algae samples from July [$n=4$] and August [$n=5$]) indicates phytoplankton community composition was similar between these two months. Furthermore, potential HAB taxa such as the diatom *Pseudo-nitzschia* sp. represented a small fraction of the overall community composition ($< 2\%$). Future work will determine the percentage of *Pseudo-nitzschia* sp. present in isopod gut contents and all samples will be tested for concentrations of the neurotoxin domoic acid produced by *Pseudo-nitzschia* sp. This research will determine the potential of isopods as a source of toxins further up the food web.

Modeling the environmental drivers of genetic structure in continuously distributed species

Ashley Rohde

Species around the world are changing their distributions and, in many cases, decreasing in abundance and range in response to anthropogenically caused changes to their habitats. Improved understanding of the environmental factors that drive changes in genetic structure is critical to mitigate the impacts of environmental changes to population viability. This goal is particularly challenging for continuously distributed species, because measures of genetic structure often assume discrete populations. *Bombus mckayi* and *B. occidentalis* are two species of bumble bees in western North America; both of which are under consideration for listing as endangered in the U.S.A. I used maximum entropy models and structural equation models to quantify relationships between genetic structure (Nei's D) and potential environmental drivers including weather, distribution, habitat change, and exposure to parasites. For both species, springtime minimum temperature was the most important predictor of occupancy likelihood, and geographical proximity to known infections of the fungal parasite *Vairimorpha bomby* was a reliable predictor of genetic differentiation, i.e., restricted gene flow across the geographic range. Although I detected significant causative relationships among environmental variables and genetic structure through these analyses, the pairwise nature of Nei's D likely confounded other, more subtle, relationships. Continued development of modeling methods to measure the environmental causes of genetic structure in continuously distributed species is needed to mitigate the impacts of ongoing environmental change.

Small yet fast water-walkers: vortex interactions during water locomotion in Microvelia

Pankaj Rohilla, Johnathan O'Neil, Victor Ortega-Jimenez, Prateek Sehgal, Chandan Bose, Saad Bhamla

Microvelia's locomotion is enigmatic because they can translate with ease on land or water using a simple alternating tripod gait, similar than terrestrial insects that perform poorly on water, but that contrast with the rowing propulsion of specialized water striders, that move clumsy on land. These millimeter-sized bugs use their hydrophobic middle and hind leg to impart momentum in the fluid via a pair of bipolar vortices. Interestingly, their hind legs seem to re-energize the majority of the vortices shed by the middle legs. Here, we unveil the biomechanics and hydrodynamics of these tiny water walkers. Using particle imaging velocimetry (PIV), high-speed imaging and digital image processing, we visualize and quantify the circulation of these vortices to find a correlation between the kinematics of these insects and the vortices shed by their legs. Furthermore, we also used a physical model of

a interacting legs to systematically study the effect of time gap between the strokes of the middle and hind legs on the vortex interactions with the hind legs. Further, we will also present our computational fluid dynamics (CFD) findings to measure the drag force on the hind legs due to their interaction with the vortices. The major aim of this study is to gain a depth understanding of the biomechanics and interfacial fluid dynamics of the locomotion of these insects on the surface of water, which can help to design bio-inspired walking micro-robots.

DeCA: A Dense Correspondence Analysis Toolkit for Shape Analysis

Sara Rolfe, Murat Maga

Concepts of “homology” and “developmental origin” are integral to many fields of biological research to establish equivalency of anatomical structures, regardless how different they may look and function in different taxa. Manually placed landmarks that delineate structures provide an easy way to capture expert knowledge of anatomy into analysis, however reliable points tend to be too sparse to capture the anatomical arrangement of complex structures such as the skull. Dense correspondence methods provide a solution to more fully capture shape information, but these techniques make strong assumptions of geometric similarity of the structures being analyzed that make more sense in context of a single species studies but may not generally be true in the context of multiple species or studies that involve organisms with perturbed development.

Here, we introduce DeCA (Dense Correspondence Analysis), an open-source toolkit for shape analysis in which expert evolutionary and developmental knowledge are incorporated via annotation of a small number of landmarks that are used to initialize dense surface correspondence analysis. This toolbox can support a variety of workflows and is published on the 3D Slicer image analysis platform where it can be installed and run through a user-friendly interface. Using a sample of mouse and rat skulls, we demonstrate that DeCA outperforms other registration methods that rely on a high level of geometric similarity.

Urbanization and air quality can impact auditory processing in an urban-adapted songbird

Kelly Ronald, Olivia Sprys-Tellner, Jacob Bergstrom, Peyton Hallemann, John Wenderski, Natalia Gonzalez-Pech

The rise of anthropogenic activities and environmental pollutants are considered a major contributor to avifauna population declines. Nevertheless, there is little understanding of how airborne particulate matter (PM) affects non-humans. The respiratory anatomy of avian species is uniquely susceptible to PM; songbirds can therefore serve as sentinel species for pollution concerns. The overall project objective is to characterize how the nanosized fraction of PM affects avian physiology and behavior. We investigated how the house sparrow auditory brainstem response is altered with exposure to urbanization and iron oxide nanoparticles (IONPs). We hypothesized that hearing sensitivity would be negatively affected by urbanization level and by IONP exposure. Our results suggest that auditory thresholds are affected by both the urbanization level of the capture site and exposure to IONPs, but uniquely across the sexes. Ultimately, alteration of hearing capabilities has the potential to impact how these species communicate to find mates, perform parental care, and avoid detection by predators. Understanding these impacts will help inform decisions for urban planning and human activities.

Monitoring sessile invertebrate biodiversity on 3D printed biodegradable materials in Colombia

Maria Rosa, David Hudson, Taegan McMahon, Gerard Gadigian, Diana Tarazona, Jaime Rojas, Alexandra Hernández-Ubaque, Julio Otero-Perez

Coastal marine environments are some of the most productive and overexploited ecosystems on earth. Coastal ecosystems, like oyster reefs and coral reefs, need physical structures to establish and habitat destruction has led to the destabilization of reef structures resulting in massive biodiversity losses. For decades, researchers have utilized Poly Vinyl Chloride (PVC) panels as

artificial larval settlement plates to study recruitment and community dynamics in these incredibly important ecosystems. Unfortunately, PVC itself is relatively toxic, the plates are smooth which makes it difficult for larval development, and it can take months to see establishment because larvae need settlement cues from biofilm development. Here, we piloted the use of 3D printed panels constructed out of plant-based biodegradable materials as an alternative to PVC panels in a tropical system. Panels constructed of three different corn-based polylactic acid (PLA) materials were tested at two well monitored sites in the Southern Caribbean Sea, in northern Colombia. Overall, the 3D printed panels showed settlement of organisms critical to coral restoration efforts in the region, such as crustose coralline algae (CCA), and wild-settled coral polyps. Beyond the application of panels for ecological assessment of local settlement, they were economically beneficial to use, which increases equity in this conservation crisis field as material expense can be cost prohibitive, particularly in scaling efforts in developing nations.

Looking for “a snail in a pond”: Investigating how eDNA contributes to eradication efforts of the in

Esme Rosas, Cassidy Reynolds, Romi Burks, Matthew Barnes, David Christie

To combat invasive species, conservationists frequently rely on environmental DNA [eDNA, i.e., the genetic material an organism sheds]. However, few studies focus on snails. *Pomacea maculata*, an invasive non-native species of apple snails, established in the southern US starting in the early 2000s. The most successful and least environmentally detrimental control strategies involve hand removals. However, limitations of eDNA detection may make it difficult to interpret results as inferred biomass or eradication. In 2020, an unauthorized aquarium dump likely released *P. maculata* into Bear Lake (Austin, TX), a small, isolated, shallow pond (~ 6 acres at full capacity) that occurs within a community green space. For the last 2½ years, a concerned homeowner removed snails and later collected monthly to bimonthly surface water samples (N=3) for eDNA analysis. We filtered 250 mL of pond water through 1.2 µm Millipore filters, performed chloroform extractions to recover apple snail DNA, and then measured eDNA using species-specific primers with quantitative PCR. We found tiny, yet detectable amounts of eDNA during winter (ranging between 1×10^{-5} and 10^{-6} ng µL Dec 2022 & Jan 2023) and then observed a period of non-detection. Despite active removal efforts, similar levels of activity reappeared in the summer months. Our current efforts involve using eDNA to detect pockets of snails left in the pond which will help inform future management efforts.

Impulse Response of Female Midshipman Swim Bladder

Owen Rosenbluth, Julian Davis, Loranzie Rogers, Joseph Sisneros, Elijah Berger

During the mating season, nesting male plainfin midshipman fish (*Porichthys notatus*) produce multi-harmonic advertisement calls to attract females to their nests by vibrating their swim bladders. These advertisement (mate) calls contain fundamental frequencies that are temperature dependent and can range from 80 to 100 Hz with harmonics (integer multiples) up to 1000 Hz. In addition, the female midshipman swim bladder is also known to play a significant role in hearing and is hypothesized to act as a resonance chamber, amplifying sound pressure signals. To explore this, we modeled eight female swim bladders, analyzing their natural frequencies, mode shapes, and response to pressure impulses from eight different orientations (45 degrees apart in the frontal plane) around the bladder. By measuring displacement of the rostral projections of the swim bladder (the horns) to pressure impulses, we identified dominant frequencies potentially transmitting motion to the particle motion sensitive saccule through swim bladder horn displacement. Preliminary findings suggest that while natural frequencies align with the male call's fundamental frequency, significant horn displacements occur at lower frequencies. This implies that most of the energy transferred to the saccule from male call-induced bladder displacement comes from low-frequency motion. This doesn't negate the female bladder's ability to respond the male's call but indicates that the female swim bladder is potentially effective at enhancing low frequency information that maybe biologically relevant for survival and reproduction.

The complex and variable behaviour of aponeurosis during dynamic contractions

Stephanie Ross, Christine Waters-Banker, Andrew Sawatsky, Timothy Leonard, Walter Herzog

Aponeurosis is a thin sheet-like layer of visco-elastic tissue that covers the surface of skeletal muscle and is a continuation of tendon. Hill-type models, which are used widely to predict muscle forces in simulations of whole-body movement, often combine the aponeurosis and tendon into a single elastic element in series with the muscle. However, because of the limited experimental data describing the behaviour of aponeurosis in response to muscle forces, it is not clear if it is reasonable to assume that the aponeurosis is mechanically in series with muscle. To address this gap, we examined the *in situ* behaviour of the medial gastrocnemius aponeurosis of 11 New Zealand White rabbits. We measured muscle belly and aponeurosis length via sonomicrometry and attached a bipolar nerve cuff around the tibial nerve to stimulate the muscle. To impose cyclic length changes and measure forces of the muscle-tendon unit, we attached the distal end of the tendon to a muscle motor. We found that, unlike tendon, aponeurosis did not consistently stretch to longer lengths when force increased and recoil when force decreased. Instead, the aponeurosis remained largely isometric with increases in force for some animals and stretched for others. These results suggest that forces applied to aponeurosis are likely more complex than those applied to tendon, so it may not be reasonable to assume aponeurosis is in series in muscle models.

Amino Acids at the Races: Vampire Bats Rapidly Fuel Running with Blood Meal Protein

Giulia Rossi, Kenneth Welch

Running mammals support this exercise primarily by oxidizing endogenous carbohydrates (glycogen). Amino acid oxidation accounts for a very small proportion (5-10%) of energy production during exercise. Vampire bats (*Desmodus rotundus*), however, specialize on a uniquely protein-rich blood diet and are, unlike other bats, capable runners. Given these natural history features, we hypothesized that these bats would rapidly begin utilizing dietary amino acids to support a majority of their running metabolism, in contrast to other mammals. We performed breath stable isotope tracking on running bats, first by feeding each bat cow's blood enriched with isotopically labeled glycine (non-essential amino acid) or leucine (essential amino acid). Bats were run at speeds of 10, 20, and 30 m min⁻¹ on a respirometry treadmill, allowing us to assess metabolic rate and track the oxidation of labeled amino acids in exhaled CO₂. During exercise, vampire bats oxidized amino acids as their primary fuel as indicated by a consistent respiratory exchange ratio (RER = ratio of CO₂ production to O₂ consumption rates) of approximately 0.9 at all speeds, with the labeled meal accounting for > 50% of oxidized fuels at peak usage. Bats did not discriminate between essential and non-essential amino acid use during exercise; peak apparent oxidation rates for both glycine and leucine were similar. Given nectar bats show an unusual ability to rely on recently ingested sugars to fuel flight, the reliance of vampire bats on blood-meal amino acids to fuel running highlights how strongly metabolism can be shaped by a specialized diet.

Caribbean upper mesophotic corals reproductively out-perform their shallow counterparts

Ashley Rossin, Gaby Carpenter, Gillian Coleman, Morgan Coleman, Benjamin Farmer, Daniel Holstein

Mesophotic depths may provide environmental refuge for corals experiencing anthropogenic stress, which is concentrated in shallow nearshore environments. A critical assessment is whether corals in these potential refuges produce viable gametes. These darker mesophotic environments may be energetically challenging for phototrophic corals, and evidence from the Indo-Pacific consistently demonstrates a negative effect of depth on coral gametogenesis. This does not appear to be the case for depth-generalist Caribbean coral species. Previous studies have found that upper mesophotic Western Atlantic environments support reproductively active populations of *Orbicella faveolata*, *Porites astreoides*, and *Montastraea cavernosa*. In this

study we assessed gametogenesis and fecundity of *O. franksi*, the dominant species and principal reef builder on extensive upper mesophotic reefs in the U.S. Virgin Islands. Upper mesophotic colonies produced larger oocytes and had a higher polyp and colony fecundity than their shallow counterparts. These results lend further credence to the hypothesis that deeper reefs may provide climate refuge to depth-generalist reef species, and that Western Atlantic corals may be more prepared to benefit from this refuge than many Indo-Pacific species.

Investigating DNA content of coding regions in Squamates

Adam Rosso, Matthew Fujita

The genome is composed of all DNA that is found in an organism. In vertebrates the DNA content of the genome has far reaching implications such as DNA folding, gene density, strength of selection, gene expression, and three-dimensional shape of the genome. Non-avian reptiles are a great group to investigate the evolution and function of DNA content because the genomes within this group are quite variable. We examine the DNA content of coding regions in representatives from major lineages of squamates to better understand the evolutionary forces that shape DNA content and the implications of the variation that exists in this order. In mammalian genomes, the position and density of the nucleotides guanine and cytosine are associated with life history traits and genome size. We expect this same result in squamates because coding regions are highly conserved and are integral to molecular processes that are fundamental to life. If the DNA content of squamate genomes shares similar nucleotide composition found in other taxa, this could provide more evidence for the molecular processes that help maintain guanine and cytosine across the tree of life.

Simulating Morphology with SlicerMorph and Advanced Normalization Tools (ANTs)

Rachel Roston, Sophie Whitehart, Sara Rolfe, Murat Maga

Three-dimensional imaging and quantitative analyses can be a powerful tool for discovering novel developmental phenotypes in reverse genetic screens. Computational approaches can support: the identification of subtle phenotypes often missed by qualitative observation; detection of novel phenotypes at earlier developmental stages; and automation of high-throughput pipelines. However, the exploratory nature of these approaches can yield results that are difficult to interpret. For example, do the results reflect real phenotypic differences, or are they showing random variation? If no phenotypic differences are detected, is it simply because the sample size was too small? Simulated morphologies offer a way to elucidate morphometric results, to explore theoretical morphospaces, and to test the sensitivity and reproducibility of morphometric analyses. Using open-access, open-source tools available in SlicerMorph and Advanced Normalization Tools (ANTs), we have developed an approach to experiment with simulated morphologies and use them to test our analytical pipeline for phenotype discovery in lethal and sub-viable knock-out mice. We have found that the discovery of subtle phenotypes can be improved by increasing sample size or limiting analyses to specific body regions. In this presentation, we will demonstrate our process for generating simulated morphologies using SlicerMorph and ANTs, and discuss its utility in developmental and comparative studies with limited sample sizes, subtle phenotypes, rare phenotypes, or machine learning.

DNA damage and repair in response to anoxia in embryos of an annual killifish

Riley Roth-Carter, Jason Podrabsky

In developing embryos insults to the genome from genotoxic stressors can cause the formation of DNA lesions that must be efficiently and accurately repaired or they can cause genomic instability, abnormal development, and cell death. The annual killifish (*Austrofundulus limnaeus*) produces embryos that can withstand many different genotoxic stressors, such as anoxia, high doses of UV-C irradiation, 3% H₂O₂, and 30Gy of ionizing radiation (IR). These stressors are known to cause a wide array of DNA lesions, including the most lethal lesion, Double Strand Breaks (DSBs). We hypothesize that killifish embryos have a greater ability to repair DSBs in response to these genotoxic

stressors when compared to other species. To test this, we used an anoxia-tolerant embryonic cell line, WS40NE, that was developed from *A. limnaeus* embryos. Cells were exposed to several different genotoxic stressors, including anoxia, H₂O₂, and ionizing radiation. DSBs were visualized using immunostaining for γH2AX and a novel assay called dSTRIDE. Initial data suggests that anoxia induces DSBs comparable to a 30Gy dose of radiation, a lethal dose to most eukaryotic cells. However, WS40NE cells appear to efficiently repair this damage. Understanding the ability of these cells to survive high rates of DNA lesions can lead to further discoveries important to human health, such as radiation resistant cancers and ischemic events.

Unveiling the role of locomotor ecology and body size on the evolution of mammalian limb morphology

Priscila Rothier, Anne-Claire Fabre, Roger Benson, Quentin Martinez, Pierre-Henri Fabre, Pedro Godoy, Vinicius Anelli, Brandon Hedrick, Anthony Herrel

In mammals, the phenotypic diversity of limbs ranges from paws to wings, flippers, hooves, and much more, and the morphology of the forearm is often proposed to be linked to the radiation of this group across numerous niches. The outstanding phenotypic variation of mammals has been shown to be impacted by a series of selective regimes, notably for the direct links between limb morphology and the environment where locomotion takes place. However, these associations have only been addressed across a somewhat limited taxonomic diversity. We tested whether locomotor mode defines phenotypic variation across nearly 800 mammal species, covering approximately 95% of the living family-level diversity of Mammalia. We show that the diversity of body size and, most remarkably, forelimb morphology is best explained by a multi-peak structure of adaptive zones driven by locomotor ecologies. Non-specialist locomotor modes exhibit nearby optima rendering transitions between ecologies simpler. Highly specialized locomotion in fluids or dense media, including flight, aquatic, and fossorial locomotion, drives the most extreme morphologies associated with the most isolated adaptive optima. We also classified species into body size bins to evaluate the impact of overall size on the diversity and evolution of limb morphology. These findings contribute to our understanding of how mammalian morphology adapted to diverse environments and highlight the significance of adaptive landscapes in elucidating selective regimes driving phenotypic diversity.

Cis-regulatory evolution of bird beak morphogenesis

Megan Rothstein, Ricardo Mallarino

The bird beak represents a striking example of evolutionary innovation within the animal kingdom. The avian bill is derived from an embryonic cell population termed the cranial neural crest, which emigrates from the dorsal neural tube to form the facial primordia. Interspecific transplantation of neural crest cells among different avian species has revealed intrinsic, species-specific properties responsible for shaping beak morphology. To elucidate how this species-specific molecular signature is encoded in the genome, we conducted an epigenomic characterization of the cranial neural crest in three different avian species: chicken, quail, and duck. By performing single-cell ATAC-seq in cranial neural crest cells isolated from these three species, we identified a large cohort of cis-regulatory regions active in neural crest cells across species. While the majority of these regions were conserved, our analysis revealed unexpected variability in the accessibility of conserved elements. To identify molecular drivers of this interspecies variability, we analyzed transcription factor binding motifs present in neural crest enhancer elements. We found an enrichment of homeodomain transcription factor motifs in the duck relative to chicken and quail, supporting a role for these factors in the differential regulation of facial primordia progenitors. Together, our work provides a comprehensive characterization of the neural crest regulatory landscape between avian species and provides a framework for the use of single cell epigenomics for comparative studies.

Predictive modelling of avian landing approaches

Tom Rottier, Ben Parslew

The ability for birds to rapidly decelerate whilst in the air may affect the structure of the legs. The mid-air decelerations greatly reduce the kinetic energy that the bird has at touchdown. As a result, the legs must do less work, requiring smaller forces and powers. This means that birds can evolve to have smaller muscles and bones, reducing the energetic cost that is incurred to carry around this additional mass while flying.

To understand how birds achieve these large decelerations a predictive, theoretical model was developed that captures the fundamental dynamics of the manoeuvre. It models the bird as a point mass under the influence of gravitational and aerodynamic forces that can be arbitrarily controlled through changes in its angle of attack. It generates trajectories of the landing approach that can be compared against empirical data.

The model shows that as the speed decreases the effectiveness of aerodynamic braking diminishes and instead upwards trajectories must be used to continue decelerating. This transition point is proportional to the wing loading, indicating that the strategy used to land scales with body mass as does wing loading. The model can be used to predict the limits of landing or the landing styles of large, extinct birds.

Promising mutant lines as a new neurogenetic tool to study the olfaction neural basis in *A.aegypti*

Angela Rouyar, Anand Rao Patil, Ming Li, Omar Akbari, Jeff Riffell

The yellow-fever mosquito is an important vector of diseases such as dengue or Zika. For this reason, a large number of studies have been conducted on the olfactory responses of mosquitoes to vertebrate hosts. However, less work has been dedicated to study of the sensory mechanisms involved in mosquito attraction to flower and nectar sources, even if nectar and sugar feeding are important for both males and females as it improves their fitness.

The antennal lobe inhibition via GABA release from local interneuron seems to be involved in the neuronal mechanisms of floral odor representation and nectar seeking. In this context, generating mutants that target the mosquito's olfactory responses and particularly the GABAergic system is essential to achieve a better understanding of these diverse processes and olfactory coding in these disease vectors.

Here we demonstrate the potential of two transgenic lines using the QF2 transcription factor, GABA-B1QF2-ECFP and GABA-B2QF2-ECFP, as new neurogenetic tools to investigate the neural basis of olfaction in *Aedes aegypti*. Our results show that the gene insertion has a moderate impact on mosquito fitness. Moreover, the lines presented here can be crossed with a QUAS reporter line expressing GFP and used to determine the location of GABA-B1 and GABA-B2, two G-protein coupled receptors. We find high expression of GABA-B1 and GABA-B2 receptors on the ALs compared to other brain loci."

Comparative Neuromuscular Biomechanics (CNB): The Intersection of Comparative and Human Biomechanics

Jonas Rubenson, Christopher Arellano, Adrien Arias, Monica Daley, Taylor Dick, Natalie Holt, Armita Manafzadeh, Christopher Richards, Gregory Sawicki, Andrew Schulz

Historically, there has been a scarcity of interaction between SICB and biomechanics societies such as the American Society of Biomechanics (ASB) and the International Society of Biomechanics (ISB). In 2016-17, only five percent of abstracts submitted to SICB and ASB were human- and comparative-based, respectively. This lack of overlap has existed despite the clear benefit of cooperation among human and comparative biomechanics fields. The Comparative Neuromuscular Biomechanics Technical Group (CNB), a 'sub-society' of the ISB, was founded on the goal of increasing interaction between comparative- and human-focused biomechanics research. Since its inception in 2018, the group has run international workshops and symposia in areas ranging from fundamental muscle mechanics to musculoskeletal modeling to bio-machine interfacing, all while strategically merging expertise in human and comparative biomechanics/neuromechanics. Our latest symposium, a full-day meeting at the ISB2023 Congress, attracted 200 scientists from diverse

disciplines including comparative biomechanics, robotics, neuroscience and clinical orthopaedics. In addition to scientific forums, the CNB organizes career development workshops, transdisciplinary technical standards, and early-career grant mechanisms, among other initiatives. We believe strongly that cross-pollination between comparative and human biomechanics fields will accelerate discovery, both of biological principles as well as novel health and engineering applications. Please check out more about the CNB here: <https://sites.psu.edu/cnbgroup/>.

A multi-experiment investigation of an elaborate anti-predator trait in moon moths

Juliette Rubin, CJ Campbell, Ana Paula Carvalho, Ryan St-Laurent, Gina Crespo, Taylor Pierson, Robert Guralnick, Akito Kawahara

When predator evasion has failed, animals are under intense selective pressure to thwart predatory attacks. Rather than outrunning or outmaneuvering a pursuant predator, some animals use elaborate traits to deceive. Within the moth family Saturniidae, many species have evolved long hindwing tails that deflect bat attack, likely by creating an acoustic illusion for these echolocating predators. Here, we present results from behavioral experiments investigating alternative evolutionary drivers shaping hindwing tails and place these results in macroevolutionary context. Using mate choice experiments with males of differing hindwing tail treatments, we show that sexual selection does not appear to be an important driver of the tail trait. Next, pitting tailed and non-tailed moth models against bird predators, we find that tail elongation is likely not constrained by diurnal hunters. We therefore return to bats as the primary drivers of long hindwing tails in moths. To better understand the relative pressure by bats, and potential countervailing constraints, we conducted macroevolutionary analyses using the best-sampled phylogeny of an entirely tailed moon moth clade to date and comprehensive insectivorous bat species distribution models. We find a signature of positive selective pressure by bats and eco-evolutionary constraint by environmental factors. We suggest that this well-studied system provides a powerful lens through which we can gain insight into the relative evolutionary pressures that may drive deceptive elaborate traits in animals.

Creepy Crawlies in the Concrete Jungle: Invertebrate Diversity Across an Urbanization Gradient

Gaia Rueda-Moreno, Kristin Winchell, Rafael Baez-Segui, Emerald Lin

Terrestrial invertebrates play many crucial roles within the ecosystem, ranging from decomposers to pollinators, all the while transferring nutrients and energy from plants and other insects to larger vertebrate predators. However, the myriad of highly specialized life histories found within terrestrial invertebrates are vulnerable to sudden environmental disturbances, such as urbanization. Urbanization presents a number of challenges to native terrestrial invertebrates, such as increasing impervious surface cover, increasing habitat fragmentation/loss, and competition with invasive species. We hypothesized that native invertebrate diversity would negatively correlate with an increase in human footprint factors (air quality, habitat fragmentation, invasive species presence), and a decrease in plant diversity. To study the effects of urbanization on terrestrial invertebrate communities, we sampled invertebrate and plant biodiversity at 7 sites across an urban to rural gradient in the New York metropolitan region, along with an array of environmental variables (canopy cover, air quality, temperature). The most frequently encountered taxa were Yellow-footed Ants (*Nylanderia flavipes*), Whirligig Mites (*Anystis* spp.), and American Winter Ants (*Prenolepis imparis*). Preliminary results suggest that sites where invasive species were present had much lower biodiversity, and sites with lower human footprint factors had greater biodiversity. Understanding the ways in which urbanization directly and indirectly impacts invertebrate biodiversity is extremely important for native conservation efforts, since they act as a trophic link between microscopic and macroscopic food chains.

Creation of artificially selected cricket lines to investigate the basis of wing polymorphism

Olivia Ruffins, Caroline Williams, Kristi Montooth, Colin Meiklejohn

Flight in insects is a complex polygenic trait reliant on morphological, physiological and behavioral traits. Flight capability is also environmentally sensitive, making it difficult to separate the genetic and environmental influences on flight. In North American Field Crickets (*Gryllus* spp.), a range of flight is observed, with long winged species, short winged species and species that are polymorphic, providing a powerful system in which to understand the genomic and evolutionary basis of insect flight. Wing polymorphism within a species is understood using the threshold trait model, where an individual has liability, existing on a spectrum that is translated into a binary phenotype. In the wing polymorphic species *Gryllus lineaticeps*, we have initiated an artificial selection experiment by selecting replicate lines for long- and short winged crickets, and measuring the evolutionary and genomic response. The creation of artificially selected wing morph lines will allow for both the deconvolution of the genetic and environmental factors that controls wing morph, and the evolutionary dynamics of flight.

After five generations, progeny from the long wing (LW) line are 75% long wing, and the progeny from the short wing lines are 15% long wing, suggesting that wing length is heritable and responds to selection. We will present data on correlated responses in flight muscle maintenance and endocrine levels. In the future, we will further explore the genomic changes that occurred during selection.

Demographic history and resilience potential of the threatened Caribbean coral, *Acropora cervicornis*

Maria Ruggeri, Iliana Baums, Macarena Blanco-Pimentel, Pol Bosch, Lisa Carne, Nichole Danser, Phanor Montoya-Maya, Megan Morikawa, Erin Muller, Andrew Baker, Ross Cuning, Craig Dahlgren, John Parkinson, Carly Kenkel

Genomic signatures can give insight into the evolutionary history of populations and their potential for future resilience. This is especially important for threatened and endangered species, where conservation genomic approaches can inform management and intervention strategies. The staghorn coral, *Acropora cervicornis*, is a foundational member of coral reefs, yet is threatened due to recurring disease and bleaching events. This study seeks to understand the demographic history of *A. cervicornis* populations and their obligate, algal symbionts to identify mechanisms of population resilience to future environmental change. Whole genomes of 37 host genotypes from wild and restored Caribbean populations were sequenced at high coverage (50x) for population genomic analyses. Genomic differentiation, migration and population structure were modeled to evaluate population connectivity and identify barriers to gene flow. Genomic diversity was also calculated to model historical population sizes over time to understand past and future population resilience. As source populations span an environmental gradient, we conducted genomic scans to identify candidate genes associated with thermal tolerance. Lastly, due to the multigenome nature of coral holobionts and the sensitivity of this symbiosis to climate change, we explored co-evolutionary history of host and symbiont. By understanding demographic history, population connectivity, and mechanisms of resilience in coral holobionts, we can better inform science-based restoration of these ecologically important reef-builders to enhance their natural adaptive capacity and fortify populations against further decline.

Decoding Chemosensory Signaling in *Aedes aegypti*: Insights from Glomerular Activity Mapping

Carlos Ruiz, Marnix Vlot, Jennifer Wei, Koen Decherer, Jeff Riffell

he yellow fever mosquito *Aedes aegypti*, one of the deadliest insects to humans, relies strongly on chemosensory cues to find hosts, sugar sources, and even substrates for oviposition. Chemical disruption of those processes can be an effective way to prevent vector-transmitted diseases. Unfortunately, our understanding of the mechanisms for the processing of odor information in the mosquito brain is very limited. We set out to characterize patterns of activity in the brain of female yellow-fever mosquitoes, in response to a set

of known attractants and recently-discovered strong repellents. To this end, we used two-photon microscopy to measure calcium activity across a set of glomeruli of interest in the antennal lobe of 4-day-old female mosquitoes in response to chemical stimuli. We found that both repellents and attractants trigger different patterns of glomerular activity at 37.5 μ m below the dorsal surface of the antennal lobe. Our findings suggest that early processing of chemosensory input happening at the level of the antennal lobe may already play a role in the interpretation of signals by the mosquito brain according to their biological relevance. Furthermore, they demonstrate that we can use glomerular activity patterns as a proxy to understand how the insect brain makes sense of the chemical landscape.

Distal wing muscle activity in a small fruit bat

Andrea Rummel, Olivia Li, Brandon Hedrick, Sharon Swartz, Richard Marsh

Bats are the only mammals capable of powered flight, using highly modified forelimbs as wings. The flight stroke is powered primarily by large core muscles like the pectoralis, with contributions from many other muscles in the shoulder and arm for propulsion and fine control of flight. Bats retain many muscles in the forearm and hand, but the function of muscles distal to the elbow has remained uncertain. It has been suggested that the largest muscles of the forearm, the extensor carpi radialis longus (ECRL) and brevis, extend the wing passively, acting more like struts and thus making active contraction and relaxation during the flight stroke less important. We hypothesized, however, that distal muscles of the wing are actively involved in control of wing extension and conformation during flight. Here, we measured muscle activity via electromyography (EMG) of the pectoralis and ECRL in *Carollia perspicillata* during steady forward flight, and paired these EMG measurements with wing kinematics. We compared the timing, amplitude, and periodicity of ECRL activity relative to the pectoralis, which sets the wingbeat frequency. We found that the ECRL is active cyclically, turning on during late upstroke and remaining on throughout much of downstroke. We suggest that its function may be to help open the wing fully after upstroke and before downstroke, and that active muscular control is important for this function.

Environmental Drivers of Movement for Marine Megafauna on the Upper Texas Coast

Madelyn Rupp, Lauren Simonitis, Bryan Gahn, Theresa Morris, Christopher Marshall

Sea turtles are highly endangered and migratory. Effective management of these cryptic species requires identification and management of all habitats occupied throughout their complex life history. The Gulf Center for Sea Turtle Research has established the first long-term, in-water monitoring of sea turtles in the Galveston Bay Estuary System (GBES) to identify the habitats used by loggerhead (*Caretta caretta*), green (*Chelonia mydas*), and Kemp's ridley (*Lepidochelys kempii*) turtles. Satellite tags were deployed on mature and immature turtles from the GBES that were either captured during in-water monitoring or cleared for release following stranding and subsequent rehabilitation. The resulting satellite telemetry data was used to identify foraging grounds, habitat connectivity, seasonal migration, and migratory corridors. Tracks from satellite tags were first fit with state-space models (SSMs) to account for the location and observation error intrinsic to the data. Environmental variables both collected from satellite tags and extracted from remotely sensed datasets were used to investigate the environmental drivers of movement for GBES turtles. Core and home ranges were identified using the SSMs to calculate the 50% and 95% Kernel Density Estimates to identify habitat use by species and age classes for turtles present in the GBES. These data provide wildlife managers the necessary information to make science-based decisions to conserve these species more effectively in the region.

Ecological and evolutionary consequences of buzz pollination

Avery Russell

Buzz pollination, a type of interaction between bees that can vibrate flowers to collect pollen and flowers that conceal pollen within tube-like (poricidal)

morphology, involves more than half of all bee species and 10 percent of all angiosperm species. In fact, the evolution and diversification of both buzzing bees and buzz pollinated plant species is thought to be strongly affected by this ancient behavior and interaction. Yet despite 120 years of research on buzz pollination, much work remains to understand the broad ecological and evolutionary consequences. We discuss our work on the evolution of buzzing behavior, its adaptive significance, and drivers of the global distribution of buzzing taxa. We also discuss our research on patterns and evolution of buzz pollinated floral morphology. Overall, we find that buzzing is a key behavior enabling flexible pollen collection from buzz and non-buzz pollinated plant taxa alike. Furthermore, we find that drivers of buzzing bee biogeography differ from those of non-buzzing taxa and are closely associated with buzz pollinated plant biogeography. Finally, we find that buzzing evolved before the earliest buzz pollinated plant species, and that buzz pollination occurs across 87 plant families and more than 635 genera, with on average 200 independent gains and 150 independent losses and is strongly associated with increased plant diversification. Finally, we present alternative hypotheses for the functional significance of floral poricidal morphology.

De-novo Genome of the Edwardsiid anemone *Edwardsia elegans*

Auston Rutledge, Adam Reitzel

Edwardsia elegans is a burrowing sea anemone commonly found on the North Atlantic coast of North America. *E. elegans* is one of ~90 species in the family Edwardsiidae, which is an informative group for research into the relationships of sea anemones and evolution in the subphylum Anthozoa (which contains corals and sea anemones). This species is a confamilial with the model cnidarian *Nematostella vectensis*, but very few sequence or genomic resources are present for species in the *Edwardsia* genus. Here we have generated the first genome of the sea anemone *Edwardsia elegans* using a combination of Illumina and Oxford Nanopore Sequencing. This genome has an N50 of over 152kb and a BUSCO score of 85.5% completeness with a low duplication of 0.8%, a quality comparable with other Anthozoan genomes. Genome annotation predicts there to be roughly 28,400 proteins, with over 95% of these predicted genes having a hit to proteins within a publicly available database. We also present information on toxin duplications, the epigenetic methylation of the genome, as well as syntenic regions of HOX and other developmental related genes. This newly generated genome will help shed light on the phylogeny of *Edwardsia*, a paraphyletic genus, and allow for improved comparative genomics, molecular, and organismal studies of both anthozoans and cnidarians as a whole.

Complex flow in stingray nostril geometry may passively enhance odor capture

Kelsi Rutledge, John Dabiri

Stingrays and their relatives (batoids) rely on their sense of smell as one of their primary senses for survival. The first step in olfaction is harnessing water and odorants from the external environment into the nostrils (nares). Batoid fishes appear to have evolved a diverse and unusual nasal morphology to aid in odor uptake. In this study, we focus on three nasal morphotypes with flap-like nasal protrusions: open, comma, and protruding morphotypes. We aimed to understand how morphologically diverse external nostril anatomy may influence odor capture potential. Models of the nasal morphotypes were 3D printed and mounted in a water tunnel with the incoming flow visualized with particle image velocimetry methods (PIV). To determine flow rate across the incurrent nostril geometries to estimate odor capture potential, a 2-D closed loop flux integral was calculated using the velocity fields generated by the PIV analysis. We found that olfactory flow is more complex and expansive than previously thought. Specifically, we document the first recorded occurrence of multidirectional olfactory flow into the incurrent nostril of a fish. The nostril flaps appear to play an important role in protruding out of the boundary layer and locally disturbing the flow to create recirculation regions around the nares. These recirculation regions funnel water into the incurrent nostril from multiple directions, greatly expanding the reach and odor capture potential of the nares.

SELECTINGS: A pipeline to detect positive Darwinian selection in large datasets

Joseph Ryan, Scott Santagata

The detection of positive Darwinian selection enables the identification of important changes in protein-coding regions of a genome that helped shape the evolutionary history of a particular lineage. Despite the availability of coding sequence data for many thousands of species, it remains a challenge to conduct large-scale selection analyses on broad collections of sequence data. To address this gap, we have developed a pipeline that allows for the analysis of an unlimited number of genes from an unlimited number of assembled transcriptomes and/or gene models gathered from genomic datasets. The SELECTINGS (Screening Evolutionary Lineages for Exceptional Coding Transcripts In Next Generation Sequence) Pipeline incorporates several existing bioinformatic packages for translating transcriptomes (Transdecoder), identifying orthologous sequences (OrthoFinder), aligning sequences (MAFFT), building gene trees (FastTree or IQTree), pruning trees (PhyloPyPruner), converting protein alignments to nucleotide alignments (PAL2NAL), detecting gene and site-based evidence of positive selection (comparable branch-site tests in PAML and HyPhy). Our GitHub site includes a set of scripts, documentation, sample data, installation instructions (all external programs can be installed via Conda), and a tutorial (<http://www.github.com/josephryan/SELECTINGS>). Compared to other similar tools, the SELECTINGS Pipeline allows for greater flexibility in terms of methodology, number of genes that can be analyzed, and the phylogenetic breadth that can be sampled.

Corticosterone manipulation alters patterns of social interaction in breeding tree swallows

Thomas Ryan, Conor Taff, Monique Pipkin, David Chang-van-Oordt, Maren Vitousek

Social interactions can serve as a source of information that helps calibrate reproductive decision making and could be especially important to organisms experiencing a challenging environment or event. Here, we assess how a common social tactic—visiting neighbors' nests by breeding female tree swallows (*Tachycineta bicolor*)—is impacted by an experimental increase in corticosterone levels. We hypothesized that birds facing challenges invest more heavily in gathering social information, and that this is mediated by corticosterone. We also hypothesized that the value of the information provided by challenged birds is lower, and thus they are visited less by conspecifics. Corticosterone levels were manipulated by a minimally invasive technique using corticosterone dissolved in a DMSO gel placed atop a fake egg adhered inside the nest. Female swallows treated with exogenous corticosterone engaged in more trips to neighbors' nests and were visited less frequently than controls. This effect was most apparent in the period when nestlings were young—immediately following the end of the treatment period—and dissipated as nestlings aged. Females that visited other nests more fed their own nestlings at a lower rate, suggesting that these social interactions may be costly. These results are consistent with a function of social information in contextualizing challenges—potentially to inform fitness-critical decisions—and suggest that corticosterone mediates social information gathering.

The interaction of tooth shape and strike kinematics in the feeding behavior of snakes

William Ryerson

Snake teeth play an integral role in the capture and subjugation of prey. However, while many have investigated the shape and structure and teeth, there are few examples examining how those shape parameters of shape function during a prey capture sequence. Using high-speed video and CT scans, I examine how tooth shape and strike kinematics interplay. Snakes fall into two categories of performance: high velocity, large gapes, and complex post-strike behaviors from species I am calling strikers; low velocity, small gapes, and large approach distances from species I call lungers. Tooth shape is also more variable than previously considered, with variation not only occurring amongst species, but within species and within individual bones of a species. Most of this variation occurs at the anterior end of both jaws, where teeth may be more upright (perpendicular to the jaw), slender, and potentially

recurved. The variation in tooth shape correlates strongly with strike performance. Strikers have teeth that are more upright at the anterior ends of the jaws, and more variation. During a strike, the upright teeth on the lower jaw penetrate the prey and serve as a fulcrum for the rest of the skull to rotate over. The curved teeth on the upper jaw slide over the prey and ensnare it. Lungers, with more curved teeth, slide over the prey with both jaws and ensnare.

Investigating the impacts of microplastics as a potential vector for chemicals on tadpoles

Anne Sabol, Alessandro Catenazzi

Microplastics, pieces of plastic under 5 mm, are becoming a pollutant of increasing concern. These microplastics are nearly ubiquitous across aquatic environments and have been found in the bodies of amphibians. In previous laboratory experiments of tadpoles, microplastics reduced survival, growth, and time to metamorphosis. Microplastics are also known to absorb and retain chemical pollutants from the environment. Chemical pollutants reduce amphibian survival and growth, and lead to physical abnormalities. Thus, when animals ingest these microplastics in nature, they may also be ingesting any pollutants the microplastics have absorbed. Therefore, instead of facing a single stressor of microplastics or chemicals alone, organisms face the combination of both stressors. However, previous studies on amphibians have only tested plain microplastics.

Therefore, we investigated the role of microplastics as a vector for other pollutants on the growth, development, survival, and telomere length of tadpoles. We soaked microplastics in water from three different locations within each category: natural, agricultural, and urban. We then exposed groups of Cuban tree frog tadpoles (*Osteopilus septentrionalis*) to these microplastics for two weeks. We tracked growth, development, and survival for two months and measured relative telomere length at the end of the experiment. There was no significant difference in growth, development, or survival between any of the treatments, suggesting that neither microplastics nor any chemicals they absorbed altered development in this experiment.

Whole forest hydraulics influences growth, drought responses and the atmosphere under climate change

Lawren Sack, Jeffrey Wood

Plant-environment interactions determine the productivity and water-use dynamics of terrestrial ecosystems and modulate the global carbon (C) and water cycles. As droughts increasingly threaten forests across the globe, it is urgent to identify the mechanisms by which plants internally regulate their gas exchange that scale up to influencing ecosystem carbon and water fluxes. The plant's internal plumbing—the plant hydraulic system—influences stomatal opening and thus the gas exchange of leaves and whole plants, and modeling studies have inferred hydraulic control of whole ecosystem gas exchange. Recently, the concept of a forest level “community hydraulic conductance” (K_{com}) has been introduced, representing the efficiency of root-to-leaf water transport through the forest. K_{com} can be measured by combining measurements of ecosystem transpiration (T) and leaf water potential. We review recent work showing that seasonal T and gross primary productivity (GPP) were constrained by internal plant hydraulics especially during drought, consistent with the sensitive hydraulic responses of roots and leaves. K_{com} is also dynamic diurnally, responding to light. Resolving the hydraulic control of gas exchange in ecosystems across the globe will improve ecological and climate forecasting through enhanced understanding of the influence of species diversity and benchmarking hydraulically-enabled terrestrial biosphere models (TBM).

Movement of weaned northern elephant seal pups during their first at-sea foraging migration

Katie Saenger, Tim Bean, Heather Harris, Lauren Campbell, Elizabeth Eby, Kate Riordan, Molly Murphy, Heather Liwanag

The northern elephant seal (*Mirounga angustirostris*, NES) is a well-studied marine mammal known for engaging in long foraging migrations at sea. Adult

male NES follow pathways along the coast towards Alaska and adult female NES follow pathways to open ocean. However, there is little to no information about newly weaned NES pup foraging behavior. This lack of knowledge is significant because NES have a mortality rate of ~50% in the first year. This project aims to better understand the migratory pathways of NES during this critical life stage. We deployed satellite tags on newly weaned NES pups at Vandenberg Space Force Base (VSFB, est. 2016, n=10) and San Nicolas Island (SNI, est. 1949, n=5). We hypothesized that weaned NES pups would [1] prioritize near-site foraging areas and thus migrate shorter distances compared to adults, [2] exhibit no sexual divergence in their pathways, and [3] have pathways that differ between the two breeding sites. We have found a high degree of individual variation in migration distance, with one seal venturing over 3000 km to the Gulf of Alaska, and another remaining near the Channel Islands of California. Examining these migratory pathways and comparing them between rookeries and years provides insight into important foraging grounds for this life stage, differences between breeding sites, and environmental influences on migration, which will inform conservation and management of this species.

Imaging snake cephalic glands and venom-delivery system components using diceCT

Ollie Safford, Kayla Hinnen, Michelle Shafer, Megan Vandenberg, Kate Jackson

DiceCT is a technology using an iodine-based stain to make soft tissue visible in a CT scan. DiceCT has been used to study a variety of animals such as fish and birds but less research has been done on its usage for imaging the cephalic glands in snakes which is a gap in knowledge we hope to fill. Several different iodine staining protocols are used by researchers for imaging soft tissues in vertebrates. We tried three different techniques of iodine staining: aqueous Lugol's iodine, ethylic Lugol's iodine, and buffered Lugol's iodine solutions, to visualize cranial anatomy of snakes. We looked specifically at cephalic glands and soft tissue components of the venom-delivery system (venom gland/Duverney's gland, venom duct, associated musculature, etc.) in a variety of colubroid snakes with front fanged (proteroglyph, solenoglyph) and rear fanged (aglyph, opisthoglyph) dentitions. We compared the effectiveness of different diceCT staining protocols for imaging snake cranial anatomy, achieving best results with buffered Lugol's iodine solution. Here we show results of different staining protocols as well as imaging of cranial anatomy of a selection of African and North American snake species. We aim to improve diceCT work flows as well as further knowledge of the anatomy of snake venom-delivery systems and cephalic glands.

Changes in gene expression and co-expression underlie heterochrony in poison frog embryogenesis

Anouka Saha, Sara Ansari, Daniela Zurita-Paredes, Andres Romero-Carvajal, Becca Young

Compared to most other vertebrates, frogs have diverse reproductive modes. In poison frogs (Dendrobatidae), many species reproduce semi-terrestrially. Associated with this reproductive mode is a diversity of early developmental patterns (e.g. developmental rates, egg sizes, formation of embryonic structures, and more). One critical process, gastrulation, is known to differ in poison frogs compared to the aquatically reproducing, well-studied *Xenopus* species. Gastrulation is a critical component of vertebrate embryonic development that forms the three germ layers – the endoderm, mesoderm, and ectoderm. In poison frogs, this stage is slower (4-6 days from fertilization to the end of gastrulation) than in *Xenopus* (less than one day). Additionally, associated processes (e.g., notochord elongation) occur after gastrulation in poison frogs. Here, we show that gene expression of candidate genes reflects known heterochrony in poison frog gastrulation. Second, we identify novel gene expression and co-expression patterns that correlate with the divergence in developmental rate and pattern of gastrulation. Specifically, we quantified gene expression in two closely related species of poison frogs, *Hyloxalus nexipus* and *Epipedobates machalilla*. We use weighted gene co-expression network analysis (WGCNA) and time series analyses to identify gene expression and co-expression patterns that differ in expression in poison frogs as compared to *Xenopus*. Because gastrulation is a critical process during

vertebrate embryogenesis, discoveries of mechanisms underlying naturally occurring variation have important implications for our understanding of human development.

Neural control of hatching enzyme release enables rapid escape-hatching in red-eyed treefrogs

Maria Salazar-Nicholls, Corey Allard, Astrid Lisondro-Arosemena, Nicholas Bellono, Karen Warkentin

Hatching is a critical embryo achievement that, for most animals, causes a major environmental change. Thus, many species adjust hatching timing based on environmental cues. The arboreal embryos of red-eyed treefrogs can hatch in seconds to escape from egg predators and other threats. These embryos develop ~3000 hatching gland cells (HGC) that release hatching enzyme (HE) to digest an exit hole in the egg membrane. However, how HE release is regulated to enable cued hatching is unknown in any frog. We combined behavioral, pharmacological, molecular, histological, and imaging techniques to test the hypothesis of neural regulation of HE release. We discovered that HE release is mediated by cholinergic signaling received by muscarinic type I receptors in HGC and rejected multiple other possibilities. This elucidates the neural regulation of a fundamental mechanism underlying an essential embryo behavior. It also shows that HE release and hatching movements have distinct regulatory pathways, which embryos must coordinate to successfully exit their egg. Integrative studies of mechanisms linking sensory cues to the hatching process can reveal developmental adaptations required to achieve escape-hatching. Anurans have great reproductive diversity, but our understanding of embryo behavior and even how hatching works is limited in most species. Our discovery in red-eyed treefrogs opens new possibilities for comparative evolutionary research to assess how shared, derived, and modified elements of hatching mechanisms enable adaptive plasticity.

Shape of the water: lizards and the tropics

Jhan Salazar-Salazar

The tropics have always amazed researchers due to their high diversity of habitats where plant and animal species have been able to adapt and diversify. Since Darwin, ecologists and evolutionary biologists have tried to understand how species evolve and adapt to those different regions and their high climatic regimes. Particularly, the tropics have several biomes where lizards have been able to adapt, both on islands and the mainland and in low and high-elevation areas. However, there are few studies trying to understand how lizard species might respond to a water deficit or scarcity in the Neotropics. This raises the question of the evolutionary liability of the water use-related traits; specifically, are closely related species or species found in similar areas going to have similar responses to water scarcity in a world that faces warming temperatures? Here, I gave a little bit of history of what we know so far about water scarcity and water use in lizards in the Neotropics, where researchers should focus future studies. Lastly, I used Colombian anole lizards, distributed from the sea level up to 2300m, to assess the question of how species might respond to those two factors of water scarcity and water use.

An insect wing's living network: structure, evolution, and bioinspiration

Mary Salcedo

Insect flight depends on an insect's living systems —hemolymph, tracheal, and nervous— and how they extend from the body into the branching wing venation network. Hydration and oxygen are critical to wing performance, flexibility, and stiffness. However, this relationship, wing functionality and its relation to a wing's branching, fluid-filled veins, is vastly unexplored. In this symposium we will explore the foundations of fluid movement in wing veins and implications towards structural proteins such as resilin, and questions of “fluid drivers” in wing evolution. We will also discuss insect wing development specifically during ecdysis, when both hemolymph and tracheal systems “switch” from their normal routines (i.e. circulation and oxygen delivery) to act as hydraulic tools, inflating and expanding the folded wing. Lastly we will posit avenues for future investigation, considering the usefulness of bio-inspired wings, using

them as platforms for learning, and applied directions in agriculture. Taken together, a wing's fluid systems dynamically contribute to an insect's behavior, its life history, and should be considered in all flight studies.

Tropical Blind Spots in Sciurid Thermoregulatory Physiology

Claudia Saldaña-DeCamillis, Eric Brown, Danielle Levesque

Historically and contemporarily, studies of thermoregulatory physiology in mammals have been heavily biased towards highly seasonal environments such as temperate and arctic zones. Squirrels (Sciuridae) are a well-represented family of rodents in seasonal environments and have served as excellent models for studying the effects of climate and speciation on physiological traits such as torpor and hibernation. Sciuridae are also found in the relatively aseasonal tropics, but we suspect that a holarctic bias in the literature exists in Sciuridae and that the thermophysiology of tropical sciurids is relatively understudied. We conducted a comprehensive literature review on the studies of thermoregulatory physiology of sciurids to determine whether such a geographic bias exists. Our review identifies important gaps in our knowledge of the thermoregulation of Neotropical, Afrotropical and Indo-Malayan squirrels, both in terms of taxonomy and biogeography. Further investigation is needed to answer important questions such as possible physiological adaptations to different environmental niches and how climate change may affect this important mammalian family.

The effect of indirect exposure to BPA during embryonic development in live-bearing fish

Yolitz Saldivar-Lemus, Saeid Panahi-Hassan-Barough, Caitlin Gabor

Bisphenol A (BPA) is a chemical primarily used in the production of polycarbonate plastics, and can cause endocrine disruption, changes in DNA methylation patterns, gene expression, behaviour, and affect axonal growth and muscle development. More particularly, in fish, exposure to BPA has had transgenerational effects and affected reproduction, movement, and behavior. In mammals, exposure to BPA during embryonic development affects reproductive tissues and the brain, and in oviparous fish, it attenuates body growth and advances the times of hatching and reproductive maturation. Many of the studies on BPA, however, are often conducted with high BPA concentrations that are not normally found in nature (not environmentally relevant), and, in the case of fish, are restricted to oviparous species. We used the viviparous and highly matrotrophic fish (where mothers provide a constant supply of nutrients during embryonic development) *Heterandria formosa* to look at the effects on offspring that were produced by an indirect exposure to BPA during embryonic development. We previously found that offspring of *H. formosa*, whose mothers were exposed to environmentally relevant concentrations BPA during pregnancy (2 ug/L) exhibited changes in behavior (motivation, solving ability and solution speed) compared to their control groups. Now we analyzed gene expression patterns in brains (heads) of the same offspring and found a list of potential genes that could be responsible for such behavioral and locomotor changes.

Both conserved and unique patterns of gene expression drive the development of lizard hemipenes

Adriana Saliceti-Galarza, Greta Keller, Marta Marchini, Ryan Cook, Tony Gamble, Thomas Sanger

External genitalia, associated with the origin of internal fertilization, evolved once among amniotes and then diversified among the major clades. Variation of the genitals consists of one midline penis, a clitoris, or either of those as a pair. Although there appears to be similarities in genital phenotypes and embryology among amniote clades, little is known about the molecular regulation of genital diversity. Mammalian penis development has been widely studied and experimentally dissected. A series of genes, including *Shh*, *Patched*, *Bmp4*, *Fgf8*, and *Hoxd13*, are critical for the proper formation of the mammalian penis. Herein, we examine the expression of those genes in the squamate model system, *Anolis sagrei*, that has paired genitalia. Many of the genes that are necessary for mammalian penis development are not expressed in early genitalia prior to sexual differentiation. At the time of oviposition, *Shh* is expressed strongly in the cloacal endoderm. Reduction of Hedgehog signaling leads to

ablation of the genitalia and cloacal opening. Our research highlights that the function of *Shh* in genital induction is conserved evolutionarily among amniotes with varying genital phenotypes. Additionally, we describe how gene expression patterns diverge among species with paired genitalia from those with a single midline phallus.

Does Developmental Photoperiod Alter Reproductive Hypothalamic Gene Expression in Dark-Eyed Juncos?

Carlie Saline, Jeffery Kittilson, Ellen Ketterson, Adam Fudickar, Timothy Greives

While natural selection has favored individuals able to align their timing of reproduction with seasonal changes in photoperiod, climate, and resource abundance, the physiological mechanisms coordinating this timing remain to be fully understood. Female birds, in particular, are crucial to study because the timing of oviposition determines the conditions under which their offspring develop. Research on dark-eyed junco (*Junco hyemalis*) populations that overwinter together in Virginia but breed at different locations (one breeding in VA, one further north) has established that differences along the hypothalamic-pituitary-gonadal (HPG) axis underpin differences in reproductive timing: more southerly-breeding birds reach reproductive maturity earlier in spring than birds breeding further north. Photoperiod is the primary cue coordinating life history stages with environmental amenability in adults. However, it remains unknown to what extent an individual's developmental photoperiod influences its seasonal reproductive timing as an adult – and, if so, where and how the HPG axis changes in response. We exposed nestlings to photoperiods reflective of growing up in southern or northern breeding grounds to test the hypothesis that developmental photoperiod influences the temporal expression of reproductive regulatory genes in the hypothalamus. Results will reveal the influence of early-life photoperiod on gene expression with potential lifelong consequences of reproductive timing and fitness.

Bioinspired reduction of tag drag

Danna Valentina Sanchez-H., Brooke Flammang

Marine organism biotelemetry is essential for the study of organismal behavioral data that informs federal conservation protections. The current state-of-the-art tag designs do not accommodate for the drag effects of the tag. As a result, tags are often lost through the pulling of the tag from drag forces, sometimes causing tissue damage to the animal. As part of a larger initiative to advance our bioinspired adhesive system, this work focused on a bioinspired streamlined housing for telemetry equipment. Our research on the fluid dynamics of attached remoras indicated that they produce little parasitic drag on their hosts. Following their body shape, we built and tested a bioinspired housing that can be scaled to accommodate the users sensor needs.

Immunological Memory or Immunological Priming in Tortoises

Franziska Sandmeier, Kiara Olson, Angelina Martin, Taylor Urban

Whether reptiles exhibit immunological memory has been contentious in the literature, possibly because some but not all classical hallmarks of immunological memory may occur in reptiles. Many species of reptiles also have been shown to have prolonged, elevated antibody responses to immunization. These elevated levels can complicate the quantification of any secondary immunological responses. Here, we re-immunized tortoises with ovalbumin (OVA) ten years after an initial, successful primary immune response. Although OVA-specific antibody levels remained elevated for years, they had returned to baseline levels by this time and animals had been kept in constant, captive conditions. In addition to quantifying increases in specific antibody titers, we also quantified relative avidity of antibodies due to this secondary challenge. Finally, we quantified antibody production and proliferation of B lymphocytes due to *in vitro* incubation with OVA prior to and after the secondary challenge. We found that antibody levels peaked more quickly due to a secondary challenge, but we did not find an increase in antibody titers or avidity. We also did not find evidence of memory B cells, which would have responded via

proliferation and antibody production due to stimulus with OVA. We propose that tortoises do show some levels of immunological priming, without exhibiting all the common hallmarks of mammalian immunological memory.

The mechanics of wings and halteres in flies

Sanjay Sane, Abin Ghosh, Tanvi Deora

The evolutionary success of insects owes largely to the evolution of flight, which enabled insects to thrive in a wide range of ecological niches. Insects possess considerable size diversity with wing spans ranging from few hundred microns in small parasitoid wasps, to 30 cm in Saturniid moths. Insects of the hyper-diverse orders Diptera, Hymenoptera, and Coleoptera possess stretch-activated myogenic flight muscles, whose action combines with thoracic mechanics to generate several hundred wingbeats a second. Dipteran hind wings evolved into vestibular structures called halteres, which provide rapid mechanosensory feedback of turning rates during aerial maneuvers. Wings and halteres of flies act in perfect synchrony, as a dual-coupled oscillator system. The musculo-thoracic architecture in flies includes a complex wing hinge, consisting of a clutch and a gearbox, composed of the radial stop (RS), pleural wing process (PWP) and pterale-C. During flight, the RS engages with the multi-grooved PWP in discrete gearbox-like configurations, enabling wings to control stroke-amplitude. Alternatively, stroke-amplitude may be largely controlled by steering muscles rather than a gearbox, requiring fine control of the RS vis-à-vis PWP. To test these hypotheses, we altered the PWP structure and measured how such flies conduct yaw turns. Inconsistent with the gearbox hypothesis, flies with altered PWP structures conduct yaw turns similar to normal flies. Thus, steering muscles may play a preeminent role in the control of amplitudes in flies.

Studying ketocarotenoid metabolism in cell culture

Amir Hosein Sanjari-Nia, Rebecca Koch, Geoffrey Hill, Matthew Toomey, Chidambaram Ramanathan, Yufeng Zhang

Carotenoid-based coloration, exemplified by redness, stands out as a potent mate choice criterion, especially in birds. Many birds transform dietary yellow carotenoids to red ketocarotenoids for their plumage coloration, however the physiological mechanisms underlying this process is still poorly understood. This study aimed to establish mammalian (HEK293) and avian (DF-1) cell lines to study ketocarotenoid metabolism. We first cloned three genes (TTC39B, CYP2J19, and BDH1L), which were newly identified for their roles in carotenoids ketocarotenoid metabolism and integrated into both cell lines using lentiviral vectors. Following the infection, cells were treated with dietary carotenoids such as zeaxanthin, β -carotenoids and lutein, and their metabolic products were analyzed using High-Performance Liquid Chromatography (HPLC). Preliminary investigations revealed successful integration and expression of the three genes in the targeted cell lines. These stably transduced in vitro systems offer a powerful tool to study mechanisms controlling carotenoids metabolism, enriching our understanding of mate choice dynamics and evolutionary biology.

The headaches of brain stereotactic surgery: Behavioral effects in green anole lizards.

Niveditha Sankar, Rachel Cohen

The amygdala is a complex brain substructure, whose role in aggression in male green anole lizards (*Anolis carolinensis*) is still unknown. Interestingly, previous work has shown that the anole amygdala has more neurons during the non-breeding season, when the animals are not territorial, suggesting a potential relationship between aggression and the amygdala in this species. This study aims to understand the role of the amygdala by injecting a neurotoxin (staurosporine) into the area and investigating impacts on aggressive behavior in non-breeding lizards. However, this first requires that the surgery itself is not associated with a change in aggression and that appropriate behavior tests are used to investigate aggression. Accordingly, aggressive behavior displays of focal lizards against mirror images (M) and intruders (I) were recorded and quantified before and 3 days after the stereotactic injection of a retrograde tracer (5% microruby in saline) in breeding male lizards. Brains were collected, fixed, and sectioned. The injected sites were visualized

by immunohistochemistry and Nissl staining. There was no significant difference between pre and post-surgery aggressive displays exhibited against M ($t(3)=0.90$, $p=0.433$) and I ($t(3)=0.94$, $p=0.418$), and I elicited a greater response ($t(6)=2.9$, $p=0.028$), suggesting that surgery does not impact aggression. We plan to perform similar stereotactic surgery using staurosporine to lesion the amygdala of non-breeding lizards and evaluate their pre and post-surgery behaviors against size-matched male intruders.

Relationship between volumic distribution and mode of undulatory locomotion in lizards

Mizuho Sano, Shin-ichi Fujiwara

Lizards employ horizontal undulatory locomotion. However, the relative amplitudes of the trunk and tail varies among species, which gives the difference in locomotor strategies among lizards. Assuming the lizard body as bidirectional pendulums (pre- and postsacral bodys) about the sacrum, yaw moment of the tail is expected to encounter the yaw moment of the presacral body to balance the body during gait. Therefore, the yaw moment of the tail in relation to the yaw moment of the body is expected to reflect the gait style. To test this hypothesis, we get CT slices 8 cadaveric lizard specimens of 8 families, and constructed 3D models of the whole body. Assuming that the density is constant, the moment inertia of pre- and post-sacral bodies (I_{pre} and I_{post} , respectively) were estimated for each specimen, and the ratio between I_{pre} and I_{post} was used as an index for the effect of tail-swing on the body undulation. Walking motions of the same lizard species were filmed from dorsal view at the Higashiyama Zoo to compare the index with amplitude ratio of the tail and body. According to the comparison, we found that lizards with larger I_{post}/I_{pre} value walked with greater waves of the body.

3D EM reconstructions of neurons in the nudibranch, Berghia reveal novel ultrastructural features

Harshada Sant, Kriti Dhiman, Ashley Glover, Brandon Drescher, Yuelong Wu, Richard Schalek, Jeff Lichtman, Paul Katz, Sarah DeAmicis

Volume electron microscopy (vEM) exposes features of nervous systems that are inaccessible through other techniques. Here we segmented and reconstructed neurons from a vEM dataset obtained from the rhinophore ganglion (rhg) of the nudibranch mollusc, *Berghia stephanieae*. The rhg contains 9000 somata whose functions and axonal projections are not known. Half of the rhg including the connective to the cerebral ganglion (ceg) was serially sectioned at 33 nm thickness and imaged using SEM, generating a dataset of 2,175 sections with 4x4 nm lateral resolution. There were distinct neuropil regions including one receiving axonal projections from a peptidergic ceg neuron. We reconstructed several neurons, including one that had a soma in the rhg and an axon that projected into the connective. Its dendrites lacked vesicles but contacted many vesicle-filled presynaptic boutons. We also reconstructed an axon terminal arbor that branched profusely in the neuropil region occupied by the terminals of the peptidergic ceg neuron, which contained many vesicle-filled varicosities - likely sites of peptide release. We also found novel structures, namely independent vesicle-filled boutons that were free of axon attachments. Some, but not all of these independent boutons were surrounded by glial cells suggesting that they might be in the process of degradation. The features that we uncovered using vEM reconstruction could help generate hypotheses about the function of this enigmatic ganglion.

Active tension driven soft coral pulsing: Exploring emergent behavior through elasticity and tension

Matea Santiago, Alexander Hoover, Alyssa Connolly, Laura Miller

Octocorals of the Xenidae family pulsate their tentacles, featuring active muscle contractions and subsequent passive expansions, similar to locomotion observed in other motile marine invertebrates. However, unlike most motile marine invertebrates, these corals are sessile animals and do not locomote. Past work has indicated that the pulsing mixes the surrounding ocean water, enhancing the symbiotic photosynthesis of their zooxanthellae, it is hypothesized that this symbiotic photosynthesis is the coral's main energy source. Prior computational studies have used laboratory data to directly prescribe

motion to the coral body, this work instead models the muscle contraction as an applied active tension. The expansion results from the passive elasticity defined on the coral body through a spatially varying elastic modulus. By varying the maximum tension and elastic modulus, the role of the tension and elasticity on the emergent kinematics and flow structures is explored. To simulate the two-way coupling between the elastic coral body and the surrounding seawater, modeled as an incompressible Newtonian fluid, the immersed finite element-finite difference (IFED) method implementation in the software library IBAMR is used.

Protecting the Farm: Widespread Biomineral Armor in the Fungus-Farming Ants

Joseph Sardina, Cameron Currie

Biomineralization, the biogenic process of forming minerals, has been a major innovation in the evolutionary history of animals. Biomineral structures, particularly calcium carbonate (CaCO₃)-based, became widespread during the Cambrian explosion, apparently due to increasing predation pressure. Given the common function of CaCO₃ biominerals in predator defense, and that Magnesium-enrichment of calcite has been shown to coincide with a significant increase in mineral hardness, the apparent rarity of Mg-enriched calcite in land animals has been surprising. Recently, a high Mg-calcite (HMC) biomineral was reported in one species of fungus-farming (“attine”) ant, the leaf-cutter *Acromyrmex echinator*, the first known calcareous structure found in any insect; the HMC forms an exo-layer covering the ants outer cuticle, and was shown to function as an armor protecting the ants from both macro-predators and micro-parasites. Here, I explore the phylogenetic breadth and morphological variation of the biomineral armor across the fungus-farming ant group, a monophyletic clade composed of 250+ species which originated over 50 million years ago. Additionally, I investigate the potential role of their symbiotic bacteria, the Actinobacteria *Pseudonocardia*, in the formation of the ant’s biomineral. More broadly, this work sheds light on the evolutionary history of a rare high Mg-calcite biomineral formed by the fungus-farming ants.

Tiny Backpacks: Experimentally Monitoring the Behavior of Radio-Tagged Hummingbirds in Colombia

Alyssa Sargent, Ana Melisa Fernandes, Rosalee Elting, Samantha-Lynn Martinez, Aeris Clarkson, Laney Hansell, Alexandra Coenen, Talwekar Yash, Miguel Angel Muñoz-Amaya, Nicolás Téllez-Colmenares, Alejandro Rico-Guevara

Though the number of movement ecology studies has risen exponentially, those of biologging ethics (the study of animals’ welfare while equipped with auxiliary markers) have not kept pace. Yet tracking devices and their attachment methods are not only an energetic and literal burden to animals, but also a possible hindrance to performing various behaviors. Given that numerous wildlife tags have recently become miniaturized enough to work with some of the smallest of vertebrates, there is a newfound urgency for biologging ethics studies, particularly affordable, field-accessible ones. In our research, we designed a time-budget experiment to answer the following question: how do radio-transmitters affect hummingbirds’ behavior? Using a large flight arena in Colombia, we individually filmed 25 Black-throated Mangos (*Anthracothorax nigricollis*)—a territorial species that relies on extreme maneuverability and energy-use for aggressive encounters—under two randomized treatments, tagged and untagged, to quantify each bird’s “degree of departure from the norm” when wearing a radio-transmitter backpack harness. We analyzed all videos using the Behavioral Observation Research Interactive Software (BORIS), to create time-budget breakdowns of our key behaviors of interest: flying, perching, preening, and feeding. Given the logistical difficulty in designing and executing rigorous biologging welfare studies, we aimed to design an experimental model that was a) relatively straightforward to fine-tune to other small taxa, and b) suitable for remote field conditions.

Learning Through Games: A Case-Study in Urban Outreach

Alyssa Sargent, June Pen, Caleb Stockham, Katharine Canning, Rachael Canada, Andrea Rockwood, Andy Clark, Alejandro Rico-Guevara

As environmental conditions and conflicts worsen, the need for effective outreach programs increases. Given the extreme losses amongst bird and pollinator populations in recent decades, we face a worrying combination of humans’ relative lack of empathy for birds and paltry media attention for the pollinator population crisis. However, creative communication approaches, spanning socioeconomic and neurodivergent divides, can combat misinformation while simultaneously promoting science equity. Though traditional lectures may be limited in their capacity to facilitate learning, interactive instruction can immerse students who process information differently, particularly those who gravitate toward tactile and kinesthetic learning. Games are especially effective for imparting complex scientific knowledge—including trade-offs pertaining to wildlife. To explore this approach, I have conceived and developed “Hummingbird Sugar Rush”: a game-based STEM curriculum in which students embody hummingbirds with different strengths and weaknesses (depending on their real-life foraging strategies) and race to collect the most nectar. Through this curriculum, which we will direct with middle schoolers from underrepresented groups across the Seattle area, we are working to instill knowledge, empathy, and excitement for the natural world.

Investigating Relationships among Microbiome, Disease Severity, and Diet in Green Sea Turtles

Christine Sarkis, Erin Seney, Brandon Hoenig, Anna Forsman

The gut microbiome is the bacterial community that inhabits the digestive tract and is essential for host health and proper immune system function. Fibropapillomatosis (FP) is a disease that causes the growth of external and/or internal tumors on green sea turtles (*Chelonia mydas*). While past work has found correlations between FP severity and broad metrics of microbiome diversity, the overarching relationship between the gut microbiome and this disease is not yet fully understood. The objective of this study was to capitalize on our large collection of colon swabs from FP-infected and uninfected individuals to provide a more comprehensive examination of the gut microbiomes’ response to FP infection. Further, as the gut microbiome of sea turtles has been shown to be influenced by diet, we also investigated how the microbiome differs based on diet composition. We analyzed 170 colon swabs collected from stranded green turtles (Florida, USA) with high-throughput DNA sequencing of the prokaryotic 16S gene to characterize the microbiome, as well as the eukaryotic COI and 18S genes for diet analysis. The sequences were analyzed using QIIME2 for quality control and taxonomic identification. The results from this study will provide critical data to inform our ongoing studies in the context of life history, ecology, and disease dynamics of federally protected green turtles and other sea turtle species.

Forces of nature: flying fish taxi and takeoff mechanics

Valeria Saro-Cortes, Brooke Flammang, Aimy Wissa

The taxi locomotion of a flying fish, where the animal uses the ocean surface as a runway for takeoff, is simultaneously the most unique and the most understudied stage of the fish’s locomotion. The caudal fin is used both during taxiing and swimming. However, the fluid-structure interaction mechanisms for both locomotion modes are different due to the proximity of the caudal fin to the air-water interface during taxiing. To investigate the differences between taxiing and swimming, we have built a biologically relevant robotic model organism (RMO) based on flying fish. In this talk, we examine the effect of the submersion depth of the caudal fin on the resulting hydrodynamic force and caudal fin kinematics. The results are compared to previous observations about the submersion depth of the caudal fin during taxiing. Moreover, we present simplified analytical models to develop hypotheses about the fin submersion depth required for taxiing and taking off. Such models and experiments will shed light on the physical requirements for this transitional mode of locomotion for flying fish and aid in developing uncrewed vehicles capable of operating successfully at the air-water interface.

Increased metabolic rate and mass loss associated with peritoneal fibrosis in threespine stickleback

Trey Sasser, Jesse Weber

The immune responses that organisms evolve to avoid or dampen the costs of parasitism are energetically costly to maintain and deploy. Quantifying these costs can inform the evolutionary dynamics of immunity. Peritoneal fibrosis is a well-studied immune response that stunts the growth of cestodes in threespine stickleback (*Gasterosteus aculeatus*) but comes at the cost of reduced host fecundity. We induced fibrosis and measured metabolic rates and body condition to quantify the energetic cost of mounting this response under laboratory conditions. We also induced the same immune response in a semi-natural mesocosm experiment to test for effects on host fecundity. Fibrosis was associated with an 11% increase in SMR and 4% decline in mass 10 days post-injection under lab conditions. Fibrosis was associated with 6% lower body mass and 34% lower ovary mass 2 months post-injection in the mesocosm. Additionally, the magnitude of fibrosis correlated positively with complementary measures of physiological cost in both the lab and mesocosm experiments. Taken together, these results provide strong evidence that fibrosis entails significant energetic costs that manifest in declines in body mass and likely underpin reductions in fecundity. We will also present the preliminary results of a survey of wild stickleback investigating potential trade-offs between life history and immune phenotypes including growth rate, lifespan, tapeworm infection prevalence, and maintaining the ability to fibrose.

Locomotion affects Patterns of Fin Integration in Coral Reef Fishes

Darien Satterfield, Bernice Yin, Sky Jung, Samantha Hodges-List, Michael Burns, Peter Wainwright

Patterns of evolutionary integration in limbs have been shown to be affected by differences in locomotor uses, such as the need to climb at an early developmental age in marsupials relative to placental mammals, and differences in flight behaviors in birds. However, in fish, diversity in fin shape has yet to be explored across species with diverse locomotor styles. In this study, we describe the morphospaces of the pectoral, caudal, dorsal, and anal fins, and test for the effects of locomotor strategy on the patterns of evolutionary integration between the fins. We cleared and stained, and photographed the fins and jaws of 119 species from the 40 most common reef fish families. Fin shapes were quantified using geometric morphometrics. We found that fish who swim predominantly with their body and caudal fin (BCF) have significantly different patterns of fin integration relative to fish that swim with their median and paired fins (MPF). Specifically, in BCF swimmers fin pairs are almost all significantly integrated, with the exception of the caudal and dorsal fin. However, in MPF swimmers, the pectoral fin has lost all integration with any other fin. Further, we find that evolutionary rate is slowest for pectoral fins, particularly in MPF swimmers. Thus, the evolutionary independence of the pectoral fin likely reflects evolutionary constraints due to the strong reliance on the pectoral as a propulsor for MPF swimmers.

Paternal behavior and disease history interact to influence offspring immune phenotypes

Erin Sauer, Sakura Roberts, Johnathan Novotny, Weston Perrine, Madeline Sudnick, Sarah DuRant

Disease imposes strong selective pressures on hosts, resulting in the evolution of behaviors that avoid exposure or boost resistance. In addition, changes to parental behaviors can shape offspring developmental environments, which can have effects on offspring phenotypes and plasticity. Thus, parental behavior can act as a mechanism for non-genomic contributions to offspring immunity, priming offspring for an environment with disease. However, it is difficult to parse physiological input (e.g. egg content, heritable traits) from behavioral input (e.g. incubation temperatures, resource provisioning). Here, we use a cross-fostering experiment to examine how maternal disease history interacts with maternal behavior to influence offspring immune phenotypes. Female domestic canaries were exposed to *Mycoplasma gallisepticum* (MG) or a sham then paired for breeding after recovering. Immediately after laying, eggs were given to foster mothers in a factorial design, maternal status

(biological or foster) by disease history (prior MG or sham exposure), to parse physiological and behavioral maternal effects. Prior to fledging, we monitored incubation temperature and nestling attendance. After fledging, offspring were exposed to MG. We found that both the biological and foster mother's disease history affected offspring outcomes and that offspring of MG-exposed foster mothers had greater MG resistance than those fostered by unexposed mothers. This study provides insight into previously unexplored aspects of behaviorally driven transgenerational effects of disease in avian systems.

Investigating potential priming of rhinophore regeneration in *Berghia stephanieae*

William Scala, Haleigh Bilodeau, James Newcomb

Recent evidence suggests that the axolotl, *Ambystoma mexicanum*, exhibits systemic priming in response to limb amputation, such that a subsequent lesion will regenerate at a faster rate. The nudibranch *Berghia stephanieae* can also regenerate body parts, such as its chemosensory rhinophores, and the purpose of this study was to investigate if this species could also exhibit systemic priming of this regenerative process. In control animals, just one rhinophore was lesioned, while in the experimental group, after initial rhinophore amputation, the contralateral rhinophore was also lesioned a week later. We took measurements at multiple timepoints over 21 days to determine the rate of growth of each rhinophore in both groups. There was no significant difference in regeneration rates between any of the groups, suggesting that *Berghia* may not exhibit systemic priming after initial injury, and that this may be a derived feature of regeneration in vertebrates, such as *Ambystoma*. The large variance in regeneration rates between individual *Berghia* suggests that it may be beneficial to repeat this experiment and increase the sample sizes to further test for systemic priming of regeneration in *Berghia*.

The subpectoral diverticulum in hawks and other soaring birds: not just another air sac

Emma Schachner, Andrew Moore, Aracely Martinez, Raul Diaz-Jr, Scott Echols, Jessie Atterholt, Roger Kissane, Brandon Hedrick, Karl Bates

Extending from the avian respiratory system is a structurally diverse series of secondary diverticula that wrap around muscles and joints, dive under the skin, and in many taxa pneumatize the postcranial skeleton. In hawks, there is a large pulmonary diverticulum (the SPD) that branches off of the interclavicular air sac, leaves the coelomic cavity, and dives between the pectoralis and supracoracoideus muscles. Here we evaluate the function of the SPD in the red-tailed hawk (*Buteo jamaicensis*; n=7) and Swainson's hawk (*Buteo swainsoni*; n=2) using gross dissection, muscle fiber analysis, microCT, 3D segmentation, and 3D multibody dynamics (MDA) models. We additionally evaluate the presence/absence of the SPD in 68 phylogenetically diverse avian taxa via CT/microCT (n=57) or the literature (n=11). Our data demonstrate that this diverticulum does not appear to be integral for ventilation as sedated birds can asymmetrically collapse the SPD, and shift inflation levels during an individual breath cycle. MDA models show that when inflated, the SPD significantly increases the moment arm of the cranial fibers of the pectoralis muscle. These data demonstrate that the SPD evolved independently in concert with dynamic or thermal soaring at least seven times in phylogenetically disparate lineages of birds, and the SPD impacts the flight ecology of hawks and other soaring birds.

Does sexual dimorphism vary across multiple populations of a socially polyandrous shorebird?

Jessica Schaefer, Tessa Patton, Alexandra Juárez, Thomas Hahn, Gail Patricelli, Misha Blizard, Sara Lipshutz

Across species, the direction and magnitude of sexual dimorphism is often associated with mating system. However, sexual dimorphism may also vary among populations within the same species, since ecological conditions modulate mating competition and parental behavior within species. Spotted Sandpipers (*Actitis macularia*) are widely distributed, socially polyandrous shorebirds that display female-biased size dimorphism and female competition for mates, yet the rate of polyandry varies among populations and years. To

explore whether this behavioral variation corresponds with sexual size dimorphism, we compared three populations of Spotted Sandpipers that differ in breeding habitat, elevation, and population density. We collected morphometrics from a population breeding in the Eastern Sierra Nevada and calculated the magnitude of sexual dimorphism for bill length, tarsus length, wing length, and body mass. We then contrasted our findings with published data from populations at Leech Lake, Minnesota and Beaver Island, Michigan. We found variation in the magnitude, but not direction, of sexual dimorphism among the three populations, suggesting the magnitude of intrasexual competition may also vary among these populations. Ongoing research incorporating genetic parentage analysis and ecological data from Spotted Sandpiper breeding sites will further elucidate the links between ecology, mating system, and sexual dimorphism.

Washington Northern Leopard Frog Recovery: Post-release Monitoring of Behavior and Predator Threats

Jonathan Schafer, Erica Crespi, Caren Goldberg, Jeff Manning, Robert Pearhill, Alexa Dulmage, Christina Kiepe, Emily Grabowsky, Adam Haines

Captive rearing programs for amphibians are designed to increase population size by mitigating the high mortality rates experienced by early life stages in natural systems. However, success of these programs has been limited, potentially because captive-reared individuals may not exhibit the behaviors necessary to survive in the wild. We assessed behavioral responsiveness and space use by captive-reared Northern Leopard Frogs (*Lithobates pipiens*, NLFs) and their predators during the early phase of soft releases in two ponds in the Columbia National Wildlife Refuge in summers 2021 and 2022. First, we conducted customized flight response assays at the release site to determine whether captive-reared frogs responded to an oncoming visual/audio cue similarly to that measured in wild juvenile frogs at a natal site. In both years, captive-reared frogs were less responsive to the visual/audio cue than wild frogs, but responsiveness improved after 5 days in soft-release enclosures. Daily post-release observations revealed predation by garter snakes and an increased presence of bullfrogs around the soft-release enclosures over time, and radiotracking of bullfrogs confirmed this pronounced spatial shift towards enclosures. These findings suggest predation risk is high at this reintroduction site, and a need to alter captive rearing conditions to increase the expression of anti-predator behavior post-metamorphosis. Future work will test the ‘match hypothesis’ by exposing captive-reared larvae to non-lethal predator cues to achieve this goal.

Effects of substrate height on arboreal locomotion in wild lemurs

Nicole Schapker, Judith Janisch, Lydia Myers, Taylor Phelps, Liza Shapiro, Jesse Young

A misstep during arboreal locomotion can lead to risky falls. By using more stable forms of locomotion on higher substrates, arboreal animals might avoid potentially fatal consequences. Primates are thought to be especially adept at arboreal locomotion, but we know relatively little about how a primate's perception of its environment, e.g. substrate height, might influence locomotor behavior. Using high-speed cameras, we recorded the quadrupedal locomotion of four free-ranging lemur species in Madagascar – *Eulemur rubriventer*, *Eulemur rufifrons*, *Lemur catta*, and *Haplorhina aureus*. We quantified the diameter, orientation, and height of locomotor supports using remote sensors and tested the influence of these parameters on gait kinematics ($N=112$). Lemurs did not adjust stride speed, length, or frequency on substrates of varying height. However, *E. rubriventer* increased the mean number of supporting limbs on higher substrates, and lemurs overall decreased the use of unstable limb support patterns and increased the use of stable ones on higher substrates. Results suggest walking is a relatively safe form of locomotion for lemurs, resulting in subtle changes in gait to increase stability on higher – i.e., riskier – substrates. Continued investigation of the impact of height on locomotion will be important to determine how primates and other animals assess risk in their environment and if these assessments are used to deliberately move more safely.

Breaking the Fourth Wall: Interrogating the obstacles to DEIJ efforts and scientific innovation

Christopher Schell

The ascension of transformative social movements, from #MeToo to Black Lives Matter and beyond, have fueled necessary and overdue calls for action to make substantive changes across all sectors. Academic institutions specifically have been compelled to increase their diversity and inclusion initiatives, hire more staff and faculty across gender, racial/ethnic, and disciplinary lines, and build decolonized and abolitionist curricula. Indeed, extraordinary strides, innovations, and essential disruptions have improved some aspects of our fields. Yet, despite the groundswell of vocal support to change how our institutions function and how research is conducted, many of the fundamental changes we hoped would materialize have stagnated. Frustrated and exhausted students, staff, and faculty – especially those from marginalized or minoritized backgrounds – are still experiencing extraordinary rates of burnout, depression, and lack of job fulfillment, forcing many to opt out of the academic space. Why has advancement been inadequate? And what must we do to fix our seemingly perpetual dilemma? In this talk, Dr. Chris Schell will address how our most monumental task as integrative biologists is to interrogate how our own academic and institutional structures operate. This task runs in parallel to diagnosing why DEIJ initiatives as currently constructed are ineffectual in achieving the very goals they were designed to achieve: improving diversity, equity, inclusion, and justice in our disciplines. In addition, Dr. Schell will discuss how incorporating his intersectional identity into his urban ecology work has helped forge new avenues of research that expand the disciplinary narrative on how we conduct our research.

Mechanical design for improved foot-ground interactions in complex terrains

Perrin Schiebel, Alyssa Hernandez, Robert Wood

Despite great advances, man-made locomotors cannot yet replicate biological organisms' ability to robustly traverse natural terrains. These unpredictable environments include irregularities such as deformable materials and random asperities that stymie locomotion by causing slipping and tripping, or even trapped limbs and falls. One observable difference between robot and animal morphology is the great diversity and often intricate design of biological “end effectors”, e.g., feet. The terrain-appropriate mechanical design of feet can enhance performance while reducing the need for costly sensing and closed-loop control. Including elements which passively mediate foot-ground interactions, like a joint which dissipates or stores energy or spines that interlock with terrain features to increase grip, can increase the probability that a step generates propulsion while simultaneously reducing the likelihood for disruptive perturbations and restrictions. I will discuss work on the study of beetle-tarsus-inspired features to improve locomotion of the insect-scale Harvard Ambulatory Microrobot in dense, random, foot-scale terrain asperities. We first explored separately the impact of compliance and spines on performance and force generation, then, using what we learned in these systematic studies, combined these elements to expand the range of terrains the robot could successfully traverse. This work is a step toward a broader understanding of foot design in biology and its application to robotics.

Characterization of the developing ventral midline in the bilaterian annelid *Capitella teleta*

Tatiana Schmid, Neva Meyer

A synapomorphy of Bilateria is a bilaterally symmetric body plan, which relies on formation of a midline during development. In some taxa these genetically-distinct cells subsequently pattern surrounding tissues. However, just because cells are positioned at the midline during development does not mean they are homologous to midline cells in other taxa. In some spiralian larvae, ventral midline cells are ciliated and express spiralian-specific genes, collectively forming a ciliated band called the neurotroch. To test homology of the neurotroch in the spiralian annelid *Capitella teleta* to signaling midline cells in other groups, we characterized expression of *Cte-slit* and *Cte-netrin*, homologs of vertebrate and arthropod secreted midline proteins. Additionally, because BMP and Wnt signaling regulate dorsal-ventral axis specification including

midline formation in some bilaterians, we incubated *C. teleta* embryos in BMP protein or the Wnt agonist azakenpaullone and assessed Cte-slit expression and neurotroch formation. Cte-slit and Cte-netrin are expressed on either side of neurotroch cells in *C. teleta*, contrasting the ventral or dorsal midline expression in insects and vertebrates, respectively. Furthermore, neither ectopic BMP nor Wnt signaling caused a loss of Cte-slit expression despite loss of the neurotroch in BMP-treated animals. These findings suggest that while neurotroch cells form the morphological ventral midline, cells on either side of the midline in *C. teleta* may be homologous to midline cells in other animals.

Evolution of scleral ring and orbit size in gekkotan lizards

Lars Schmitz, Adam Ost, Bianca Howell, Molly Bradshaw, Alexandra Towers, Aaron Bauer, Juan Daza

Gekkota is a species-rich clade of squamates with a complex evolutionary history of diel activity pattern (DAP). Geckos are originally nocturnal, which may have been facilitated by the loss of the postorbital bar, allowing for the evolution of proportionately larger eyes. The majority of the Gekkota remain primarily night-active, but evolutionary reversals to diurnal activity have occurred multiple times. DAP, as an important determinant of photic environments, has been interpreted as a major explanatory factor of the phenotypic diversity of eyes. Using high-resolution CT scans, we collected morphometric data on scleral ring and orbit proportions for 111 gekkotan species to test whether the diversity of these visual performance features was impacted by DAP. Phylogenetic comparative analyses suggest that most diurnal geckos have smaller relative orbit size than nocturnal geckos. Limb-reduced pygopods drive this pattern to the extreme, which could be a combined effect of their DAP, habitat, and transformations towards an elongated skull. The orbit and scleral ring shape of diurnal gekkotans, including pygopods and many sphaerodactylids, are convergent and different from nocturnal geckos. We observed several exceptions to this pattern, with some primarily diurnal species clustering in the nocturnal area of the morphospace. Our results document the adaptive impact of DAP on eye shape, but also suggest that gekkotan eye morphology is variable, and evolved different trait combinations even when occupying similar photic environments.

Smallmouth startles: Extreme temperatures reduce responsiveness and increase escape latency

Molly Schneider, Grace Johnston, Emily Volpe, Katrina Whitlow

Escape response performance is often used to predict successful evasion of predators in the wild, and therefore has important implications for survival and fitness. Both the behavioral and musculoskeletal aspects of an escape are impacted by ambient water temperatures, though the relationships are complex and differ across species. Micropterus dolomieu (smallmouth bass) are a fish species native to many freshwater regions of the United States. This species is predicted to be particularly vulnerable to temperature shifts due to their limited thermal tolerance. This study examined how temperature impacts escape response in smallmouth bass. Fish were collected from the St. Joseph's and Elkhart River in South Bend, Indiana and slowly acclimated to the trial temperatures (15, 20, 25, and 30°C) for at least seven days before testing. Escape responses were elicited using a rubber stopper and were recorded using a high-speed camera. Preliminary results suggest responsiveness is maximal at 25°C. Interestingly, mean latency (time from stimulus to response) did not differ across treatments, but extreme temperatures elicited more variable response times. This study will help illustrate how smallmouth bass could respond to the rising water temperatures from climate change, as well as provide more information about species fitness under these changing conditions.

Muscle activation patterns of multiple feeding strategies in chameleons

Nikole Schneider, Christopher Anderson

Chameleons are notorious for their extremely specialized feeding mechanism, where the tongue is ballistically projected out of the mouth to capture prey items more than two body lengths away. However, chameleons have also been documented approaching slower or nonmobile prey items and capturing them

using only their jaws (i.e., direct prey capture). It is unknown whether these two strategies utilize the same underlying muscle activation patterns or if direct prey capture necessitates the use of independent motor control patterns during feeding. We compared the muscle activation patterns of chameleons feeding by ballistic tongue projection and direct prey capture, quantifying the duration of muscle activity, the latency between muscle activity onset to prey contact, and muscle activity intensity (root mean squared of muscle activity) in five muscles associated with feeding and tongue movement. We found that the duration of activation was much more variable for direct prey capture than ballistic projection and the latency period was shorter during projection than direct prey capture for all muscles. Additionally, the intensity of muscle activation was on average greater during ballistic tongue projection than in direct prey capture, with some muscles rarely active. These results provide insight into the mechanisms by which highly specialized behaviors can be utilized to perform additional, disparate behaviors, and on the principle of neuromotor conservation during the evolution of novel behaviors.

BMP signaling instructs dorsal vs. ventral cell lineages in a marine annelid

Stephan Schneider

How early signaling events determine cell fates in spiral-cleaving embryos like the annelid *Platynereis dumerilii* is still not understood on a single cell level. In this embryo the first two asymmetric cell divisions generate four progenitor cells with the smaller A and B, and the larger C and D cells. Each of these four progenitor cells will divide to form one smaller animal-pole daughter cell or micromere (1a, 1b, 1c, 1d), and one larger vegetal-pole daughter cell (1A, 1B, 1C, 1D). The progeny of these four micromeres will form the ventral (1a, 1b) and dorsal (1c, 1d) head region of the developing larva. We identified an early acting BMP signaling event that is required to instruct the progeny of animal micromeres 1c and 1d to form cell lineages that adopt dorsal cell fates. Inhibition of BMP signaling causes the cell progeny of 1c and 1d to adopt ventral cell fates similar to ones of the micromeres 1a and 1b in normal development, and as a consequence the head region of the larva is ventralized in these embryos. Thus, we will present evidence for a BMP activity instrumental to establish dorsal vs ventral cell fates that acts perpendicular and overlapping to a beta-catenin-mediated specification system along the animal-vegetal axis creating a Cartesian coordinate patterning system in this early annelid embryo.

Examining the relationship between habitat and color diversification in darter fishes

Natalie Schroth, Maya Stokes, Jessica Arbour

Coloration serves many important functions among clades, including mate selection, kin recognition, and predator avoidance. Although ecological pressures act upon the color pattern evolution within groups, the impact these pressures have on said evolution is poorly understood. Within family Percidae, the often brightly colored subfamily Etheostominae (lotic “darter” fishes) serves as an excellent study group with more than 200 species. To better understand the macroevolutionary relationships between darter coloration and habitat, we used adjacency analysis to quantify color variation from photographs of wild caught specimens. We gathered habitat data for each species in the dataset from GIS databases and species accounts. Using the R package phytools, phylogenetic canonical correlation analysis was used to identify and measure any relationships between habitat and coloration characteristics. Cluster analysis was used to group major habitat types, and Multivariate BM models were used to test for heterogeneity in color diversification rates between macro- and micro-habitat types. We found a weak but significant relationship between habitat types and coloration. Evolutionary modelling revealed rapid diversification in rivers with large drainages, high discharge, low slope, and soft substrates. However evolutionary rate variation associated with micro-habitat data (i.e., riffles vs pools) showed the greatest support and demonstrated faster diversification in riffle-associated species. Our results suggest that although habitat is not strongly associated with specific coloration, it is a driver of diversification in Etheostominae.

Resilience to high temperature and hypoxia in fish: view from the genome and epigenome

Patricia Schulte

In this era of anthropogenic climate change, assessing the resilience of populations, species, and ecosystems is a critical task. However, understanding the factors that determine resilience is complex because resilience at one biological scale is determined by complex interactions of factors at lower levels of biological organization. Here, I explore how standing genetic variation and various types of plasticity, including epigenetic effects, influence the resilience of fishes to climate-change relevant stressors such as high temperature and low oxygen (hypoxia). Examples from killifish and rainbow trout demonstrate that there is substantial genetically based variation in both upper thermal tolerance and hypoxia tolerance within and between populations that could allow rapid adaptation to environmental change. There is also substantial plasticity in both of these traits, and using examples from stickleback and white sturgeon, we have shown that these species demonstrate substantial resilience to increased temperature, including exposure to heatwaves. This plasticity is also associated with improved cross-tolerance to additional acute stressors and involves rapid responses in both epigenetic and transcriptional mechanisms. Taken together, these data suggest that there is potential for both evolutionary adaptation and plasticity in the face of increased temperature and decreased oxygen, but also that the capacity for resilience has clear limits. Thus, as climate change progresses, more and more populations and species will be approaching these limits to resilience.

Beyond Thermoregulation: UV and Temperature Regulation by Tree Lizards along an Ecological Gradient

Andreanna Schultz, Matthew Lattanzio

Historically the maintenance of physiological homeostasis in ectotherms via basking and shuttling behavior has been understood through the lens of body temperature regulation alone. However, lizards also behaviorally regulate their exposure to ultraviolet (UV) light, which is crucial for organ function and bone development. And, while lizards may be able to sustain preferred body temperatures in the shade, exposure to direct sunlight is necessary to manage their UV needs. This disparity, coupled with evidence that lizards may even prioritize UV over temperature needs, supports the hypothesis that trade-offs in effectiveness at regulating both conditions should occur across distinct microhabitats as well as ecological gradients. We combine field surveys and laboratory behavioral data to address this hypothesis using adult tree lizards (*Urosaurus ornatus*) captured along an elevation gradient. Based on prior work, we predict that the ability of *U. ornatus* to regulate its body temperature effectively will increase with elevation, but their UV regulation effectiveness will be more strongly impacted by microhabitat use. Our findings largely aligned to these predictions, but microhabitat use also partly influenced temperature regulation. In general, lizards occupying living trees regulated both conditions better than lizards using dead trees, regardless of elevation. Overall, ecological factors long assumed to influence homeostasis mainly via thermoregulatory constraints may actually have stronger fitness implications for a species' ability to regulate its UV effectively.

Adapting a high-fidelity simulation of human skin for comparative touch sensing in an elephant trunk

Andrew Schulz, Gokhan Serhat, Katherine Kuchenbecker

Skin is a complex biological composite consisting of layers with distinct mechanical properties, morphologies, and mechanosensory capabilities. This work seeks to expand the comparative biomechanics field to comparative haptics, analyzing elephant trunk touch by redesigning a previously published human finger-pad model with morphological parameters measured from an elephant trunk. The dorsal surface of the elephant trunk has a thick, wrinkled epidermis covered with whiskers at the distal tip and deep folds at the proximal base. We hypothesize that this thick dorsal skin protects the trunk from mechanical damage but significantly dulls its tactile sensing ability. To facilitate safe and dexterous motion, the distributed dorsal whiskers might serve as pre-touch antennae, transmitting an amplified version of impending contact to the mechanoreceptors beneath the elephant's armor. We tested these

hypotheses by simulating soft tissue deformation through high-fidelity finite element analyses involving representative skin layers and whiskers, modeled based on frozen African elephant trunk (*Loxodonta africana*) morphology. For a typical contact force, quintupling the stratum corneum thickness to match dorsal trunk skin reduces the von Mises stress communicated to the dermis by 18%. However, adding a whisker offsets this dulled sensing, as hypothesized, amplifying the stress by more than 15 at the same location. We hope this work will motivate further investigations of mammalian touch using approaches and models from the ample literature on human touch.

Whiskers that don't whisk: Unique structure from the absence of actuation in elephant whiskers

Andrew Schulz, Lena Kaufmann, Michael Brecht, Gunther Richter, Katherine Kuchenbecker

Whiskers are so named because these hairs often actuate circularly, whisking, via collagen wrapping at the root of the hair follicle to increase their sensing volumes. Elephant trunks are a unique case study for whiskers, as the dorsal and lateral sections of the elephant proboscis have scattered sensory hairs that lack individual actuation. We hypothesize that the actuation limitations of these non-whisking whiskers led to anisotropic morphology and non-homogeneous composition to meet the animal's sensory needs. To test these hypotheses, we examined trunk whiskers from a 35-year-old female African savannah elephant (*Loxodonta africana*). Whisker morphology was evaluated through micro-CT and polarized light microscopy. The whiskers from the distal tip of the trunk were found to be axially asymmetric, with an ovular cross-section at the root, shifting to a near-square cross-section at the point. Nanoindentation and additional microscopy revealed that elephant whiskers have a composition unlike any other mammalian hair ever studied: we recorded an elastic modulus of 3 GPa at the root and 0.05 GPa at the point of a single 4-cm-long whisker. This work challenges the assumption that hairs have circular cross-sections and isotropic mechanical properties. With such striking differences compared to other mammals, including the mouse (*Mus musculus*), rat (*Rattus norvegicus*), and cat (*Felis catus*), we conclude that whisker morphology and composition play distinct and complementary roles in elephant trunk mechanosensing.

Effects of temperature acclimation on metabolism and behaviour in sheepshead minnows

Madison Schumm, Kerri Ackerly, Andrew Esbaugh

Marine fish are expected to cope with environmental stress through modifications to their physiology and behaviour. Thermal stress is of particular concern for fish because increased temperatures lead to elevated baseline metabolic demand. Increases in metabolism must be supported by concomitant increases in resource uptake and supply, and thus, may be coupled to the expression of behavioural traits that contribute to resource acquisition. Energy demand may further increase risk-taking and reduce behaviours that diminish predation risk in fish (e.g., shoaling and scototaxis). On this background, our goal was to evaluate changes to behavioral traits, metabolic rate and the relationship between behaviour and metabolic rate across two temperatures in an estuarine model teleost, the sheepshead minnow (*Cyprinodon variegatus*). Fish were acclimated to 22°C or 32°C for 14-days and standard and routine metabolic rates (SMR and RMR, respectively) were measured. Fish were subsequently subjected to a suite of behavioural assays to assess activity, exploration, sociability, and anxiety. As expected, warmer temperatures increased in SMR, but surprisingly RMR was not affected by warming. Interestingly, the warm acclimation altered the relationships among behavioral traits and significantly increased measures of activity and anxiety. There was no correlation between behaviours and metabolic traits in individuals acclimated to either temperature. Our data indicate that while both metabolism and behaviour show flexibility with warming, there is no apparent link between the two.

A Chordate Model for the Origins of Cardiac Regenerative Capacity

Keaton Schuster

The human heart cannot regenerate, yet several vertebrate species can regenerate the heart after damage. While progress has been made in understanding the mechanisms of cardiac regeneration, several open questions remain: How did it evolve in the first place? How does the heart acquire its regenerative capacity during development? Are there alternative cell sources required for repopulating the damaged heart? Given these questions, *Ciona robusta* is an ideal model to study the cellular, developmental, and evolutionary origins of heart regeneration. *Ciona* adults and juveniles have prodigious regenerative abilities, yet they cannot regenerate as embryos. Therefore, *Ciona* is an excellent model to understand how regenerative competence is acquired during development. As a chordate, *Ciona* is a key model to study the evolutionary origins of vertebrate traits. Using the NTR/MTZ ablation system, we discovered that the heart can regenerate. We also found that several signaling pathways are conserved in their requirement for heart regeneration. This regenerative capacity is acquired during the mid-late stages of metamorphosis, when the heart becomes mature enough to start beating. The heart regenerates primarily through proliferation of surviving cardiomyocytes, yet in animals where the entire heart is ablated, we found that the heart can still regenerate. This is accomplished by an endoderm-derived cell population. In conclusion, we have demonstrated that *Ciona* is capable of heart regeneration, and is an emerging model to study the origins of heart regeneration.

Three is one, one is three: Upheaval of “Toxopneustes” species

Chloe Schwab, Abigail Uehling, Gustav Paulay

Rapid biodiversity assessments in under-researched areas have become urgent in the midst of a global biodiversity decline. Such efforts have already shown that marine invertebrate diversity is much higher than previously documented. By utilizing DNA barcoding, this project aims to reevaluate the known diversity of the sea urchin genus *Toxopneustes*. Commonly known as the flower urchin, *Toxopneustes* is found across the tropical Indo-Pacific Ocean. A total of 30 individuals, across eight locations, were sequenced. Eight out of the 30 individuals were collected in Oman, an under-researched area defined by unique environmental parameters. These parameters, the geological history of this region, and the observation of distinct morphological features suggest that Oman may have undescribed endemic species. To test this hypothesis, two mitochondrial (COI and 16S) and two nuclear markers (ITS and H3), were sequenced and phylogenetic trees were constructed. Phylogenetic analysis revealed that *Toxopneustes* species were quite different from what was previously thought: three recognized *Toxopneustes* species were revealed to be one, one species was split in two, and a possible endemic, Tethyan relict species was discovered in Oman. Investigating *Toxopneustes* helps create a genus-specific biodiversity baseline, which may aid in conservation efforts in a time of intense environmental threats.

Computational and Physical Models in Research and Teaching to Explore Form-Function Relationships

Marie Schwaner, S. Tonia Hsieh

The application of modeling approaches in biology has the potential to revolutionize our understanding in multiple ways. First, computational and physical models make it possible to investigate cross-scale dynamics and explore in detail the complex interactions of system components. Second, the templates and anchors approach popularized in the late 1990s emphasizes the power of using simplified templates and increasingly complex anchors to understand the often non-linear, dynamically coupled interactions within a biological system. Third, models allow for iterations and high-resolution parameter sweeps, which are often unrealistic while collecting empirical data, due to time constraints or simply because a combination of certain characteristics does not exist in nature. Effective implementation of models requires us to cross disciplines and research silos, seeking expertise from scientists with diverse backgrounds and skill sets. Therefore, in order to move modeling

initiatives forward, we must start and nurture cross-disciplinary dialogues and collaborations, to use these computational and physical modeling tools to their full capacity. The goals of this symposium is to inspire wider adoption of model-based research and to discover new, impactful opportunities to apply modeling approaches. Within this introduction, we will present a brief history of computational and physical modeling approaches in the context of the themes of the symposium.

Intrinsic factors –not activation– are main drivers of variation in muscle force and work in vivo

Marie Schwaner, Kiisa Nishikawa, Monica Daley

Agile locomotion requires fine-tuned coordination of mechanics and sensorimotor control. Yet, our understanding of how muscle intrinsic mechanics and neural activation interact to influence muscle force and work output remains limited. Here we recorded in vivo muscle length, force, and activation of the main ankle extensor, the lateral gastrocnemius in 6 guinea fowl (*Numida meleagris*) as they were subjected to speed perturbations (accelerations and decelerations) while walking and running on a treadmill. We used path analysis with multiple linear regression to probe for the strongest predictors of variation in muscle force and work. Across multiple speeds, we found that 50-55% of the variation in work during stance is explained by intrinsic factors, most notably fascicle length and velocity near the stance swing transition, and 5-8% of the variation is explained by muscle activation factors (EMG area, duration, timing). Intrinsic factors explain ~60 % of variance in peak force across speeds, with activation factors explaining less than 2%. Activation factors explain slightly more variance in force and work in walking compared to running, which may drive the higher variability observed in walking kinematics. Yet, our data clearly indicate that intrinsic factors are important predictors of muscle mechanical output across speeds. In future work, we will explore model-based predictions of variation in vivo muscle dynamics, which could help reveal nonlinear interactions between activation and strain trajectory.

Zooplankton species richness estimates are increased with environmental DNA sampling at FHL

Megan Schwartz, Michael O'Mahoney, Johanna Cannon, Jennifer Brave, Maddie Emerson, Annika Johnson, Christopher Meyer

Despite their critical importance in marine food webs, energy transfer, and nutrient cycling, zooplankton communities are understudied by as much as 70-90%. Environmental DNA (eDNA) sampling with metabarcoding is a method that promises rapid species richness assessment. The goal of this study is to compare two methods, zooplankton samples hand sorted to morphospecies and directly sequenced for a DNA barcode, and eDNA samples bulk filtered and metabarcoded, for their ability to recover species richness. Zooplankton were collected during the day and night from the dock in front of Friday Harbor Laboratories (FHL), an area with a robust set of COI DNA barcodes. Students in the FHL summer 2021 marine invertebrate zoology course sorted half the samples to morphospecies for DNA barcoding, while the other half was filtered and metabarcoded. We captured 198 unique animal OTUs from both methods of which only 63% represent named species with DNA barcodes. Of the recovered OTUs, eDNA sampling with metabarcoding were successful at capturing 70% of OTUs but hand sorting to morphotype and direct COI barcoding netted an additional 17% of total species richness, with 13% overlap between methods. OTUs from day and night zooplankton samples overlapped only by 20%. Our results demonstrate that eDNA is an excellent tool for biodiversity discovery, yet hand sorting with direct sequencing is helpful for the most complete inventory of species richness.

Dynamic Light Filtering Over Dermal Opsin as a Sensory Feedback System in Fish Color Change

Lorian Schweikert, Laura Bagge, Lydia Naughton, Jacob Bolin, Benjamin Wheeler, Michael Grace, Heather Bracken-Grissom, Sonke Johnsen

Photoreceptor proteins (opsins) had once been thought to occur only in eyes, but are now being discovered elsewhere at a rate that is far outpacing gains in understanding their functional significance. In vitro studies of opsin in skin

have implicated dermal photoreception in the ability of certain animals to change skin color (i.e., dynamic coloration). In live animals however, support is lacking for the capacity of dermal photoreception to initiate color change apart from eyesight, leading to questions of how and why dermal photoreception is coupled to color change. Here, we used approaches in immunohistochemistry, confocal and transmission electron microscopy, sequenced-based spectral sensitivity estimation, and microspectrophotometry (MSP) to investigate the physical and optical relationship between SWS1 opsin and skin color-change cells (i.e., chromatophores) in hogfish (*Lachnolaimus maximus*). Our results show that SWS1-opsin expression is localized to a morphologically-specialized population of cells existing beneath chromatophores, which contain pigment that selectively absorb the wavelengths of SWS1 peak spectral sensitivity. As SWS1 receptors appear subject to light changes from pigment activity (aggregation and dispersion), the predicted function of dermal photoreception in hogfish is to detect these shifts in chromatophore pigment in order to obtain sensory feedback about skin color change.

Predicting Mouse Perception from Olfactory Neurons with Decision Trees

Jadyn Scott, Benjamin Cowley

Mice rely on their sense of smell to transform perceived odors into behavioral actions. To better understand this transformation, we can avoid presenting many chemical odors—requiring the collection of many chemical compounds—by instead optogenetically stimulating many different patterns of glomerulus activity in the olfactory bulb. We focus on mapping these glomeruli activation patterns to the observed behavior of a mouse trained to discriminate between different activation patterns (i.e., different odors). To fit this mapping, we use the machine learning model of a decision tree, chosen for its simplicity and ability to fit almost any function with discrete valued inputs. We find that using multiple decision trees—called a random forest—increases prediction by taking a consensus across many trees. An added benefit is to use the disagreement across trees as a training signal to more efficiently train the random forest. We show in simulations that this closed-loop process, called active learning, greatly reduces the number of stimulation patterns needed for training (i.e., recording time). This is of particular importance for our setting: A mouse has over a thousand olfactory glomeruli resulting in 2^{1000} possible activity patterns. Thus, we provide a framework to model olfactory perception and a viable way to obtain the model's parameters in a small amount of recording time.

Shared epigenetic controls link post-natal development and aging in mammals

Karen Sears, Steve Horvath, Amin Haghani, Joseph Zoller, Ishani Sinha, Aidan Couzens, Clive Lau, Meghety Manoyan, Yadiamaris Ruiz, Annais Talbot

Aging's universality across mammals has engendered much speculation on its causes. We generated genome-wide DNA methylation (DNAm) profiles for 58 tissues from 185 mammalian species and used them to develop pan-tissue aging clocks. These clocks' ability to estimate age across mammals suggests that mammalian aging might result from defined mechanisms that are largely shared across tissues and species, rather than the random accrual of damage. The clocks also provide clues to the nature of these mechanisms; the cytosines that comprise them, i.e., those whose DNAm increases with age in all mammals, save one, are near genes enriched in development, PRC2 binding, and H3K27me3. This tandem enrichment is likely not coincidental; PRC2 maintains genes in a repressed state during development and in adults through H3K27me3 marks. We found only one exception – the opossum (*Monodelphis domestica*). Age-related DNAm increases in opossums differ in that they are enriched in cell cycling and senescence, rather than PRC2 activity, and mostly fall outside of CpG islands. In contrast, we found that age-related CpGs in post-natal mice and opossums are similar; both are near genes associated with developmental and PRC2 processes, among others. While further investigation is needed, our current hypothesis is that postnatal development and aging are generally coupled across mammals because of their shared reliance on PRC2 activity, except in opossums, the "exception that could prove the rule."

Blue and Humpback Whales Use Upper Jaws as Delta Wings to Provide Stabilizing Lift During Feeding

Sophia Sebo, Frank Fish, Paolo Segre, Jean Potvin, Jeremy Goldbogen

Ability to maintain stability while feeding can be important for lunge feeding baleen whales. As the lower jaw is depressed to engulf prey-laden water in the expanding throat pouch, increased drag below the center of gravity can produce a destabilizing downward pitch. To counteract this condition, the pectoral flippers are abducted and pitched upward to provide a lift to counter the pitching moment. Foraging whales will also raise their upper jaw, which has a planform like a delta wing. To examine the delta wing effect of the upper jaw, 2-D plexiglass planforms of the upper jaw of the blue whale and humpback whale were constructed for hydrodynamic testing. Models were tested with a six-axis force transducer in a water tunnel at Reynolds number up to 192,000 at angles of 0°-25°. Jaw planforms were compared to equivalent delta wing shapes. Neither delta wing models nor whale jaw planforms showed stall. Delta wing shapes had higher lift coefficients than the jaw planforms for both species. The lift-to-drag ratio (L/D) for the blue whale planform was higher than the humpback whale planform. Maximum L/D for the blue whale occurred at an angle of 14°, whereas maximum L/D for the humpback planform occurred at 16°. The upper jaws of both whales could act like delta wings during feeding and provide lift for stability.

A Dance of Feathers: The Fluid Dynamics of Feather-Inspired Flow Control Devices

Girguis Sedky, Ainy Wissa

Covert feathers located on the suction side of a bird's wing have been observed to passively deploy during high-angle-of-attack maneuvers. This observation has sparked the idea of integrating bioinspired flaps onto the suction side of aerial vehicles, aiming to enhance flight performance at high angles of attack. During flight, the flaps of a wing inspired by these covert feathers dynamically oscillate around an average deflection angle, resulting in an augmentation of lift particularly in post-stall angle-of-attack configurations. The extent to which the enhancement can be attributed to the dynamic behavior of the flaps, as opposed to the average deflection angle of the flaps at relevant Reynolds numbers, remains unknown. In this talk, we will present time-resolved force and flowfield measurements of two covert-inspired wing configurations. The first configuration features fully passive covert flaps free to deploy and oscillate in response to the aerodynamic flow. The second configuration involves static flaps set at deflection angles equal to the mean angles of the first configuration. Both setups are tested at a Reynolds number (Re) of 200,000, which is representative of bird flight. Based on these experiments, we quantify the influence of the flap dynamics on the lift enhancement and determine the applicability of using static flaps to capture the dominant flow physics of the aerodynamic system.

Sled dogs don't synchronize

Benjamin Seleb, Matt Bull, Saad Bhamla

The sled dog team offers a time-tested system for studying cooperative transport and collective behavior in multi-agent teams. Harnessed into a synthetic network of tethers, these dogs comprise an artificial collective where cohesion is enforced and arrangement is decided by the driver, or musher. Utilizing custom animal-borne sensors, we examine the movement dynamics and force production within a team of sprint sled dogs—an exemplar artificial collective designed for speed. Considering the dogs in their tether network as a system of coupled oscillators, we find that dogs rarely synchronize their strides. Challenging prevailing beliefs, our findings also suggest that greater coherence does not necessarily equate to enhanced performance. Building on these findings, we aim to shed light on the nuanced relationship between synchrony and performance, not just in the context of sled dogs, but in physically-connected teams more broadly.

On the variability of human leg stiffness across strides and data analysis consequences

Alessandro Maria Selvitella, Kathleen Lois Foster

Typically, animal locomotion studies involve consecutive strides, which are frequently assumed to be independent with parameters that do not vary across strides. This assumption is often not tested. However, failing in particular to account for dependence across strides may cause an incorrect estimate of the uncertainty of the measurements and thereby lead to either missing (over-estimating variance) or over-evaluating (underestimating variance) biological signals. In turn, this impacts replicability of the results because variability is accounted for differently across experiments. In this paper, we analyse the changes of a couple of measures of human leg stiffness across strides during running experiments, using a publicly available dataset. A major finding of this analysis is that the time series of these measurements of stiffness show autocorrelation even at large lags and so there is dependence between individual strides, even when separated by many intervening strides. Our results question the practice in biomechanics research of using each stride as an independent observation or of sub-selecting strides at small lags. Following the outcome of our analysis, we strongly recommend caution in doing so without first confirming the independence of the measurements across strides and without confirming that sub-selection does not produce spurious results.

Molecular evolution and development of the mammalian gliding membrane

Harsha Sen, Ricardo Mallarino

Understanding how genomic changes over evolutionary time lead to developmental and phenotypic changes is a central question in evolutionary developmental biology. While many loci responsible for evolutionary trait loss (e.g. flightlessness in birds, loss of limbs in snakes) have been identified, little is known about gene regulatory network (GRN) changes that underlie the evolutionary and developmental bases of new traits. Furthermore, the degree of cellular and molecular convergence underlying instances of convergent trait gain is unclear. We use the sugar glider (*Petaurus breviceps*) as a model system to study the development and evolution of the mammalian gliding membrane, or patagium, a specialized tissue that has independently evolved in multiple groups of mammals, to address these questions. Using single-cell approaches and single-molecule imaging techniques, we identify a patagium-specific cell type and its associated histological phenotype in sugar gliders. Ongoing experiments to knock down expression of *Wnt5a*, a patagium-upregulated gene, will help us dissect the GRNs controlling patagium outgrowth. We have also generated corresponding single-cell datasets from field-collected Seba's short-tailed bat (*Carollia perspicillata*) embryonic samples, and compare cell-type specific transcriptomic signatures in the lateral patagia of both species. By studying the lateral patagia of two distantly related mammals that have evolved this novel trait, we can better understand how key developmental pathways are redeployed to pattern novel morphological traits, giving rise to the diversity we see in nature.

When Squid Fly: Encapsulating the anatomy of the flying squid in a working robotic model.

Yohan Sequeira, Jake Socha

Flying squid are capable of multi-modal locomotion through water and air. Pelagic squid species such as *Todarodes pacificus* and *Ommastrephes bartramii* have been reported to jet from the sea's surface and fly for over 20 m with fins and arms extended. However, little is known about how the squid produce aerodynamic forces and control their body in the air. We hypothesize that: (1) flying squid use their arms and fins to generate lift and increase aerial distance, (2) flying squid eject water from their siphon while jetting to create thrust, and (3) flying squid open and retract their fins and arms to control their trajectory in air. In the absence of kinematic data, we are developing a robophysical model to better understand the functional implications of squid anatomy on aerial trajectories. To produce a morphologically accurate model, we are using three-dimensional scans of preserved specimens, from which we extract measurements of mantle and arm length, fin and arm-membrane area, and siphon position. To mimic the behavior of the flying squid in its flying

pose, the model will include a mantle cavity that expels water to simulate jetting. Finally, the arms and fins of the model will fold and unfold, altering the aerodynamic forces on the body. Ultimately, we aim to create a launch system to investigate the aerodynamics and trajectories of this model in flight.

Adapting to seasonal changes: A study of flexible breeding strategies in poison frogs

Shirley Serrano-Rojas, Lauren O'Connell

The unprecedented scale of rapid environmental change is altering the seasonality of tropical forests, causing shifts in rainfall patterns, and accentuated drought events. The reproduction and survival of many species will be shaped by the plasticity of their behavioral responses. Our understanding of these behavioral adjustments between seasons remains constrained. In this study, we investigated seasonal variation in social behavior of three poison frog species (Family Dendrobatidae) that vary in behavior and breeding duration. *Ameerega macero* and *A. shihuemoy* breed year-round whereas *Allobates femoralis* reproduces mainly in the wet season. We tested the hypothesis that *A. macero* and *A. shihuemoy* have different space use and more flexibility in resource use strategies that enable breeding all year round compared to the seasonal breeder species. We combined telemetry and high-resolution GIS mapping with behavioral observations and quantified androgen and cortisol concentrations. We found that home range size was larger for the seasonal breeder in the wet season, but smaller than the home range of the year-round breeder species in the dry season. Similar substrates were shared between both species but at different elevations. Offspring caring was observed at a higher frequency for the year-round breeder species in the dry season. Current work includes processing hormone samples. Our findings contribute valuable insights into the behavioral adjustments used to successfully adapt to seasonal changes in resource availability for reproduction.

The Effects of Wear on the Morphology and Puncture Force of Shark Teeth

Ryan Sesler, Lisa Whitenack

Sharks frequently replace their teeth throughout their lives from once per week to roughly every three months. Past studies on the biomechanics of shark teeth indicate that teeth are structurally and mechanically strong, indicating that frequent tooth replacement is not due to tooth breakage. Instead, it has been hypothesized that teeth are replaced due to wear and its effects on tooth function. Teeth from five species of shark (*Carcharhinus limbatus*, *C. leucas*, *C. plumbeus*, *Sphyrna mokkaran*, and *I. oxyrinchus*) were subjected to 400 punctures each in teleost prey; tooth wear and puncture force were measured throughout the series of punctures. Measurable tooth wear happens within 20 punctures, although the rate of wear differs among species. Puncture forces steadily increase as the teeth wear, with significant differences from initial puncture force after 10 punctures. However, puncture forces at 400 punctures were still below theoretical bite force estimates of sharks of *C. leucas*. These results together indicate that tooth wear may affect the ability of teeth to function efficiently, particularly for puncturing soft prey. Unfortunately, data on tooth replacement rates or how many uses each tooth undergoes before it is shed do not exist for the vast majority of shark species. We are currently expanding this study to include lateral head shaking to understand the link between wear, tooth performance, and tooth replacement.

Recalculating: Can Sea Turtles Account for Gradually Changing Geomagnetic Parameters?

Jadyn Sethna, Dana Lim, Isabelle Sechrest, Catherine Lohmann, Kenneth Lohmann

When hatchling loggerhead turtles from the southeastern U.S. leave their nesting beaches, they embark on a multi-year pelagic migration, exploiting a series of regional magnetic fields as navigational markers to help them remain within the North Atlantic Subtropical Gyre. While these magnetic signposts vary spatially across the globe, the geomagnetic field gradually changes over time. Thus, to use regional magnetic fields in navigation, the loggerhead population presumably must update responses to regional magnetic fields that exist

along the migratory route. In a previous experiment conducted in 2007, hatchlings responded to a magnetic field that existed near Puerto Rico by swimming in a northeasterly direction, a response that aligns with their migratory route. Here we tested how hatchlings respond to the magnetic field that currently exists near Puerto Rico as well as to the magnetic field that existed at the same location in 2007. Hatchlings responded to the 2023 magnetic field in Puerto Rico by swimming in a northeasterly direction. By contrast, the 2023 cohort of hatchlings responded to the 2007 magnetic field by orienting strongly northward, a response that is significantly different from both the 2023 response and the original 2007 response. These findings suggest that hatchling loggerheads can update their orientation responses to regional magnetic fields and can do so in under one generation time. The mechanisms that underlie this updating ability remain enigmatic.

Expression and function of *Six3* supports the homology of the sea spider proboscis and the labrum

Emily Setton, Benjamin Klementz, Hugh Steiner, Grace Hareid, Guilherme Gainett, Sophie Neu, Ethan Laumer, Charlotte Wood, Georg Brenneis, Prashant Sharma

The arthropod “head problem” is a century-long, ongoing debate regarding the homology of different regions of the panarthropod head. One outstanding element of the arthropod head problem lies in the protocerebral (anterior-most) segment and the origins of its appendage-like structure, the labrum. In insects it has been shown that the gene *Six3* (*Optix*) plays a role in proper labral specification, but functional data within arthropods are limited to Holometabola. One outstanding group of arthropods that continues to defy resolution viz. the arthropod head problem is Pycnogonida, also called sea spiders. Sea spiders do not have a labrum, but instead bear an elongate proboscis, whose evolutionary and developmental origins are disputed. Here, we investigate the function of *Optix* in Chelicerata via RNAi in the harvestman *Phalangium opilio*. Comparable to the insect phenotype, *Optix* diminution in the harvestman is associated with loss of anterior protocerebral structures, including the labrum. These data support a conserved role for *Optix* in patterning the labrum in insects and arachnids, and by extension, across Arthropoda. With the validation of *Optix* as a labral selector gene, we deployed fluorescent gene expression protocols for the sea spider *Pycnogonum littorale* toward understanding the developmental origins of the proboscis. We demonstrate that the proboscis develops in an *Optix*- and *Sp6-9*-positive territory of the embryonic head, supporting the homology of the labrum- and the sea spider proboscis.

Challenges and progress on rearing the common eastern firefly, *Photinus pyralis*

Lauren Shaffer, Aidan Sullivan, Sarah Lower, Moria Chambers

During distinct life stages, an organism’s immune system is expected to be regulated in different ways based on the optimal allocation of their energy and resources, e.g. larvae may allocate to growth and survival, while adults allocate to reproduction. The common eastern firefly, *Photinus pyralis*, is an intriguing system to study investment in survival in the face of infection due to its relatively long-lived larval stage. *P. pyralis* spend 1-2 years in the larval stage, and only two weeks in the adult stage. However, *P. pyralis* larvae are difficult to find in the wild, and there has been limited success with rearing larvae in the lab. Here, we describe methods for rearing *P. pyralis* from 26 unique mated pairs collected from two different locations near Lewisburg, Pennsylvania during summer 2023. Not only will these methods be able to inform immune investment studies, but also conservation efforts for these charismatic creatures.

Age and Growth Patterns of North Pacific Olive Ridley sea turtles: A Skeletochronological Approach

Hassan Shaikh, Calandra Turner-Tomaszewicz, Jeffrey Seminoff

Olive ridley sea turtles (*Lepidochelys olivacea*) are globally distributed and are one of the most abundant sea turtle species. However, per IUCN Red List, there has been a 30–50% reduction in global population size of olive ridleys in the last decade. Effective management strategies require basic demographic data, yet much remains unknown about olive ridleys. To address the

knowledge gap on size-at-age and growth rates, we performed skeletochronology (the study of bone growth layers), on the humeri of 20 North Pacific olive ridleys originating from the East and West Pacific nesting sites, recovered as a result of bycatch or strandings from the central and east Pacific between 2004–2021. Previous genetic analysis identified the stock origin (nesting location) as either East ($n=12$), or West ($n=8$) Pacific. Body sizes (curved carapace length, CCL) ranged from 27.5–68.5 cm (mean $\pm$ SD: 55.79 $\pm$ 9.3). These preliminary results are comparable to Zug et al. 2006 study; estimated age ranged from 3–24 years (10.5 $\pm$ 6.5), and estimated age- and size-at-maturity was 16 years and 62 cm CCL, respectively. Similar age-at-size and growth trends were observed regardless of nesting stock, and additional samples, currently being processed, will help further characterize these demographic parameters and patterns. Findings will aid ongoing conservation and management efforts, and serve as a foundational resource for future investigations on the species’ population dynamics.

Characterizing melatonin receptor expression in the green anole lizard brain

Nicholas Shankey, Rachel Cohen

Seasonal breeders become reproductively active during specific times of the year, exhibiting changes in physiology, behavior, and morphology. Green anole lizards (*Anolis carolinensis*) breed seasonally, becoming reproductively active during late spring and regressing to a non-breeding state during late summer. Generally, shorter photoperiods stimulate higher levels of melatonin secretion and, although previous work supports the notion that melatonin regulates reproduction, it remains unclear what function melatonin has in regulating anole breeding transitions. To examine this, we collected tissue from male and female lizards during the breeding and non-breeding seasons. We also treated lizards with subcutaneous blank or melatonin capsules during the early breeding season for 5 or 10 weeks. At the conclusion of each treatment, brains were collected and melatonin receptor (MT1 and MT2) mRNA expression was determined using qPCR. Results in unmanipulated brains revealed that MT1 expression is higher in breeding compared to non-breeding males ($H_3 = 12.50$, $p=0.006$) while MT2 expression was higher in females compared to males ($F(1,25) = 7.32$, $p=0.012$), indicating that MT1 and MT2 expression follows a seasonal pattern. This result suggests that melatonin signaling may intensify during certain times of the year, potentially exerting increased effects on reproduction. We continue to perform analysis on receptor expression in lizards treated with melatonin. Understanding the regulation of melatonin receptors in treated anoles may reveal how melatonin signaling in the absence of shorter photoperiods influences reproduction.

Origin and development of synovial joints

Neelima Sharma, Yara Haridy, Neil Shubin

Synovial joints, where articular surfaces move relative to each other, are hypothesized to have evolved from fibrous joints to increase mobility and improve load bearing. Current studies place the origin of synovial joints at the common ancestor of osteichthyans. However, our micro-CT scans reveal that the chondrichthyan *Leucoraja erinacea* has cavitated and articulated joints in the pelvic fins and jaws, but only fibrous joints exist in the cyclostome *Petromyzon marinus*, urging a revisit to the topic of the evolution of synovial joints. In the developing embryos of *Leucoraja erinacea*, we find the expression of growth differentiation factor-5 (*gdf5*) in the joint interzone and a lubricating protein (*prg4*) at the articular surfaces, suggesting similarities with mammalian synovial joints. *Petromyzon marinus* also expresses *gdf5* and *prg4* in their cartilaginous skeleton, implying that the genetic machinery required to form synovial joints is ancient and predates true synovial joints. Next, we analyzed the fossils of the major extinct taxa between cyclostomes and osteichthyans. Preliminary data shows that the placoderm *Bothriolepis canadensis* has reciprocally shaped articular surfaces separated by a cavity in the articulation between the pectoral fin and the shoulder; thus, the first cavitated joints likely appeared in stem gnathostomes. Our results have consequences for understanding how the evolution of cavitated joints in ancient vertebrates impacted behaviors like feeding and locomotion.

Task-Relevant Multisensory Representation in a Population of Descending Neurons

Varun Sharma, Simon Sponberg

To achieve agile movement animals often combine multiple sensory modalities across a dynamic range of inputs. *Manduca sexta* is a hawkmoth which uses vision and proboscis mechanosensation in linear combination to track moving flowers across frequencies. These sensory cues must pass through a bottleneck of 1000 neurons in the descending connective to affect locomotion. We hypothesized that there are multimodal descending neurons, exhibiting the same frequency-dependence and linearity observed in free behavior. Alternatively, integration may happen downstream from the descending connective, and behavioral frequency response may be reflected only at the population level.

We used behavioral data from flower tracking experiments to playback moths' visual and mechanosensory experiences with a two-part robotic flower. Using 32-channel extracellular electrodes we found distinct populations of proboscis and flower-face responsive units. Most were unimodal, but a few were multimodal. Single multimodal units did not show stimulus-response linearity, in terms of spikerate. A few units responded differently when moths actively moved their proboscis. These results show that some sensory integration for flower tracking is combined in descending neurons, but unimodal information is also sent to the motor system. Nonlinear multimodal integration in individual units means that these alone are insufficient for a task-level representation of combined cues. Population representations may be necessary to capture behavioral dynamics. Alternatively, motor circuits might compensate for descending neuron nonlinearities, resulting in behaviorally observed simplicity.

The Urgent Responsibility of Biologists to Defend Intersex Variation and Human Rights

Sam Sharpe

Diversity is understood as inherent and myriad in the biological sciences, yet conceptualizations of sex are frequently presented as a presumed universal sex binary. This widespread presumption underappreciates the extant diversity of reproductive and developmental manifestations of sex across taxa and foment the erasure and oppression of intersex variation in human populations. Human sex diversity beyond binary dimorphism has been largely disregarded or pathologized in the biological sciences, contributing to eugenic, surgical, and political attempts to eradicate intersex traits from the population. This harmful trend has been both facilitated by and further perpetuates widespread ignorance of the biological, medical, and sociological realities of intersex individuals, who make up at least 2% of the global human population and are present across all demographics. As with many types of prejudice, claims that intersex variation does not exist or is psychosocially inferior to endosex phenotypes originated from cultural biases against sex differences, with supposed biological arguments subsequently developed to justify pre-existing biases. Given these insidious histories, it is essential that the biological sciences confront and address the ongoing legacies of harm towards intersex individuals. Dismantling the incongruous perpetuation of an assumed universal sex binary in the face of contradicting material evidence and substantial material harm is an urgent responsibility in our work as biologists throughout our research, educational, and outreach capacities.

Neuronal coding of navigation and spatial position in the cane toad, *Rhinella marina*

Daniel Shaykevich, Grace Woods, Daniela Pareja-Mejia, Chloe Golde, Lauren O'Connell

Many animals perform tasks requiring the processing of spatial information. Modern neuroscience tools have allowed for in depth characterization of space coding neurons (e.g. place and grid cells) in the mammalian hippocampus and entorhinal cortex, enabling a rich understanding of how organisms navigate and remember environments. Much less work has characterized cells with similar functions in other vertebrates, especially in amphibians. We are performing field- and lab-based research in the cane toad, *Rhinella marina*, a large and globally abundant amphibian, to study how the amphibian brain

supports navigation and encodes spatial information. Our main target is the medial pallium, the proposed amphibian homolog of the mammalian hippocampus, which we hypothesize is activated during navigation and contains spatial coding neurons. First, we completed a translocation-homing study of invasive cane toads in Hawai'i and demonstrated that toads can return home from distances exceeding 1 km. We collected brains from toads and quantified cells immunoreactive for a marker of neural activation and identified pallial brain regions that are activated during homing. In addition, we developed tools that allow for recording of spatial neural coding in amphibians. Here, we show preliminary results for the implementation of flexible mesh electronics, a novel recording probe, in toads, and an experimental plan for using these recording units to study neuronal coding of spatial orientation.

DNA methylomes reveal sex-specific epigenetic aging patterns in a squamate with dimorphic lifespan

Ethan Shealy, Aaron Reedy, Tonia Schwartz, Robert Cox, Benjamin Parrott

Among other life-history traits, average lifespan tends to vary between the sexes in most species. Recent evidence suggests that in wild brown anoles (*Anolis sagrei*), female maximum lifespan is significantly greater than in males. However, it is not known whether this difference is driven by extrinsic factors like predation, or intrinsic physiological mechanisms. Epigenetic clocks use DNA methylation frequencies at specific genomic locations to predict chronological age, and this method has been used to accurately estimate the age of individuals across a wide variety of taxa. Additionally, residuals between predicted and actual ages appear to be responsive to factors which are known to influence mortality risk, such as toxicant exposure and calorie restriction - potentially providing a robust estimate of individual longevity. We used whole-genome DNA methylation sequencing to develop an epigenetic age estimator for brown anoles, and determine whether the observed sex differences in mortality risk across the lifespan are reflected at a molecular level. Our best model produced estimates with a median error of 3 months, but did not show consistent biases in error between sexes. However, female juveniles tended to show more "robust" initial methylation states at age-associated sites, along with a faster rate of change regardless of directionality. This result suggests that early life methylation patterning may be more relevant than the rate of change in shaping organismal lifespan.

Montane grasshopper fitness constraints in changing environments

Monica Sheffer, Caroline Williams, Lauren Buckley, Sean Schoville

The ability to predict how populations and species will respond to climate change is a central goal in the modern era of biology, and requires empirical data on fitness responses to climate means, variability, and extremes. Given that fitness is the product of fecundity and survival, it is critical to consider how/whether these two fitness components separately shift in response to the environment. To this end, we performed a two-year reciprocal transplant experiment on two montane grasshopper species that differ in their dispersal propensity and phenology. We hypothesized that fecundity constraints would be strongest at high elevation due to lower temperatures and a shorter season, while survival constraints would dominate at low elevation, where the incidence of extreme temperatures is higher. We transplanted grasshoppers across elevations, measuring development time, growth, fecundity, and survival. In the first year, we found that survival to adulthood was lowest at low elevation, while time to reproduction was longest at high elevation, consistent with our hypothesis. Size clines were opposite to our expectation from wild grasshoppers, although this may have been driven by food limitation at low elevation. Transplant elevation, not elevation of origin, was the strongest factor influencing phenotypic outcomes, providing little evidence for local adaptation. Overall, we found evidence supporting different responses of fitness components to environmental conditions.

Assessing the paleoecology of fishes from the Mazon Creek fossil assemblage using morphometrics

Edward Shelburne

The Mazon Creek fossil assemblage is a lagerstätte from the Middle Pennsylvanian (307-315.2 Ma) Francis Creek Shale Member. These fossil beds represent a deltaic-to-coastal marine ecosystem preserving a diversity of ancient vertebrates—particularly fish. The purpose of this study was to examine the paleoecology of actinopterygians from this site, as well as the unique and enigmatic Tully monster (*Tullimonstrum gregarium*), using landmark-based geometric morphometrics. The body shapes of 490 extant marine fish species were quantified and used alongside their established ecologies to generate an ecomorphospace. Twelve Mazon Creek taxa were then morphologically compared with the extant fishes to infer paleoecological preferences. Several parameters were assessed, including lifestyle, preferred diet, and preferred substrate. Results indicated actinopterygian niche occupation within the Mazon Creek system was more restricted than in modern marine systems. Absent from this assemblage are definitively benthic fish, with most species occupying morphospace consistent with a benthopelagic or pelagic lifestyle. Diets varied, but most bore morphological affinities with modern omnivores, planktivores, and generalist carnivores. Surprisingly, soft bottoms appeared to be the least suitable substrate, with morphospace occupation indicating adaptation for harder and more vegetated bottoms. The Mazon Creek paleoenvironment is typically reconstructed with a uniform, muddy bottom, making this result notable. This study provides a more comprehensive analysis of the Mazon Creek paleoenvironment and offers insight into the development of marine fish communities through time.

Effect of warming water temperatures on local gastropod growth, reproduction, and mortality

Alaina Shepardson, Abigail Cahill

For our research, we studied two species of local gastropods, the native species *Physa acuta*, and the invasive species *Bellamya chinensis*. We wanted to test how warming water temperatures caused by climate change would affect the growth, reproduction, and mortality of these species. Our hypothesis was that rising water temperatures will create a new stress factor for gastropods and in turn will affect their growth, reproduction, and mortality.

We separated the two species into four tanks, two of which were kept at room temperature (20°C), and used aquarium heaters to heat two tanks to 27°C. Weekly, we would measure the growth, reproduction, and survival of adult snails. We kept juvenile snails in custard dishes in a heated water bath of 27°C or left them in dishes at 20°C. At the end of our research, we found that our hypothesis was supported for *Physa acuta* but not supported for *Bellamya chinensis*: the heated treatment caused a significant difference in survival rate and egg mass reproduction in *Physa acuta*, but not in *Bellamya chinensis*. Our results could help shine some light on how climate change will affect the abundance of two local gastropod species, as well as how these two species will do in a changing environment.

Early life diet-induced corticosterone reprograms juvenile behavior in spadefoot toads

Alex Shephard, Sarah Lagon, Kate Millar, Sydney Jacobsen, Cristina Ledón-Rettig

It is well understood that early life exposure to elevated glucocorticoids – such as corticosterone (CORT) in vertebrates – can have lasting effects on behavior and physiology later in life. These CORT-driven carryover effects are known to be induced by a wide breadth of stressful conditions, such as dietary restriction, predator presence, and cues from the social environment. However, it is not known whether CORT is involved in carryover effects induced by natural variation in dietary resource use. American spadefoot toads of the genus *Spea* present an excellent system for testing this question, since larvae of these species have evolved to use two alternate diets: an ancestral detritus-based diet and a more novel diet consisting of live shrimp. In this study, we used the plains spadefoot toad (*Spea bombifrons*) to test whether treatment of larvae with exogenous CORT results in carryover effects on juvenile behavior that mirror those effects induced by the novel shrimp-based diet. We found

that larvae consuming a live shrimp diet displayed elevated CORT levels. Additionally, treatment of larvae with exogenous CORT affected juvenile prey striking behavior in a manner similar to the behavioral effects induced by the shrimp-based diet. Our study supports the idea that glucocorticoid mechanisms contribute to the expression of carryover effects induced by the use of novel dietary resources.

Blood hematological and transcriptome responses to cortisol in blue rockfish

Janae Shew, Henry Marden, Hayley Mapes, Sean Lema

Glucocorticoid hormones have several physiological roles in fishes as in other vertebrates by regulating carbohydrate metabolism, modulating the immune system, and helping individuals respond to environmental stressors. Given the importance of those functions, the ability to rapidly and non-lethally assess a fish's physiological state related to recent glucocorticoid status could provide useful information about how fish are experiencing or responding to environmental stressors. Blood transcriptomics provides an underutilized technology for identifying qualitative or quantitative 'biomarkers' of physiological state. Unlike mammals, teleost fishes have nucleated erythrocytes. Those erythrocytes are transcriptionally-active and change gene expression with environmental factors. Other cells common in blood like leukocytes also can change patterns of gene transcription, and thus may also contribute information about immune status via the blood transcriptome. Here, we aimed to get an overview of glucocorticoid-regulated genes in fish blood using blue rockfish (*Sebastes mystinus*) as a model. Adult rockfish were treated with a single intraperitoneal injection of either cortisol or vehicle solution. Blood was then collected 24 h after injection and analyzed for hematological parameters, and the cellular portion was preserved for transcriptomic analyses using RNAseq. While hematocrit and blood cell counts did not vary between cortisol- and vehicle-treated fish, preliminary analyses indicate cortisol modulated expression profiles of the blood transcriptome including the abundances of mRNAs encoding genes related to immune system functioning and circadian rhythm.

Impact of dynamic changes in flow rate on infant feeding function in an animal model

Hannah Shideler, Elska Kaczmarek, Max Sarmet, Kendall Steer, Thomas Stroud, Alexane Fauveau, Ani Smith, Skyler Wallace, Maressa Kennedy, Alex-Ann Velasco, Morgan Blilie, Christopher Mayerl

All infant mammals suckle, which is a complex process involving extensive sensory processing to elicit appropriate motor output. Yet, we do not fully understand the relationship between variation in sensation and variation in neuromotor function. This inhibits the development of interventions grounded in physiologic mechanisms and limits interventions to being purely compensatory. One common means of variation in oral sensation is variation in milk flow rate. Here, we used infant pigs as an animal model to evaluate how dynamic changes in nipple hole size (flow rate) resulted in changes in oromotor function using high-speed X-ray video synchronized with intraoral pressure generation and muscle activity. We found that infants did not modify their sucking rate during feeding. However, when milk flow was restricted, pigs generated more suction, took more sucks between swallows, and had smaller boluses than when flow rate was not restricted. We found correlated changes in muscle firing amplitudes and sucking kinematics. Our results suggest that temporal patterning of suckling (sucking rate) is not responsive to variation in flow, but the mechanisms driving milk acquisition are. Dynamic modulation of nipple hole size is thus a promising avenue for exploring the use of motor learning principles in neurorehabilitation for infant feeding difficulties.

Tail Use for Postural Stabilization in Captive Cheetahs during Routine Transportation

Stacey Shield, Gavin Foster, Azraa Valley, Naoya Muramatsu, Ardian Jusufi, Amir Patel

In addition to having the highest top-speed of any terrestrial animal, cheetahs are also highly manoeuvrable. It is theorised that their tails contribute to their manoeuvrability by stabilizing the body during rapid changes in speed

or direction, but this has yet to be confirmed through detailed biomechanical analysis of the animal's motion. One reason for this is that it is very difficult to obtain the necessary footage to perform such an analysis on the motion of wild cheetahs, as reconstructing the animal in three dimensions requires high-quality video from multiple perspectives. Further, isolating the effect of the tail within the complicated galloping motion is challenging.

In this study, we captured unique footage of captive cheetahs during routine transportation to investigate the use of the tail in a context that mitigates these difficulties. During transportation, it was observed that the cheetahs would swing their tails when sudden changes in the motion of the vehicle would cause them to lose their footing. We filmed the animals using three high-speed cameras and tracked the motion of the vehicle with an inertial measurement unit (IMU). We then reconstructed the three-dimensional skeletal motion of the animal. By comparing the rotation of the tail to the perturbation of the body, we confirm that the motion of the tail is consistent with postural stabilisation.

The Fish Bites Back: A Developmental Analysis of Feeding Biomechanics in Danionin Minnows

Collin Shinkle, W. James Cooper

Ecological roles are often closely related to the food organisms consume, and investigating the developmental control of feeding morphology and biomechanics contributes to a broader understanding of how ecological roles evolve. Changes in thyroid hormone signaling are thought to be involved in the evolutionary diversification of feeding mechanics in many fish lineages, including danionin minnows such as zebrafish (*Danio rerio*), a common model organism. Recent work suggests that changes in thyroid hormone signaling may have influenced the trophic divergence of fishes in the genus *Danio* and those in the genus *Devario*, including the giant danio (*Devario aequipinnatus*). We examined developmental series of wild-type zebrafish, transgenic zebrafish rendered incapable of producing thyroid hormone (hypothyroid zebrafish), and giant danio. To test whether eliminating thyroid hormone would cause the form and functional abilities of zebrafish skulls to converge toward those of the giant danio, we tracked development of form and function in all three types from early larval stages when exogenous feeding begins to the point of normal sexual maturity. Clearing and staining techniques were used to visualize the cranial skeleton so that changes in skull form could be analyzed using geometric morphometrics to statistically compare developmental trajectories in morphology. We also used high speed video to record and kinematically compare fish feeding strikes at multiple developmental stages.

Heat waves and flash drought: interactive effects during embryonic development in the painted turtle

Ayley Shortridge, Fredric Janzen

Climate change is leading to rising temperatures and precipitation extremes worldwide. One consequence is the increased co-occurrence of heat waves and drought. While many studies have investigated the ecological impacts of thermal and hydric stress, more research is needed on their compound effects. Reptiles, including turtles, are highly sensitive to temperature and soil moisture conditions experienced in the nest. We conducted an experiment to determine the interactive effects of flash drought and heat waves during embryonic development in painted turtles (*Chrysemys picta*). We manipulated temperature and soil water potential during egg incubation in the laboratory, then recorded weekly egg mass, incubation time, and hatchling morphometrics. Our data reveal that changes in egg mass during late development are driven by the interaction between heat wave and flash drought events. Heat waves magnify changes in egg mass; either increasing growth in a typical hydric environment, or intensifying water loss in drought conditions. Furthermore, both stressors decrease total incubation time. Finally, flash drought and heat waves interactively affect hatchling body size, producing smaller-than-expected individuals. As compound heat wave and drought events become more common, we expect to observe these phenotypic effects in wild turtle populations, but the consequences on phenotypic evolution are unknown. Future directions include investigating the impact of heat waves and drought on hatchling survival during migration from the nest.

Nematostella vectensis Methylation Response to the Biotic Stress of Cell Free Supernatant

Rachel Showers, Adam Reitzel, Auston Rutledge

Organisms present in estuarine environments experience daily variation in abiotic and biotic factors. *Nematostella vectensis*, the starlet sea anemone, is a species commonly found in estuaries and is used as a model organism due to its adaptability to abiotic and biotic factors as well as having a high-quality genome. The adaptability of *N. vectensis* can be attributed not only to genetic recombination and natural selection, but potentially to DNA methylation, which is defined as a heritable change in DNA expression where a methyl group is added to the five position carbon on a cytosine ring. Previous research has been focused on *N. vectensis* tolerance to changes in abiotic factors such as temperature and salinity, however, research focused on changes in biotic factors is understudied. Our work is focused on understanding microbial metabolite interactions with marine invertebrates, using a liquid containing secreted metabolites from bacterial growth known as cell-free supernatant (CFS). Multiple populations of *N. vectensis* were placed into a 10% concentration of CFS for a week and then extracted using RNA-Seq and MethylRad to determine how DNA methylation in *N. vectensis* was affected by the presence of CFS including how methylation varied between populations and control groups. Additionally, we examined CFS's impact on egg-to-adult development in *N. vectensis* by exposing different life stages to CFS and measuring the survival and development of individuals after exposure. Overall, our work will provide insights into the role of within estuarine environments and its potential harmful or beneficial effects on marine invertebrate survival.

Zoo Biomechanics and Biomimicry Day: One event for thousands to learn about zoo research

Cassie Shriver, Audra Davidson, David Hu, Young-Hui Chang, Staci Wiech, Joseph Mendelson, Andrew Schulz

Zoo-academic collaborations in the past has been common with collaborations on ecology, evolution, behavior, and conservation. Recently there has been an increase in comparative biomechanics and biomimicry collaborations between Georgia Tech and Zoo Atlanta in Atlanta, GA. The fields of comparative biomechanics and biomimicry researchers often do not find about these fields until potentially graduate school. In this work, we sought to capitalize these new collaborations to display comparative research at the Zoo with an outreach day known as Biomechanics Day: Animals in Motion. This event hosted multiple universities from the Midwest and south that were hosted at the zoo displaying animal locomotion, bio-inspired robots, and human physiology. In total, we hosted over 8,000 participants to this Zoo Biomechanics and Biomimicry Day. These participants were able to learn and re-create how a moth controls flight, how a flamingo stands on one leg, or even how an elephant trunk works. We are already learning about next steps and in the future are looking to collaborate with non-profits as well as scientific companies to increase the impact and potentially work to expand this to zoos across the nation. This is the first zoo biomechanics outreach event of its kind and we hope to see this expanded across the nation. On this poster we provide the steps taken several months out all the way to day of execution to help others plan and execute their own versions of Biomechanics Day: Animals in Motion.

Defining Mammalian Climbing Gaits and their influence criteria including morphology and mechanics

Cassie Shriver, Andrew Schulz, Dylan Scott, Jennifer Elgart, Joseph Mendelson, David Hu, Young-Hui Chang

In comparison to terrestrial locomotion, climbing presents a couple unique challenges. First, organisms must move upwards, meaning they lack a "restoring force" to bring them back into contact with the climbing surface as they continuously overcome gravitational forces. Second, organisms must possess morphology capable of gripping the climbing surface and perform appropriate contact patterns to prevent falling. While recent studies have examined climbing via van der Waals forces and capillary adhesion, these are often limited to non-mammalian species less than 500 grams. Even amongst the studies for mammals, many are focused on primates, which take advantage of highly specialized opposable thumbs, elongated digits, and/or

prehensile tails. Despite the phylogenetic diversity of mammalian climbers, basic concepts like climbing gaits are still limited to insects, primates, and robots. In this work, we attempt to translate the foundational descriptions of terrestrial gaits, e.g. horses trotting, to mammalian climbing gaits. We performed kinematics analyses to identify common mammalian climbing gaits and discerned some underlying criteria influencing these gaits. Due to the aforementioned biomechanical constraints specific to climbing, we predict non-primate, mammalian climbing gaits will all fall within and occupy a smaller subspace of the known terrestrial gaits described by Hildebrand.

Improving fish and shark ecological and evolutionary histories from microfossil ichthyoliths

Elizabeth Sibert

Ichthyoliths, microfossil fish teeth and shark scales (also called dermal denticles), present a unique, high-resolution view into fish and shark communities in deep time. Ichthyoliths are commonly preserved in deep-sea sediment cores, which also preserve diverse environmental proxy records within precisely dated sediment cores, allowing for comparison of fish evolutionary and ecological changes within the context of the past local and global climatic conditions. However, while ichthyoliths are the most numerically abundant fossil record of marine vertebrates, and preserve not only individuals, but whole assemblages at high time resolution, there are significant challenges to using them to study fish and shark evolutionary trends. Most notably, ichthyoliths are preserved outside of any traditionally used biological context: there are rarely body fossils of deep-sea species preserved with in-situ teeth or scales, so the majority of ichthyolith identification is done based on comparing the fossils with teeth and scales from extant species to make inferences about taxonomy and ecology of ichthyoliths based on morphological similarities. Here I present updates from an ongoing multi-year effort to develop and apply quantifiable and repeatable morphological frameworks for both shark denticles and fish teeth, to maximize the potential of the ichthyolith microfossil record. I will share updated R package functionality, as well as present several vignettes featuring the ecological and evolutionary history of several charismatic and ecologically significant deep-sea taxa.

Diverse jaw muscle orientations provide clinically relevant correlations with occlusal grinding

Amira Siddique, L Odette Herrand, Alyssa Stringer, Emily McParland, Peishu Li, Courtney Orsbon, Nicholas Gidmark

Mammalian jaw muscle orientation drives jaw function and movement. These movements can be restricted when pathology of the jaw joint (temporomandibular disorders, TMD) occurs. A wide range of model organisms (mice, rats, rabbits, monkeys, sheep, pigs) are employed for clinical TMD experiments. Here, we measured the lateral and anterior angles of the four main masticatory muscles (masseter, temporalis, and medial and lateral pterygoids) in relation to the occlusal plane across these models. We found the masseter is more dorsally-oriented in sheep, pigs, monkeys, and humans, which all use lateral chewing motions. However, it is rotated anteriorly in rodents, correlating with their distinct anterior-posterior jaw translations. The sheep medial pterygoid and pig lateral pterygoid are the most laterally-oriented muscles of those observed, likely because laterally-oriented pterygoids aid in transverse power strokes. In humans and monkeys, the lateral pterygoid is not as laterally-oriented as the ungulates, but it falls along the dorsal plane. These findings are based on observations when the mouth is in centric occlusion. Current studies explore how these orientations change across the occlusal cycle. Our comparative results provide a framework to help pre-clinical researchers select the most appropriate model organism when studying TMD.

Pushing a system to the limit: *Mus musculus* mastication at the highest XROMM magnification

Sacha Sides, Kelsey Stilson, Josiah Guynes, Susan Williams, Erika Tavares, Anna Wolff, Elizabeth Brainerd

Mice (*Mus musculus*) are an essential study organism in medical and developmental studies, including studies of the head and mastication. Yet

comparatively little is known about the cranial and hemimandibular movement (kinematics) of this system because of the small size of the animal in proportion to current x-ray methods of kinematic quantification. For example, the mandible is composed of two bones (hemimandibles) that move semi-independently, yet they are often modeled as one solid mass.

To examine the movement of the mouse skull in greater detail, we implanted 0.25 mm tantalum markers in the cranium and mandibles of both living and non-living standard lab mice (C57BL/6J). Mice were then trained at the Brown University Keck Research Facility to feed in a biplanar-radiography machine recorded under the highest magnification of the system (M3; focal spot of 0.6 mm). We used the X-Ray Reconstruction of Moving Morphology (XROMM) workflow to analyze the data. Mean intermarker distance for two markers within the cranium was found to be 8.35 ± 0.012 mm (under ideal conditions). Rigid body error (a measurement of whole bone movement) was found to be 0.02 mm for the cranium and 0.095 mm for the left hemimandible. While marker implantation is still an extremely technical process, careful fluoroscope placement and a high frame rate makes measuring of these extremely small systems possible.

Multiple Tropical Insect Families Form Partnerships with Internal Fungi Related to Parasites

Ruby Siehl, Shana Goffredi

Sap-sucking insects within the suborder Auchenorrhyncha fail to obtain necessary vitamins, amino acids, and sterols from the plant sap they consume. Instead, internal microbial symbionts provide these nutrients, missing from the diet of their insect hosts. Within this suborder, some insects within the Fulgoromorpha, commonly known as “planthoppers,” also associate with fungal endosymbionts, which either accompany or replace the anciently-associated bacterial partners. Planthopper-symbiont surveys, however, have only been conducted in temperate regions, thus necessitating investigations into these relationships in the tropics. Here, based on molecular techniques, at least five closely-related tropical planthopper families host yeast-like fungal endosymbionts, related to parasitic fungi within the Hypocreales. Two of these insect families were not previously known to associate with fungi, and in four of the five families, a complete replacement of bacterial endosymbionts (both *Sulcia* and *Vidania*) by fungal endosymbionts was observed. This is particularly noteworthy given the comparatively recent acquisition of the fungal symbionts, based on past estimates. Phylogenetic analyses indicate that fungal endosymbionts generally mirror host insect phylogenies, suggesting an intimate relationship with possible vertical transmission from parent to offspring. To our knowledge, this serves as the most comprehensive tropical planthopper-symbiont survey to date, which adds to the roster of fungal alliances that confer the remarkable success of this diverse insect group.

Developmental changes underlying the evolutionary expansion of the mushroom body in ants

Bogdan Sieriebriennikov, Yuwei Zhong, Hunter Whitbeck, Sergio Cordoba, Maria Ahmed, Claude Desplan

Brains of several animal lineages have become larger and more complex during evolution. We aim to explore the underlying developmental mechanisms by focusing on the mushroom body (MB) in insects, a crucial sensory memory center primarily constituted of Kenyon cells (KCs). In *Drosophila melanogaster*, each of the four MB stem cells sequentially produce seven KC subtypes grouped in γ , $\alpha\beta$, and $\alpha\beta$ classes. These follow an intrinsic temporal patterning program influenced by external cues. In comparison, hymenopterans, such as ants and bees, have more MB stem cells which generate considerably more KCs. Utilizing single-cell RNA-sequencing on the brains of the jumping ant *Harpegnathos saltator*, we found that roughly 40% of all ant neurons are KCs, and categorized them into 17 transcriptionally distinct cell types. Remarkably, these ant KC types align closely with the *Drosophila* KC classes, as evidenced by marker gene expression, statistical label transfer techniques, and birth order inferred from the soma position. Importantly, the late-born class in ants contains many more cells and a greater number of constituent cell types. A detailed look into the birth order of these cell types suggests that they are produced sequentially. This suggests that each of the

ant stem cells generates a longer and more diverse cell lineage, thus resulting in a larger and more complex structure. This may be a general mechanism to increase brain complexity.

Exploring the Impact of Mitochondrial Haplotypes on Physiological Function

Samantha Sierra-Martinez, Douglas Crawford, Marjorie Oleksiak

Mitochondrial interactions constitute a complex network between the mitochondrial and nuclear genomes that is essential for cellular function. These interactions, while known to play pivotal roles in various physiological processes, have yet to be fully understood, even in the metabolic pathway whose enzyme complexes are encoded by both mitochondrial and nuclear genes, the Oxidative Phosphorylation (OxPhos) pathway. We investigated how mitochondrial variations impact OxPhos metabolism using admixed *Fundulus heteroclitus* populations with distinct Northern (N) and Southern (S) mitochondrial haplotypes (mt-haplotypes). Specifically, we quantified State 3 OxPhos metabolism in brain homogenates from three admixed *F. heteroclitus* populations. Additionally, to gain an in-depth understanding of how OxPhos metabolic processes respond across varying temperatures, we quantified State 3 OxPhos metabolism across a 12°C to 28°C thermal performance curve. Through the integration of genetically variable natural populations, specialized physiological measurements, and the innovative application of a thermal performance curve, this research seeks to expand our understanding of the importance of mitochondrial haplotype variations in shaping metabolic responses and provide valuable insights into ongoing discussions about the genetic basis of metabolism and adaptation.

Do silkmoths and butterflies fly alike?

Usama Sikandar, Brett Aiello, Simon Sponberg

Many Saturniidae (wild silkmoths) display erratic flight with pitching and bobbing body undulations occurring at wingbeat frequency. This is kinematically similar to their close cousin Papilionoidea (butterflies) and may help both groups be more elusive to predators. Differently, the silkmoth's sister clade in the Bombycoidea superfamily, Sphingidae (hawkmoths), does not show these kinematic features characteristic of silkmoths and butterflies. Instead, hawkmoths, which rely on similar wing kinematics and morphology to the hummingbirds, also display a hover feeding behavior. This raises the question of whether silkmoths and butterflies also share broad similarities and their kinematics and wing morphology, and if so, what are the aerodynamic consequences of this flight package? We find that these groups have similar wing loading, aspect ratio and wingbeat frequency – smaller in values compared to hawkmoths. Wing loading and flapping frequency are correlated with the undulation magnitude because of coupled wing-body dynamics. Body pitch oscillations make the stroke plane more vertical during downstroke than upstroke, thus specializing one half-stroke in weight support and the other in forward thrust. Our blade-element aerodynamic analysis shows that this strategy reduces the drag force in a subtle way hence reducing the aerodynamic power requirements. Together these results suggest that the flight strategies of sister clades hawkmoths and silkmoths have diverged from one another to be more akin to hummingbirds and butterflies respectively. Ancestral Bombycoidea have wing shapes intermediate to both sister clades, but different from butterflies. This, together with the likely existence of a limited number of flight strategies available for survival, suggests that flight strategies in butterflies and silkmoths are evolutionarily convergent.

Characterizing Ecological Interactions Between Fruit Bats and Piper Plants Across Different Habitats

Sneha Sil, Flo Visconti, Sharlene Santana

Bats are important seed dispersers of hundreds of plant species in the tropics, but their patterns of plant use across habitats are still relatively unknown. Piper are pioneer plants essential to forest regeneration that benefit from a mutualistic relationship with short-tailed fruit bats (*Carollia*), which disperse Piper seeds via consumption and defecation during flight. Since the types and diversity of Piper frugivores are thought to be influenced by the primary habitat of different Piper species, we aimed to characterize the interactions between

Carollia bats and Piper plants across forested and open habitats. We analyzed nightly acoustic ultrasonic recordings and 24h camera trap data collected in La Selva, Costa Rica. The presence of *Carollia* (search-phase echolocation calls, bats flying by or inspecting fruits, bats consuming fruits) was noted across 45 plants and 12 Piper species (6 forest, 6 gap). Additionally, since *Carollia* utilize olfaction to find Piper, fruit scent volatiles data were analyzed to reason whether chemical cues play a larger role in these interactions. We found significant differences in forest and gap Piper scent compositions and patterns in *Carollia*-Piper interactions that suggest habitat effects; however studying visitation patterns requires substantial consideration to biomonitoring modality. Documenting ecological interactions between bats and plants across habitats not only allows us insight into plant-animal mutualisms, but can assist us in developing conservation strategies to preserve tropical ecosystems.

Garter Snakes are Bringing the Heat; Temperature Preferences and Behavior Changes Pre- and Post-Shed

Alex Sills, Jamie Marks, Anne Bronikowski

Understanding animal behavior is a crucial component to maintaining captive animal populations used in research. Incorporating findings from these animal studies helps mitigate unintended variables in future experiments. Garter snakes (*Thamnophis elegans*) exhibit many different behaviors throughout the day, such as bathing and burrowing in their bedding. By using their heat tape, they are able to thermoregulate by moving around the thermal gradient in their tanks. The goal of this study is to understand how behaviors change when the snakes are in the shedding process, because we are unable to take blood samples needed for other experiments. Handling is known to cause a stress response in snakes; thus, this helps us minimize this response and overall improves snake care. We used an ethogram to collect the behavior data. Shedding dates were recorded to compare the frequency of each behavior prior to and after shedding. We made three specific hypotheses; garter snakes do not exhibit different behaviors prior to shedding; garter snakes do not have a temperature preference prior to shedding; and wild caught and captive born individuals will not differ in behavior prior to shedding. We found that the snakes are more likely to perform behaviors on the warm side of their enclosure in the days leading up to their shedding. Furthermore, there is a difference in behaviors prior to shedding between our wild caught individuals and our captive bred individuals. These outcomes can help us better care for our snakes by knowing when to avoid taking their blood, handling them, and manipulating their enclosure. Our goal is always to have the best husbandry practices possible, so these studies can help us to keep our snakes healthy for other projects they are involved in.

Epigenetic variability in wild populations of mangrove rivulus exhibiting genetic diversity gradient

Frederic Silvestre, Valentine Chapelle, Ryan Earley, Kristine Marson, Jérôme Lambert

An increasing number of studies are investigating the role of epigenetic mechanisms within evolutionary processes. Understanding the origins and patterns of epigenetic variation from an evolutionary point of view is challenging as it necessitates the natural control of genetic variation among individuals to avoid genetic interference. The mangrove rivulus *Kryptolebias marmoratus*, presents a mixed mating reproductive strategy that can naturally produce isogenic populations through self-fertilization and generates genetic diversity gradient by controlling the balance between selfing and outcrossing rates. Using Reduced Representation Bisulfite Sequencing (RRBS) on brains and livers, we investigated DNA methylation variation within and among four wild mangrove rivulus populations in Florida and in Belize along a genetic diversity gradient. We also assessed individual and consistent behavioral variation as an endpoint to represent the capacity of DNA methylation to generate phenotypic variability. We found similar level of epigenetic variation within all four populations, regardless of genetic heterogeneity. Moreover, the functional enrichment analysis of genes differentially methylated showed shared pathways between the four populations, while a smaller proportion were population-specific. Another intriguing result is that individuality emerged in populations with very low genetic diversity, but not in the most genetically diverse population. We brought evidence that different sources of epigenetic

variation can be jointly used in a population-specific way, influencing methylation level of genes involved in common pathways with ecologically relevant implications. Our results suggest that generation of phenotypic variation through epigenetic alterations could be a potential explanation for the persistence of genetically limited populations in heterogeneous environments.

Extrinsic stressors impact North American bat cellular immunity during reproductive periods

Molly Simonis, Sarah Ciarrachi, Kristin Dyer, Meagan Allira, Riley Bernard, Matthew Chumchal, Catherine Haase, Jeffrey Foster, Daniel Becker

Wildlife face a number of extrinsic stressors such as habitat loss, pathogens, and contaminants. Extrinsic stressors can increase the energy needed to maintain health and survival, especially during periods of elevated intrinsic stress such as reproduction. Bats can be used as bioindicator species for extrinsic stressors of wildlife, given their long lifespans, high mobility, diverse habitat use, and—for insectivorous bats—occupancy of high trophic levels. To better understand the combined physiological effects of extrinsic and intrinsic stressors, we initiated a pilot study using North American bats from Oklahoma, Arizona, Tennessee, and Wyoming. We collected blood and fur from six bat species, and recorded their reproductive status from spring to fall 2023. From blood samples, we assessed bat cellular immunity and *Bartonella* sp. infection, as well as micronuclei intensity (representing genotoxic effects of pesticide exposure). We also quantified mercury concentrations from fur and assessed land-use proportions at capture sites from species-specific home ranges. Using principal component analysis and general additive models, we will determine how extrinsic stressors shape bat immunity during their reproductive periods. This work will continue to expand by incorporating more locations, immune markers, and extrinsic stressors (e.g. white-nose syndrome infection) to better understand how stressors impact bat immunity across spatial gradients. This ongoing research is crucial for the protection of wildlife populations and identifying where and when to allocate resources for conservation.

Flowing Your Nose: correlation between sensory tissue and water flow in Chondrichthyan noses

Lauren Simonitis, Aubrey Clark, Elizaveta Barskaya, Tricia Meredith, Marianne Porter

To smell, fish rely on passive water flow into their olfactory organs to detect chemical signals in their aquatic environment. The nasal morphology of cartilaginous fishes varies widely and this diversity in shape is hypothesized to correlate to differences in water flow patterns. Previous research has shown a connection between flow regimes and sensory microstructure in hammerheads (family Sphyrnidae). Here, we investigate the continuation of this pattern in members of other Chondrichthyan families including Carcharhinidae, Squalidae, Alopiidae, and Chimaeridae. Using a suite of biological imaging tools such as contrast enhanced microCT, scanning electron microscopy, and histology we describe the morphology of olfactory organs and where the sensory microstructure is concentrated along the medial to lateral gradient of the olfactory rosette. We also use a combination of existing and new research on nasal fluid flow patterns to correlate sensory structure density with water movement in the olfactory organ.

Heat vs. Humidity: novel methods for studying microhabitat use of small lizards in the lab

Amber Singh, David Ensminger

Under standard conditions, thermoregulation is currently understood to be the main driver for microhabitat selection in lizards. The exact preferred temperature range is relatively easy to study in a laboratory setting as thermal gradients are easy to control over a prolonged period. Humidity is assumed to be an important secondary driver of microhabitat selection. However, humidity is difficult to study in a prolonged, controlled setting due to the quick evaporation and dissipation of water molecules in a small area, as well as the direct relationship between temperature and humidity. Here we present our novel approach to studying the interactive effects of temperature and humidity on the microhabitat selection of a small species of lizard, *Sceloporus occidentalis*.

Sectioned gradient enclosures were constructed to allow for a continuous temperature gradient to exist along one axis (short side) and a segmented substrate humidity gradient to exist along the perpendicular axis (long side). This construction allowed for the free movement of individuals between the gradients while limiting the movement of humidity, so conditions remain relatively constant over a 36-hour period. In this way, we can tease apart the importance and range of both humidity and temperature on Western Fence Lizard microhabitat selection. Currently we are investigating how maternal stress alters juvenile microhabitat selection using these methods.

What is ctenophore jelly made of?

Sara Siwiecki, Alison Sweeney, Amit Datye

Gelatinous zooplankton are one of the most common groups of marine animals, found in diverse worldwide locations. Among marine animals, there is a wide spectrum of soft and hard materials. Gelatinous zooplankton have been characterized by their seemingly “simple” but successful bodies that have evolved multiple times. Yet despite their commonality, the biochemistry that builds this namesake gelatinous structure is understudied. Our work explores the biochemical and biophysical mechanisms that form the gelatinous tissue (mesoglea) of ctenophores, one of the most widespread groups of gelatinous zooplankton. Interestingly, previous reports found that ctenophore genomes seem to lack most of the typical collagens that usually make up metazoan gelatinous tissues. So, we are investigating this unique biochemical content and the dynamics of ctenophore mesoglea in two species: *Mnemiopsis leidyi* and *Pleurobrachia pileus*. Using a thermogravimetric analyzer, we measured the dry and ashed weight of mesoglea. We identified that the total sample weight for both species is made of ~97% water, ~1.5-2.5% salt, and ~0.5-1.5% organic material. Since only ~1% of mesoglea is actual biological matter, we are looking into how the mesoglea macromolecules organize their watery body and how mesoglea differs from seawater. To do this, we are using differential scanning calorimetry to determine amounts of bulk and bound waters throughout the body. In this ongoing work, we hope to understand how the gelatinous body of ctenophores functions to make them successful in diverse marine environments.

North Pacific larval fish differ in their biochemical responses to environmental stressors

Emily Slesinger, Ben Laurel, Erik Thuesen, Samantha Mundorff, Mary Beth Hicks, Paul Iseri, Thomas Hurst

Changing environmental conditions, including ocean warming and ocean acidification, may lead to novel species interactions, largely driven by differential responses among individual species. At the Pacific-Arctic interface, Pacific cod and Arctic cod both spawn pelagic free-swimming larvae during late winter, and warming in the region may facilitate overlap between the species. We assessed larval metabolic capacity through metabolic enzyme activity assays of citrate synthase (CS; aerobic metabolism), lactate dehydrogenase (LDH; anaerobic metabolism), and β -hydroxyacyl CoA dehydrogenase (HOAD; fatty acid metabolism) in specimens raised under routine husbandry conditions and elevated temperatures and pCO_2 . Pacific cod enzyme activities were ~2x higher than Arctic cod, but Arctic cod relied more heavily on fatty acid metabolism; these may reflect the more active larval lifestyle of Pacific cod. Pacific cod enzyme activities were all elevated when raised at colder temperatures, while only CS in Arctic cod was elevated. Arctic cod LDH was elevated relative to CS under warmer temperatures suggesting high temperature stress. Both species were mostly unaffected by pCO_2 , but Pacific cod HOAD activity decreased at control temperatures, potentially due to lipid dysregulation, while Arctic cod CS and HOAD activity increased at control temperatures which suggests increased enzymatic activity to match higher energy demands. Overall, enzymatic activities of larval cod were much more influenced by changes in temperature than changes in pCO_2 .

Investigating the influence of thermal fluctuations on developmental efficiency in a TSD species

Christopher Smaga, Samantha Bock, Anthony Breitenbach, Benjamin Parrott

The energetic cost of embryonic development, defined as the product of development time and metabolic rate, represents the efficiency by which maternal resources are utilized by developing embryos. According to developmental cost theory (DCT), organisms evolve thermal dependencies of metabolic rate and incubation duration to minimize that cost, resulting in a narrow, optimal range of temperatures at which efficiency is maximized. Thermal fluctuations, as occur in nature, can alter developmental cost: fluctuations about the optimum temperature are predicted to increase developmental cost, as temperatures above and below both reduce efficiency. In comparison, the effect of fluctuations about a sub-optimal temperature are predicted to be reduced, since fluctuations in one direction decrease cost while fluctuations in the opposite direction increase cost. However, this has not been tested empirically. Here, using the American alligator (*Alligator mississippiensis*), we test the influence of fluctuating incubation temperatures on developmental efficiency, defined as the residuals of the relationship between egg mass and hatching mass. Alligators utilize temperature-dependent sex determination (TSD), and male-promoting temperatures minimize developmental cost. Using a combination of constant and fluctuating male and female-promoting temperature treatments, we compare developmental efficiency across groups, predicting that fluctuations about a male-promoting temperature will decrease developmental efficiency to a greater extent than fluctuations about a female-promoting temperature. Finally, we discuss the potential implications of DCT for the evolution of TSD in this species.

The role of rove beetle odorant receptors in the evolution of symbiotic lifestyles

Hayley Smihula, Julian Wagner, Sheila Kitchen, Joe Parker

Although animals must interact across species boundaries to recognize, evaluate, and respond appropriately to other organisms, the neural mechanisms underlying these interactions remain poorly understood. Most rove beetles (Staphylinidae) are free-living soil-dwellers equipped with defenses against ants, their primary predators. But within this family, hundreds of lineages have convergently evolved symbiotic relationships with ants and exhibit host-seeking, rather than defensive, behaviors. This dichotomy facilitates comparative studies between free-living and “myrmecophile” beetles to address how neural circuits evolve to produce vastly different behavioral outputs to similar stimuli. Here, we focus on the olfactory basis of detecting host ant semiochemicals. We show that the myrmecophile *Sceptrorhynchus lativentris* is attracted to its host's pheromones, while the free-living *Dalotia coriaria* finds the cues aversive, and that this aversion is mediated through chemoreceptors located on the antennae. Using single cell-RNA sequencing, we find that a small number of orthologous odorant receptors (ORs) are more highly expressed in both *Sceptrorhynchus* and *Dalotia*, and we couple heterologous expression systems and RNAi paired with behavior assays to determine whether these ORs are responsible for detecting the relevant ant pheromones. We also assemble putative OR repertoires across Staphylinidae species and perform selection analyses to determine if conserved expansions and reductions within the OR family correspond with lifestyle and the shift from free-living to myrmecophily.

“Tell me why”: open-ended responses explain perceptions of biological statements used in biomimicry

Dimitri Smirnoff, Anita Schuchardt, Mary Guzowski, William Weber, Jessica Rossi-Mastracci, Alan Love, Ruth Shaw, Mike Travisano, Amanda Hund, Mark Borrello, Gillian Roehrig, Emilie Snell-Rood

Biomimicry is one of many schools of thought that turn to biological organisms as a source of inspiration for solutions to technical challenges. To help tap into the wealth of biological knowledge, frameworks distilling biology into accessible statements have been developed to educate and inspire professionals and students. However, these statements' biological accuracy and design utility have not been systematically assessed. Our study surveyed

people of different professional backgrounds and career stages to assess their perception of the biological accuracy and problem-solving utility of two different frameworks used in bio-inspired design, Life's Principles and Nature's Unifying Patterns. This poster builds on our quantitative study with an exploration of why survey participants assigned particular statements particular ratings. Preliminary results suggest biologists question the generalizability of statements, take issue with the use of the term “nature”, and critique the oversimplification of biological concepts. Non-biologists express confusion with the phrasing of some statements, critique the vagueness of some statements, and question their relevance to their particular fields of work. They also identify why certain statements hold utility for them in their problem-solving process. Both biologists and non-biologists highlight what makes a statement accurate or useful and make recommendations for how to improve those that are not there yet. These results help us begin to understand why certain statements are perceived as accurate and/or useful and how inaccurate or unhelpful statements might be revised.

A Quest for Biological Accuracy and Design Utility in Biomimicry Frameworks: a quantitative study

Dimitri Smirnoff, Anita Schuchardt, Mary Guzowski, William Weber, Jessica Rossi-Mastracci, Alan Love, Ruth Shaw, Mike Travisano, Amanda Hund, Mark Borrello, Gillian Roehrig, Emilie Snell-Rood

The natural world is increasingly seen as a source of inspiration for solutions to socio-environmental challenges, a practice known as bioinspiration or biomimicry. Yet, practitioners without a background in biology can struggle to tap into the wealth of biological knowledge. To address the difficulty, frameworks distilling biology into accessible statements have been developed to educate and inspire professionals and students. However, these statements' biological accuracy and design utility have not been systematically assessed. Our study surveyed people of different professional backgrounds and career stages to assess their perception of the biological accuracy and problem-solving utility of two different frameworks used in bio-inspired design, Life's Principles and Nature's Unifying Patterns. Differences in perceptions emerged between biologists and non-biologists, and between biology professionals and biology undergraduates. Notably, non-biologists rated statements as more accurate than biology professionals, suggesting current frameworks may foster biological misconceptions. Simultaneously, specific statements were rated as more useful for inspiring designs by non-biologists. This study forms a foundation for exploring factors that enhance statement biological accuracy and relevance in bioinspired design. Ultimately, our work contributes to accessible biology education for practitioners of bioinspiration and as well as students from various grade levels.

Vibratory Signaling in Japanese Rhinoceros Beetles

Aidan Smith, Brook Swanson

Japanese Rhinoceros beetles (*Trypoxylus dichotomus*), known primarily for their large horns, are a classic example of ornate weaponry produced through sexual selection. The male beetle's prominent horns are used in male-to-male combat for dominance and access to females. Observations in the lab and the field suggest that multiple forms of signaling are also involved in both the aggressive interactions and female mate choice. One such signal seems to be the songs created through male abdominal stridulation. Males perform both an alarm-style chirp (also seen in aggressive interactions) and rhythmic “purring” prior to copulation attempts. Several questions arise in relation to this behavior and its effect on mating outcomes: Is there a relationship between song characteristics and morphological characteristics? Can vibrations be transmitted through the surrounding substrate? Is there a relationship between song characteristics and courtship outcomes? To analyze these songs in the field, a Polytech VibroGo VG-200 laser vibrometer was used to measure the velocity of both the male's elytra and surrounding tree bark during courtship. Vibrational amplitude and periodicity, corresponding location, beetle characteristics, and courtship details were collected. Male courtship song characteristics will be compared to morphological variables, as well as courtship outcomes. Substrate vibration transmission and attenuation will also be discussed.

Impact of Chronic Use of Viscous Milk on Infant Feeding Physiology: Sucking

Ani Smith, Elska Kaczmarek, Max Sarmet, Kendall Steer, Thomas Stroud, Alexane Fauveau, Maressa Kennedy, Hannah Shideler, Alex-Ann Velasco, Skyler Wallace, Morgan Billie, Christopher Mayerl

Infant mammals acquire food by suckling, during which the tongue seals around a nipple and moves in an anteroposterior wave-like pattern to generate suction. Sensorimotor integration helps to regulate suckling and allows an infant to respond to various sensory inputs, including milk viscosity, which is often prescribed as an intervention for infants who struggle with feeding. However, we have little insight about the neuromotor response to thickened milk. To address this, we raised three infant pigs on regular formula and three on thickened formula. At the end of infancy, we exposed each group to both regular formula and thickened formula while recording synchronous video-fluoroscopic video and intraoral pressure generation. We found no significant differences in suck rate across groups and milk types. The viscous-raised pigs generated less suction and acquired less milk per suck when feeding on regular milk compared with control pigs. The suck volume of control pigs was lower when feeding on thickened milk than standard milk, but was similar to the suck volume for viscous-raised pigs when feeding on thickened milk. This was correlated with differences in tongue movement between groups and milk types. These results suggest that being raised on thickened milk reduces the ability to respond to variation in milk viscosity and feed effectively on regular milk, and inhibits the development of oromotor function.

Neurophysiological Alterations in Crayfish Using Common Inhibitory Drug

Grace Smith, Daniel Bergman

Gabapentin (GBP) is one of the most prescribed medications in the United States today. Its original use was for treating partial seizures; however, it gained popularity when it became approved for treating fibromyalgia and neuropathy. Many providers have grown to accept GBP more for its ability to treat various pain syndromes off label than for its FDA accepted uses. The drug is continuing to gain popularity with new off label uses suggesting therapeutic effects in mood disorders. Current research suggests 95% of gabapentin is prescribed off label. It is known that GBP gains its anticonvulsant properties from its ability to slow electrical conduction within the brain, however, its mechanisms for pain and mood stabilization remain unknown. Proposed is an experiment in two parts: a neurophysiological model and a behavioral model. The first will assess the contractibility of muscle fibers in the presence of gabapentin using electrophysiology to confirm neuronal inhibition. Second, an assessment of variations in aggressive interactions among crayfish. We hope to establish a model system that allows for the better understanding of GBP's mechanisms of action.

Diel thermoregulation in grasshoppers: where to be, when to feed

Julia Smith, Lauren Buckley, Monica Sheffer

Ectotherms' ability to acquire and process food and the rate at which they burn energy are highly temperature dependent. While ectotherms do not internally regulate their body temperatures, they can alter their body temperatures and subsequently performance and energetics via microhabitat selection and temporal partitioning of activities. Food processing (digestion) has a higher thermal optima than food acquisition (locomotion) for my focal grasshopper species, *Melanoplus boulderensis* and *M. sanguinipes*. I analyze field observations to test the hypothesis that grasshoppers' choices of activity and microhabitat throughout the day reflect the differing thermal optima of locomotion and digestion. I do not find a strong relationship between time of day and activity or microhabitat, but a next step is to examine how activity timing and microclimate selection vary with grasshopper body temperatures. The observations will be used to construct an energy budget and analyze shifts in estimated discretionary energy over the past 60 years. Understanding behavioral thermoregulation and how it mediates energy gain may be key to predicting the fitness effects of climate change on many ectotherms.

Cultural evolution of mountain white-crowned sparrow song: Drift or cross-dialect contamination?

Katie Smith, Thomas Hahn, Carly Hawkins

Regional song dialects in songbirds are an example of culturally-evolving behavior patterns. Prior work on temporal stability of song dialects in Mountain White-crowned Sparrows (*Zonotrichia leucophrys oriantha*) indicated that in small, isolated groups of males, temporal changes in dialects were consistent with abrupt additions and losses of song components. In contrast, in larger groups, drift in the details of existing notes and phrases was the norm. Here we revisit the structure of songs in the Tioga Pass (high Sierra Nevada, CA) dialect area of Mountain White-crowned Sparrows 25 years after the previous analysis. In this area of numerous breeding birds, some males still sing song versions indistinguishable from those in the 1990s. However, many males now sing a "new" version of the Tioga Pass dialect that differs from the "old" version only in minor details in one of the five song phrases. This finding supports the earlier interpretation that song changes in large groups proceed more by gradual accumulation of small modifications than by major, rapid changes owing to introduction or extinction of components. We discuss implications of song learning patterns (e.g., vertical versus horizontal transmission during the sensory phase of song development, and prevalence-based song-type selection during the crystallization phase) to the observed patterns of song dialect evolution.

How does trabecular structure influence the mechanical properties of tiny mammalian vertebrae?

Stephanie Smith, Myleen Amendano, Saniya Patel, Kenneth Angielczyk, C. Tristan Stayton

The relative contributions of trabecular (spongy) and cortical (compact) bone to bone strength and stiffness, although investigated in humans, is mostly unclear. As a result, we do not understand how the skeleton of small animals, especially the axial skeleton, has evolved to deal with the particular challenges of life at tiny size. In mammals, some small species have notably reduced their vertebral trabecular bone structure, resulting in mostly hollow medullary cavities. To assess the importance of trabecular structure to the mechanical properties of small mammalian vertebrae, and incorporate the effects of both trabecular and cortical bone structure, we conducted finite element analysis on the lumbar vertebrae of 15 species of shrews (Mammalia: Soricidae). We analyzed two sets of models: vertebrae with the trabecular structure intact, and vertebrae with all trabeculae excised from the centrum. In all models, the cranial end of the centrum was immobilized, and a 5N load was applied to the caudal end of the centrum, parallel to the craniocaudal axis. Results indicate higher peak stresses and larger displacements in models lacking trabeculae. Ongoing work will assess how bone morphofunctional characteristics change as body size increases, and to validate these analyses with empirical materials testing.

Paranasal spaces and nasal glands in bats: Too big to fit?

Tim Smith, Abigail Curtis, Thomas Eiting, Vaibhav Chhaya, Nicholas King, Sarah Downing, Veronica Rosenberger, Valerie DeLeon, Sharlene Santana

Bats are notable for their great variation in nasal anatomy, including paranasal cavities that vary from absent to large, cavernous spaces rivaling the central airway in size. In most mammals, these chambers house the lateral nasal glands (LNG) or olfactory turbinates. Here, we studied 78 bat species from 15 families using histology and iodine-enhanced CT to assess the relationship of the LNG to the paranasal spaces. We noted presence or absence of paranasal recesses and nasal respiratory glands, and made developmental inferences using several neonatal specimens. Some bats of the suborder Yangochiroptera, including all vespertilionids, lack any paranasal recesses. In contrast, all bats of the suborder Yinpterochiroptera possess paranasal spaces, including a maxillary recess and its rostral continuation, a cul-de-sac called the anterolateral recess. Notably, the LNG is partly or completely outside the confines of the paranasal recesses in most bats of both suborders. It extends far caudally in pteropodids, a bat family that lacks the septal nasal gland. Our developmental samples suggest that bats have deficient growth or

loss of parts of the nasal capsule. Notably, the lamina semicircularis is deficient rostrally, which may allow expansion of the LNG beyond paranasal spaces. Our findings indicate that bats may have fundamentally reorganized the nasal bauplan, and suggest paranasal spaces have lost function in some bats, and perhaps evolved novel functions in others.

STEM Educators as Civic Educators

David Smyth

Spurred by twin crises of COVID-19 and climate change, and an uncertain employment horizon, the goals for STEM education have shifted from developing a STEM capable workforce to broader and more durable undergraduate learning outcomes, such as evidence-based and informed civic engagement, life-long learning, and systems thinking. At the same time, our polarized and weakened democracy has generated new demands for greater attention to civic education. For over 20 years Science Education for New Civic Engagements and Responsibilities (SENCER) has supported curricular and institutional change to improve civic capacity and STEM learning through context and problem-based teaching. Designated a “community of faculty transformation” and a “lever for change” in literature on STEM reform, SENCER’s programs help faculty re-frame traditional course content by teaching disciplinary concepts through real-world, relevant, civic challenges. Gen Bio becomes “Biomedical Issues of HIV-AIDS,” or “COVID-19” Environmental Sci 100 becomes “The Future of Natural Resources,” Gen Chem becomes “Assessing Exposure to Toxic Chemicals,” Cellular and Molecular Biology becomes “Cancer,” Math 102 is “Statistical Analysis of Community Challenges.” Learning research confirms the effectiveness of SENCER’s problem-based, inquiry driven pedagogies, as well as the importance of social and community relevance to retention and persistence of traditionally underserved students. This talk will describe strategies employed by SENCER faculty to integrate civic issues into their courses than span the disciplines and engage all students with the goal of not only increasing STEM content knowledge but also STEM engagement, understanding of STEM relevance and a love of lifelong STEM learning.

Phylogenetic Niche Models Offers Insights into Past Refugia and Niche Evolution in *Plethodon* spp.

Anthony Snead, Kristin Winchell

While adaptive radiations serve as model systems to quantify rates of diversification. Diversification driven by vicariance or allopatric speciation is considered to be the most frequent form of diversification. Salamanders within the *Plethodon* genus exhibit few morphological differences between taxa and are suggested to be a nonadaptive radiation, even though their ranges differ significantly. Hence, the woodland salamanders offer an interesting system to interrogate the rate of niche differentiation when speciation is assumed to be driven by largely neutral processes. By combining a time-calibrated phylogeny with publicly available occurrence and climatic data, we develop ecological niche models for 46 of the 54 recognized species in the genus and use the response curves as traits to understand how ecological niches changed over macroevolutionary timescales. Our results suggest that evolutionary change in response curves differ significantly depending on the environmental variable and taxon being compared. Furthermore, we find dramatic differences between groups in their ecological niche even though the radiation is suggested to be non-adaptive. Using our ancestral niche reconstruction, we project onto paleoclimatic data from eleven different time-points to identify potential refugia for woodland salamanders during previous climatic fluctuations. Hence, we offer insight into the ecological divergence between woodland salamander groups while uncovering changes in their distribution across macroevolutionary timescales.

Single-Cell Characterization of Coral Phagocytic Cells

Grace Snyder, Anthony Bonacolta, Javier del-Campo, Benyamin Rosental, Nikki Traylor-Knowles

Stony corals are pivotal for reef ecosystems yet are globally threatened with climate change and have drastically decreased in coverage. Despite the anthropogenic stressors they now face, much is still unknown about their

innate immune system and how it is being impacted by a changing environment. Regardless of the numerous transcriptomic studies on innate immune responses in corals, little work has been conducted on the cellular level; only recently were immune cells characterized in stony corals. However, doubt still remains on whether these phagocytic cells can truly be classified as immune cells. To address this, single-cell sequencing was used on functionally identified phagocytes in stony branching coral *Pocillopora damicornis* and shown corresponding immune gene upregulation upon exposure to various stimulants, as well as multiple subpopulations that align with morphological observations. Beyond toll-like receptor signaling and tumor necrosis factor receptor pathway upregulation, we also showed that these cells are distinct from those undergoing dextran-induced pinocytosis. This highlights the uniqueness and specialized immune role of these phagocyte populations. The combined functional and molecular description of immune cells in this early-diverging metazoan single-cell study is novel and provides valuable insight into its innate immune defense system and resilience strategies against rising threats of increased ocean temperatures, acidification, disease outbreaks, and other anthropogenic stressors.

Watch your step!: Stingray Sting Prevention

Jacob Sobol, Anthony McGinnis, Trinity Lozano, Cassandra Donatelli, Benjamin Perlman

Every year off California’s coast, there are roughly 2500 people being stung by stingrays, with Seal Beach being among the highest locations of reported injuries. The primary culprit of these injuries is the round stingray (*Urolophus halleri*), which is the one of the common stingrays found within California beaches. The stings administered by these rays create a puncture wound and deliver venom that amplifies the pain received by the victim. They are also sharp enough to penetrate most beach outerwear, including neoprene wetsuits. One preventative measure from getting stung is the “stingray shuffle”, an act of shuffling your feet while entering the surf zone. To better understand the forces stingrays generate when striking, sting resistant (neoprene) material can be created to resist these strikes. With the collaboration of material science engineers, we prototyped neoprene-based footwear to withstand these stingray strikes. We tested the point at which the material failed. The forces we measured that round stingrays generated in-situ were less than the point of failure for the material, so we determined that our material was strong enough to withstand most round stingray strikes. By acquiring new knowledge on the striking forces that *U. halleri* can generate, we can continue to improve beachwear that provides protection from stingray strikes.

Chironius: a new jumping snake from South America

Jake Socha, Jeffery Anderson, Beckett Socha, Amalia Moore, Taylor Mortensen, Amalia Moore, Josh Taylor, Emerson Torres Pacaya, Alex Marsh

Jumping is a rare behavior in snakes, currently known in only a few taxa. Two genera exhibit jumping in an arboreal setting: flying snakes (*Chrysopelea*) use jumping to initiate glides and to cross gaps, a behavior also found in their sister taxon, the bronzebacks (*Dendrelaphis*). This dynamic behavior is only elicited experimentally when the gap is sufficiently large, but relatively few species have been tested. Do other arboreal snakes jump, and if so, how does it differ from previously recorded species? We examined multiple genera of neotropical snakes wild-caught in Peru, including *Chironius*, *Drymoluber*, *Imantodes*, *Oxyrhopus*, *Oxybelis*, and *Siphlophus*. Snakes were placed on an origin perch and encouraged to cross a gap to a target perch. In successive trials, the gap size was increased until the snake jumped or refused to cross. For most species tested, only non-jumping behaviors were observed. However, jumping was found in a single specimen of *Chironius exoletus* (m=62 g, SVL=75 cm), elicited at gap sizes of 50 and 55% SVL. Additionally, a single jump was observed in the wild from a second specimen (m=450 g, SVL=139 cm). Like *Dendrelaphis* and *Chrysopelea*, *Chironius* formed a loop and then accelerated to initiate the jump. This finding demonstrates that arboreal jumping in snakes evolved independently at least twice, and may be more prevalent than previously recognized.

Escalating summer temperature-minima drive collapse of a mountain insect community

Keith Sockman

Mountain ecosystems have disproportionately high rates of climate change and disproportionately high numbers of endemic species. Like most ecosystems, they require insects to function. In order to determine how climate change is impacting mountain insects, I quantified their abundance during 13 seasons spanning 18 yr at a remote, subalpine meadow in Colorado, U.S.A., where weather data were recorded for the past 36 yr. During the period of insect sampling, this location showed no evidence of environmental change, other than climate change and presumably ecological succession. I discovered that the abundance of aerial insects declined at an average rate over 10% annually, yielding an 84% decline over the study. An information theoretic analysis of nearly 800 combinations of 39 weather-related factors revealed that mean temperature-minima in the 2 wk preceding sampling and mean temperature-minima the summer before sampling interacted as the primary predictors of insect abundance. Specifically, within-season abundance increased with recent temperature-minima, whereas between-season abundance declined with the prior summer's temperature-minima, which have risen an average of over 0.1°C annually. Given the ecosystem services insects provide, the collapse of this subalpine community augurs poorly for high-elevation ecosystems generally. Curbing climate warming is critical for the long-term persistence of these organisms and ecosystems.

Sensory Salience Modulates the Tracking Response of Zebrafish During Rheotaxis

Sewal Solmaz, Mehmetcan Gokce, Elif Can, Ismail Uyanik

Sensory salience influences multisensory behavioral control profoundly. Here, we explore the impact of sensory salience on the rheotaxis behavior of Danio rerio (zebrafish) hovering still against a flow. In a custom-built speed-controlled swim tunnel, a D-shaped tube was placed to alter the flow. It produced mechanosensory cues upon movement, while its red inner stick elicited visual stimuli, tracked by the fish. Specific scenarios varying the tube's diameters optimized or reduced visual and mechanosensory salience, depending on light conditions. An outer transparent tube remained fixed while the inner red stick diameter was decreased sequentially, maintaining mechanosensory cues but lessening visual cues under light, with no changes under dark conditions. Removing the outer tube while varying the inner red stick's diameter further under light diminished both visual and mechanosensory stimuli, but under dark conditions it only hampered mechanosensory receptions. Using N=5 fish, frequency response were estimated between fish and stimulus movements. The critical role of mechanosensory information was affirmed in the fish's tracking performance, with significant enhancement from visual stimuli. The study underscores the import of sensory salience in rheotaxis behavior management for zebrafish. Supported by TUBITAK (120E054).

A multi-factor meta-analysis of the determinants of variation in thermogenic capacity in deer mice

Derek Somo, Grant McClelland, Graham Scott

Physiological studies of wild-caught animals bred in captivity offer a powerful system for dissecting the contributions of the environment (developmental plasticity, acclimation, etc.) and evolutionary adaptation to phenotypic variation and fitness. Previous studies of deer mice (*Peromyscus maniculatus*) have indicated that the aerobic capacity for thermogenesis ($\dot{V}O_{2,Summit}$) is a key fitness-related trait in the cold hypoxic conditions at high altitude, and that environment and evolutionary adaptation contribute to adaptive increases in this trait in high-altitude populations. However, it remains poorly understood whether sex or epigenetic changes in later generations of captivity affect this variation. We hypothesized that $\dot{V}O_{2,Summit}$ would be reduced in later generations and would vary between sexes. We tested our hypothesis using a linear mixed modelling approach to analyze a large dataset (N=760) of $\dot{V}O_{2,Summit}$ in high- and low-altitude native deer mice and low-altitude restricted white-footed mice (*P. leucopus*). We assessed the effects of population/species, acclimation condition, breeding generation, sex, and age. As previously demonstrated, native altitude and acclimation condition

are strong determinants of aerobic thermogenic capacity, with increases of 41% and 10.5% in thermogenic $\dot{V}O_{2,Summit}$ associated with acclimation to cold hypoxia and high-altitude ancestry, respectively. In contrast with our hypothesis, $\dot{V}O_{2,Summit}$ did not vary with sex or generation. These results suggest that adaptive variation in thermogenic $\dot{V}O_{2,Summit}$ in high-altitude deer mice persists in captivity and does not differ between sexes.

Diversity of 3D flight patterns of insects entrapped by artificial light

Yash Sondhi, Akito Kawahara, Huai-Ti Lin, Samuel Fabian, Pablo Allen

Artificial light can dramatically entrap night-flying insects by corrupting their dorsal light response and leading to unstable flight modes, like looping, stalling and inverting. However, insects are hugely diverse, and not all may react in the same manner. To examine the variety of responses to artificial light, we analyzed 3D flight patterns of different nocturnal insects flying to light. We find that a range of insect taxa (7 orders) including flies, wasps, moths, and beetles and bugs show similar light entrapment effects, with some differences in frequency of motifs across different orders, and showcase 3D flight paths for these different taxa. Unstable modes of flight predominantly occur within each order in the presence of upwelling or downwelling point sources, and extended tube sources, but dramatically reduce with diffuse downwelling light, reaching similar frequencies as complete darkness. We also compare flight path metrics like tortuosity, speed and as a function of distance from light to see if this approach can yield some estimates of how far from a light these unstable modes leading to light entrapment start to appear. We find similar motifs across the different orders with variation seemingly reflected by the flight styles and also found higher tortuosity under point light than diffuse sources.

Activity of the insect NaCCC2 sodium transport proteins

Hemmi Song, Grace Neuger, Ryan Yarcusko, Peter Piermarini, Christopher Gillen

The NaCCC2s are an insect-specific group of transport proteins with sequence similarity to the Na-K-2Cl cotransporters. Prior work indicates that NaCCC2 orthologs from *Aedes aegypti* (aeCCC2) and *Drosophila melanogaster* (Ncc83) are electrogenic Na transporters, in contrast to the electroneutral Na-K-2Cl cotransporters. We have further characterized aeCCC2 and Ncc83 by expressing them in *Xenopus* oocytes and evaluating Na⁺ and K⁺ transport with the tracer ions Li⁺ and Rb⁺. Li⁺ uptake was greater in unstimulated oocytes expressing aeCCC2 and Ncc83 than in oocytes injected with water or with the *Drosophila* Na-K-Cl cotransporter Ncc69. Moreover, following hypotonic swelling, Li⁺ uptake increased in oocytes expressing aeCCC2 and Ncc83 by approximately 100% and 150%, respectively. Li⁺ uptake saturated with increasing external Li⁺ concentration in aeCCC2 and Ncc83 oocytes, with an apparent affinity (K_m) in the range of 20 mM. In the presence of ouabain, resting [Na⁺] was 48% greater in aeCCC2 and 36% greater in Ncc83 oocytes compared to water-injected controls. These results are consistent with previous electrophysiological measurements and provide further insight into the novel functions of the NaCCC2s. Funding: R15-GM139088 and Kenyon College.

Glial cells in *Lumbriculus variegatus* nervous system regeneration

Meilin Song, Veronica Martinez-Acosta

Lumbriculus variegatus is known for its remarkable capacity for regeneration. Essential to this process is regeneration of the central nervous system (CNS). The annelid CNS consists of a bilobed cephalic ganglion and a ventrally placed nerve cord (VNC). The VNC contains three giant fibers, made up of gap-junctionally fused giant axons that run the length of the animal and contain lamellar sheathing thought to consist of glia. Observations of decreased lamellar compaction during regeneration suggest a potential role for glia in regeneration and recovery of function. Because little is known about the function of annelid glial cells, we first identified potential cell markers. Antibodies for glial fibrillary acidic protein (GFAP) and S-100 protein labeled specific cell populations around the ventral nerve cord and within the body wall musculature.

Using a pharmacological approach to disrupt glial reconstruction, we treated worm fragments with rapamycin (10 μ M to 100 μ M), a known inhibitor of the mTOR pathway. Rapamycin-treated fragments demonstrated reductions in segmental regeneration and recovery of rapid escape reflexes. One week post-amputation, fewer regenerated tail segments were observed in fragments treated at higher concentrations. Some fragments also demonstrated reductions in giant fiber conduction velocity, while electron micrographs revealed more abundant lamellar formations around the giant axons in treated fragments. Together, these data offer preliminary insight for the identity and function of glia during regeneration in *L. variegatus*.

Collagen entanglement in elephant skin gives way to strain-stiffening mechanisms

Sophia Sordill, Andrew Schulz, David Hu, Claire Higgins

Form-function relationships often have tradeoffs: if a material is tough, it is often inflexible, and vice versa. This is particularly relevant for the elephant trunk, where the skin should be protective yet elastic. To investigate how this is achieved, we used classical histochemical staining and second harmonic generation microscopy to describe the morphology and composition of elephant trunk skin. We report structure at the macro and micro scales, from the thickness of the dermis to the interaction of 10 μ m thick collagen fibers. We analyzed several sites along the length of the trunk, to compare and contrast the dorsal-ventral and proximal-distal skin morphologies and compositions. We find the dorsal skin of the elephant trunk can have keratin armor layers over 2mm thick, which is nearly 100 times the thickness of the equivalent layer in human skin. We also found that the structural support layer (the dermis) of elephant trunk contains a distribution of collagen-I (COL1) fibers in both perpendicular and parallel arrangement. The bimodal distribution of collagen is seen across all portions of the trunk, and is dissimilar from that of human skin where one orientation dominates within a body site. We hypothesize that this distribution of COL1 in the elephant trunk allows both flexibility and load-bearing capabilities. Additionally, when viewing individual fiber interaction of 10 μ m thick collagen, we find the fiber crossings per unit volume are five times more common than in human skin, suggesting that the fibers are entangled. We surmise that these intriguing structures permit both flexibility and strength in the elephant trunk. The complex nature of the elephant skin may inspire the design of materials that can combine strength and flexibility.

Cognitive abilities of both a native and an invasive species of Anolis lizard

Mahaut Sorlin, Simon Lailvaux

Invasive species constitute a major, and increasing, ecological problem, both in terms of their economic costs and through their negative impact on biodiversity.

The invasion process is complex, each step facing different challenges. Among them, cognitive abilities and more specifically cognition flexibility is thought to play an important role. Therefore, studying the evolution of cognition in the context of invasive species is necessary if we want to understand the general mechanisms facilitating species invasions. Although these findings are seemingly consistent across vertebrates, studies have mostly focused on avian and mammalian species, thus, making it difficult to infer evolutionary patterns across taxa.

Squamates in particular have received little attention regarding the role of cognition in facilitating success of invasions. Two species from the Anolis radiation, the brown (*Anolis sagrei*) and the green anole (*Anolis carolinensis*) are of particular interest, given that both have a similar invasion path, albeit separated by several million years.

We investigated the cognition flexibility potential along the recent invasive path of *A. sagrei* between populations of both anole species using an array of cognition flexibility tests. We tested the hypothesis that *A. sagrei* will overall exhibit higher cognitive flexibility than *A. carolinensis*. We also expected to see increasing cognitive flexibility in populations of *A. sagrei* as towards the invasion vanguard, but no differences among populations of *A. carolinensis*.

The WPU Honors Track as model for integrating research into undergraduate biology curricula

Joseph Spagna, Emily Monroe

In recent decades, faculty-mentored research has been recognized as a high-impact practice in undergraduate education, particularly in the natural sciences. Explicit models integrating curricular and co-curricular field and/or bench-based research work vary widely across institutions. Here I present the William Paterson University Honors College's co-curricular Biology Honors Track as one such model. This model, successfully implemented since 2015 in the context of a minority-serving, comprehensive Master's-degree granting university, integrates both classroom and laboratory elements. These include coursework in literature review and statistical methods, while the Track also sets research expectations and a general timeline to production of a thesis for participants and mentors. In the past eight years, Track students have produced 51 individual mentored thesis projects and nine co-authored peer-reviewed publications; graduates have secured positions in research M.S. and Ph.D. programs (38%), biotech and other technical fields (30%) and professional schools (medical, dental and veterinary, 13%) as well as K-12 education. Here I discuss both implementation of the curricular model and a variety of strategies for building buy-in from faculty and administrators, both of which have been critical to the remarkable success of this program.

Decentralized visual processing in a chiton with shell eyes

Daniel Speiser, Alexandra Kingston, Daniel Chappell

Animals with distributed visual systems have arrays of numerous photoreceptive organs dispersed across their bodies. Distributed visual systems have evolved separately in lineages distinguished by different body plans and yet united by relatively decentralized nervous systems. How do photoreceptive organs and nervous systems co-evolve in these lineages and how may spatial information be processed within decentralized neural circuits? We are exploring these questions in chitons (Mollusca; Polyplacophora), particularly in a species, *Acanthopleura granulata*, that has hundreds of image-forming eyes embedded in its shell plates. We have learned that optic nerves traveling from the shell eyes of *A. granulata* innervate a body-circling ring of neuropil and that their overlapping arborizations form a decentralized visuotopic map. Further, the structure of this neuropil ring may be co-evolving with the shell-embedded sensory organs: it is double-layered in *A. granulata*, but single-layered in chiton species that lack eyes. We also report, for the first time, recordings from the ring-shaped optic neuropil of *A. granulata*. We found spiking responses to the dimming of light and, from the temporal dynamics of these responses, we infer the visual system of *A. granulata* has a sampling rate of at least 18 Hz. We propose chitons with shell eyes acquire, integrate, and process visual information in fundamentally different ways from other animals, highlighting the importance of comparative approaches to the study of distributed visual systems.

Orchestrating a comprehensive, spike-resolved motor program for agile insect flight

Simon Sponberg, Joy Putney, Brett Aiello, Usama Sikandar, Leo Wood

Agile animals must parse complex sensory scenes to extract representations of actionable features relevant for behavior. Such task-relevant stimuli are often encoded in spike rate codes in descending neurons from the brain. But such representations must be translated into the animal's motor program – the set of temporally coordinated patterns of spikes across the many muscles that mediate movement. Hawkmoths provide a compact set of relevant muscles and enable us to record a comprehensive, spike-resolved motor program of nearly every action potential that controls the wings. These motor programs are highly coordinated in time, can be decoded into both discrete and continuous representations of behavior with nearly perfect accuracy, and utilize spike timing precision down to a millisecond. Unlike *Drosophila*, which has segregated phasic and tonic muscles, hawkmoths achieve flight control with fewer muscles, but with each utilizing changes in both spike rate and spike timing. All muscles are involved in every turn. Yet functionally interpreting the coordinated changes across the motor program remains challenging. Using high-density multielectrode arrays we can record the activity of many

neurons in the neck connective and capture population activity that encodes sensory perceptions. These representations sometimes capture task-relevant sensory cues such as specific flower features in rate codes, but such traditional approaches cannot yet account for the coordination of the whole motor program or the dynamics of behavior.

Integration of visual and olfactory cues by foraging bumble bees is state-dependent.

Jordanna Sprayberry, Katelyn Graver

Bumblebees are essential pollinators, utilizing sensory information to locate flowers while foraging, including visual and olfactory cues. Unfortunately, climate change and human activity have impacted the sensory signals they use to forage for floral resources. Thus, a comprehensive understanding of how floral sensory signals are used to find resources is a crucial component of conservation efforts. The majority of existing research exploring the relationship between visual and olfactory floral cues is performed at local spatial scales. This research is thus highly applicable to understanding floral selection, but an understanding of floral cue-use during search behavior is still poorly understood. This study uses associative learning and wind-tunnel paradigms to investigate how the bumblebee *Bombus impatiens* uses visual versus olfactory information from flowers across behavioral states and spatial scales. Findings indicate that multimodal cue use depends largely on the physiological state of an animal, further demonstrating the complexity of insect foraging behaviors and underscoring the importance of studying such effects using integrative paradigms.

Uncovering principles of long timescale behavior in sensory evoked navigation

Gautam Sridhar, Antonio Costa, Massimo Vergassola, Claire Wyart

Animals employ short timescale locomotor bouts to construct long timescale navigational strategies, reflecting interactions between environmental cues and internal states. Understanding the principles driving these strategies necessitates quantifying behavior across timescales, a challenge we address using larval zebrafish, known for their complex yet stereotypical stimulus responses. We establish a maximally predictive state space using only the tail pose of the fish and study the time evolution of behavior using transfer operators. A hierarchy of behavior along timescales emerges with three driving modes: the first involving steady/rapid changes in orientation called cruising/roaming strategies; the second encoding overall speed; and the third encoding directional preference. This approach then allows an understanding of how sensory stimuli impact navigation along these modes simultaneously. Looming expanding spots induce fast roaming behavior, while chasing dots induce lower speeds. During hunting, eye convergence events elicit slow cruising, whereas inter-hunt behavior is dominated by roaming strategies. We then compare the dynamics of individual fish in the same stimulus condition, revealing that behavior individuality sets a preference for certain strategies throughout the experiment. This highlights latent states possibly influencing behavior regardless of sensory conditions. Our approach provides insights into how the brain generates and modulates motor strategies, illuminating the multiscale dynamics that underlies the impact of sensory stimuli and individuality in animal behavior.

Finite element model validation in biomechanics using a 3D scanning Laser Doppler Vibrometer

Ananth Srinivas-Nurani, Arend von-der-Lieth, Jen Bright, Emily Rayfield, David Goldsby, Lauren Sallan

Paleontologists are often interested in understanding the mechanical performance of biological structures, and finite element (FE) modeling is now widely used for this. As FE models are highly sensitive to the information used in constructing them (e.g. material properties), and given the incomplete nature of the fossil record, it is essential to thoroughly validate FE outputs to understand whether modeling reflects the biological reality. We investigate the use of a novel non-contact technique, 3D scanning Laser Doppler Vibrometry (LDV), an optical instrument that evaluates vibration velocity and displacement

data from a set of points on a structure, to measure vibration and displacement patterns in bone and to compute full field dynamic strain values to validate biological finite element models. Strain data was collected from two alligator mandibles under varying loading conditions, and compared against finite element simulations. Material properties of bone and teeth were also measured using well established techniques and were included in the models. The experimental results highlight localized high strains around the tooth row and on the ventral surface and qualitatively match the numerical simulations. These patterns would not be resolved by traditional strain gauges and the dynamic nature of the technique overcomes the immobility required by DSPI. This work opens the door for more full field dynamic strain measurements and help to establish better confidence in our modeling of paleobiomechanics.

Prolonged Diapause Enhances Insect Persistence in Microclimate Refugia

Robert Srygley

Microclimates provide a refuge for species to persist in changing climates. Cowan hypothesized that Mormon crickets persist in centers of endemism located in montane or piedmont areas where microclimates provide refuges for hatchlings. Eggs from the same genetic stock buried across an elevational gradient (1200-2800 m) had a median hatching time of two years to eight years, depending on the elevation and microhabitat. Consequently, soil temperature is a critical factor in determining the persistence of Mormon cricket eggs in egg banks. The thermal performance curve for embryonic development peaked at 27°C, and critical thermal minimum and maximum equal to 20°C and 34°C. Due to aestivation in later stages of development, the optimum temperature declined to 24°C and the performance breadth narrowed to 10°C. These thermal constraints on development have major impacts on the embryos buried across the elevational gradient. At high elevation sites, the season for embryonic development is short and embryonic growth can only occur when the soil heats up during the day. Hence, slow embryonic development is a natural consequence of temperatures at high elevation. Consistent with Cowan's centers of endemism hypothesis, mountains and canyons provide a patchy thermal environment where multiple generations of eggs are likely to be banked in the soil and from which the right conditions can synchronize development and hatching.

A 300-million-year-old ray-finned fish evolved maxillary mobility independently of extant clades

Jack Stack, Michelle Stocker

Evolution of excessive species diversity in a clade when compared to its close relatives may arise from adaptations imparting ecological versatility. For instance, the ~30,000 species of neopterygian ray-finned fishes (Actinopterygii) represent 99% of living actinopterygian diversity. The extreme abundance of neopterygians is thought to be linked to an upper jaw (maxilla) with reduced bony fusions to the cheek elements, allowing for greater mobility and versatility of the feeding apparatus. However, *Platysomus schultzei*, an extinct ray-finned fish from a 300-million-year-old (Pennsylvanian) estuary deposit in New Mexico, had a maxilla separated from the cheek 50 million years prior to the first recognized neopterygian fossil. *P. schultzei* was originally assigned to the extinct "paleoniscoid" grade of ray-finned fishes without a phylogenetic analysis. We aim to test if *P. schultzei* is an unrecognized neopterygian, or if it independently evolved a similar upper jaw. We examined specimens of *P. schultzei* to integrate it into a phylogenetic matrix of 76 actinopterygian species scored for 222 discrete morphological characters. A parsimony analysis in TNT 1.5 and a Bayesian search in MrBayes 3.7.2a found *P. schultzei* within a clade of "paleoniscoids", separate from neopterygians. We also found that other "paleoniscoids" possess similar upper jaw morphologies but resolve as either sister to neopterygians (parsimony) or sister to acipenseriformes (i.e., sturgeons and relatives; Bayesian). Our analyses indicate that maxillary mobility evolved independently of neopterygians in at least one, potentially more extinct sub-clades of actinopterygians.

Agent-Based Modeling to Assess Optimal Conditions to Minimize Airborne Transmission

Lyndsy Stacy, Ashley Teufel, Davida Smyth

SARS-CoV-2 is a virus that is spread through aerosol transmission, meaning it can stay suspended in the air for longer durations when in an aerosol. To study how the virus disperses through aerosol we used the bacteriophage Phi6 as a surrogate for SARS-CoV-2. We released an aerosolized bacteriophage into the air using a nebulizer and placed plates containing a host organism at different distances and durations of exposure that the phage would kill when encountering. This allowed us to calculate rates of transmission, which were then used to create an agent-based model. This model is used to further analyze how the virus spreads and make inferences about the distance particles flow under different conditions. It can do this by either testing specific intervention strategies or by examining the parameters that impact transmission. The goal of this model is to provide insights into optimizing responses to pathogens transmitted through the air, with regard to room layout, ventilation, and humidity.

Determining the causes of mass mortality events in Purple Martins

Maria Stager

Mass mortality events are phenomena that can rapidly and indiscriminately remove a substantial proportion of a population. In spite of their potential magnitude, such events can be especially challenging to study due to their unpredictable nature. We made use of a unique series of >300 Purple Martin specimens to inform our understanding of the causes of avian mass mortality events. Aerial insectivores, including martins, are particularly susceptible to mass mortality events. This large group of individuals died in Texas and Louisiana during Winter Storm Uri in February 2021. With these carcasses, we asked: (1) How anomalous the particular environmental conditions were that led to this mass mortality event? (2) Were certain classes of individuals disproportionately affected by this event? And (3) what physiological mechanisms contributed to their death? To do address these questions, we paired morphological measurements and assays of body composition for martin carcasses with interpolated weather data. Ultimately, the answers to these questions will help us address whether physiological tipping points exist past which we can expect to observe avian MMEs in the future.

The genetic architecture underlying avian flexibility in a complex physiological trait

Maria Stager

When environmental fluctuations occur, the ability to reversibly modify trait values can allow individuals to optimally match their phenotype to the environment. Such phenotypic flexibility is ubiquitous across the tree of life and determining the causes of variation in flexibility may be critical to our understanding of individuals' capacity to cope with accelerating global change. However, the genetic basis of variation in flexibility has rarely been characterized — a pursuit which necessarily requires linking genotype to phenotype. Birds exhibit substantial flexibility in physiological traits and can therefore serve to help characterize these connections. For instance, Junco populations vary in their physiological flexibility and this variation correlates with the temperature variability of their native environment. Utilizing tissue samples collected during laboratory acclimation experiments, I performed whole-genome resequencing and muscle transcriptome sequencing to quantify genomic and transcriptomic patterns of variation associated with differences in physiological flexibility for 95 Junco individuals. I then identified allelic variants related to physiological flexibility using association methods and validated these sites with gene expression profiles and additional phenotyping. Taken together, this work helps to provide a mechanistic understanding of the ability of natural populations to respond to environmental change.

Ectoparasite infestation alters alpha and beta diversity in the skin microbiome of an altricial bird

Keegan Stansberry, Kaitlin Couvillion, Tosha Kelly, Allison Cannon, Melanie Kimball, Christine Lattin

Development of a healthy skin microbiome is critical for nestling birds; however, not much is known about how skin microbial species abundance and community composition change with age and in the presence of ectoparasites. We characterized the alpha and beta diversity of the skin microbiome in European starling (*Sturnus vulgaris*) chicks (n=120) by swabbing skin 3 and 9 days post-hatch. We predicted that alpha diversity of the skin microbiome would increase with age, but that chicks in nests naturally infested with Northern fowl mites (*Ornithonyssus sylviarum*), a hematophagous ectoparasite, would have reduced alpha diversity because of mite-induced disruptions to microbiome development. We also predicted that microbial community composition (beta diversity) would differ between chicks from mite infested and non-infested nests. We found that nestlings without ectoparasites maintained stable alpha diversity in the skin microbiome from day 3 to 9 post-hatch; however, nestlings with mites generally had reduced alpha diversity over time. We also saw significant differences in beta diversity with age and infestation status, as well as an interaction between the two, suggesting mites alter skin microbiome community composition in songbird nestlings. If these microbiome effects persist into adulthood, they could affect future skin health and the skin's function as an essential immune barrier.

Examination of Age Class Niche Variation of Sevengill Shark Through Stable Isotope Analysis

Kimberly Stauffer, Meghan Holst, John Durand

We seek to determine the ecological roles of different age classes of sevengill sharks in the San Francisco Bay ecosystem using stomach contents and stable isotope analysis. We will collect tissue samples during Spring and Summer seasons from several prey species of sevengill in addition to previously collected stomach contents of sevengill. Tissue samples will then be sent to the Stable Isotope Facility at the University of California, Davis where the Carbon ($\delta C13$) and Nitrogen ($\delta N15$) isotopes will be identified. We will graph $\delta C13$ against $\delta N15$ outputs from tissue samples, use the SIBER ellipses package in R to visualize and analyze possible niche partitioning among sevengill shark age classes by diet. This will be supplemented by data collected from stomach content. This will allow us to gain a better understanding of ontogenetic shifts in niche occupancy of sevengill sharks. These results will facilitate recommendations for improved fishing regulations and strategies to protect the conservation of the San Francisco Bay ecosystem, and the services they provide.

When does phenotypic integration promote convergent evolution?

C. Tristan Stayton

From a macroevolutionary perspective phenotypic integration is commonly imagined as “channeling” phenotypic change along a limited number of evolutionary trajectories. As a consequence higher levels of shared phenotypic integration are typically conceived as being more likely to promote convergent evolution within a group. However, this prediction has not yet been tested using contemporary phylogenetic comparative methods, and in particular never with measures which quantify integration and convergent evolution. Here I investigate the correlation between integration and convergence using multiple data sets, including turtle shell shape, fish body shape, and squirrel mandible shape. Integration is quantified using a range of techniques; convergence is quantified using the convervol suite of measures. Surprisingly, clades which show higher levels of integration are not disproportionately likely to show high levels of convergent evolution. Higher levels of integration seem to be equally likely to promote evolutionary stasis or parallel evolutionary trajectories which, while interesting, do not lead to large decreases in phenotypic dissimilarity among lineages. However, these findings are likely highly dependent on the definitions of both “integration” and “convergence” used, and in particular on the operationalizations of those definitions in quantitative measures. Researchers should be explicit and consistent regarding their

framing of similar studies, and should be aware that, at a minimum, high levels of phenotypic integration need not lead to elevated levels of convergent evolution within clades.

The flapping kinematics of forward flight aerodynamics in a large beetle (*Betocera rufomaculata*)

ori stearns, Roi Gurka, Gal Ribak

Due to their small size and high flapping frequency insects tend to fly at low-advance ratios (flight speed/wing flapping speed) where the unsteady flows associated with wing flapping dominates the aerodynamics. The aerodynamics of hovering insects has been the subject of intense research, however, less is known about the aerodynamics of fast forward flight, where the contribution of flight speed to flight aerodynamics cannot be ignored. We studied the flapping kinematics of large beetle (*Betocera rufomaculata*) during free, forward-flight. We captured the flight of the beetles with three high-speed cameras and extracted the 3D flapping kinematics from the movies. The beetles flew at advance ratios ranging between 0.05 and 0.35. Increased advance-ratio was associated with lower flapping amplitudes and lower body pitch (flight) angles. The wing incidence angle varied over the flapping cycle and showed a consistent asymmetry between the upstroke and downstroke. While the wing rotated continuously during the downstroke it paused and reversed direction during the mid-upstroke. This translated to differences in angles-of-attack between the upstroke and downstroke within each beetle. The angle-of-attack was lower during the downstroke and higher during the upstroke, reaching a peak at mid-upstroke. Consequently, the higher drag operating on the wings at that phase propelled the beetle forwards. Such changes to angle-of-attack kinematics explain how beetles modulate flapping to match the different flow conditions required during forward flight.

Developing *Parhyale hawaiiensis* as a comparative model of olfactory navigation.

Theresa Steele, Katherine Nagel

In arthropods, olfactory navigation behaviors and the brain structures that generate them are similar across aquatic and terrestrial species that inhabit dramatically different olfactory environments. Understanding how olfactory behaviors and brain regions involved in navigation have evolved across these environments may reveal fundamental principles of nervous system evolution. However, prior studies of olfactory navigation strategies in aquatic arthropod species have largely been limited to high level descriptions of locomotion in genetically inaccessible model systems. To address this gap, I am developing a new crustacean model of olfactory navigation in water using the genetically tractable amphipod, *Parhyale hawaiiensis*. I constructed a behavioral imaging apparatus capable of tracking locomotion and posture in freely moving *Parhyale* while foraging for food. *Parhyale* exhibit increased turning and reduced movement speed in the presence of odorous food, consistent with olfactory search behaviors exhibited in terrestrial species. Foraging behaviors are disrupted after selective removal of *Parhyale* antennules (the homologue of the insect antennae), suggesting that these structures are important for food localization, while removal of the antennae (which are thought to mediate contact chemoreception) disrupts locomotion more generally. The development of this foraging assay paves the way for further investigation of how behavioral responses to odor, and the crustacean chemosensory system have evolved to facilitate olfactory navigation underwater.

Four Eyes, One Vision: Shedding Light on the Function of Decapod Accessory Eyes

Nicholas Steichmann, Daniel Speiser

Eyes are physiologically costly organs to produce and maintain, making the development of secondary eyes intriguing. Caridean Shrimp possess reflecting superposition primary eyes, with some species across fourteen families adding a pair of eyes called accessory eyes along the dorso-posterior margin of the primary eyes. We propose these four-eyed shrimp improve their visual system's efficiency by using accessory eyes to communicate directly with endocrine organs in their eyestalks. The X-organ sinus gland complex

in the shrimp eyestalk releases hormones which serve a variety of functions, including regulating pigment dispersal within chromatophores, a light influenced behavior. The function of accessory eyes is not known, however we have shown using electroretinography (ERG) that they have a broad spectral response range between 380-700 nm in *Saron marmoratus*. We are using histology and optic nerve tracing to ask how the accessory and primary eyes connect to the X-organ sinus gland complex and the optic neuropils of the brain in *S. marmoratus*. We predict that the nerve tracts carrying information from the accessory eyes lead directly to the sinus gland and from there to a direct hormonal response, whereas information proceeding from the primary eye is integrated separately in the brain. We propose that this is used by the shrimp to quickly alter their chromatophores to their environment without interfering with the tasks of the primary eyes.

The Effect of Substrate Roughness on Self-Cleaning of Ant (*Camponotus pennsylvanicus*) Adhesive Pads.

Evan Steinberg, Alyssa Stark, Stephen Yanoviak

Ants utilize adhesive tarsal pads coated in a glue-like substance to climb a variety of substrates when active. While foraging, the "Carpenter Ant" *Camponotus pennsylvanicus* traverses surfaces which may impact their adhesive performance. Specifically, in ants and other adhesive insects, surfaces covered in fine particulate including dirt and other debris adhere to the sticky pad and reduce adhesion due to a loss in contacting surface area. Despite this adverse interaction, ants maintain adhesion after fouling by removing stuck particles. The mechanism for "self-cleaning" is not fully understood and may depend on the physical interaction between substrate roughness and the adhered particles. To test the effect of substrate roughness on self-cleaning, we measured the adhesive recovery of *Camponotus pennsylvanicus* after walking on variably rough substrates. Adhesive recovery was measured as a percent of adhesive performance regained when compared to clean (uncontaminated) same-substrate adhesion. We fouled the adhesive pads using hydrophobic and hydrophilic particles of 10um diameter tested independently on all substrates. To vary substrate roughness, we used glass and three sandpaper types (P2500/2000/1500) which have particle sizes below, equal to, and above the 10um fouling particle size. We hypothesized that P2500 sandpaper will demonstrate greater self-cleaning due to matching particulate and substrate roughness sizes. This study will clarify if the self-cleaning mechanism is hindered under ecologically relevant surfaces that differ from the lab (i.e., glass substrates).

Dew or Die: Unraveling the Wet vs. Dry Adhesive Performance of Diving Bell Spider Attachment Discs

Bernd Steklis, Todd Blackledge

The adhesive performance of diving bell spider (*Argyroneta aquatica*) attachment discs (ADs) in wet and dry conditions was investigated. Diving bell spiders are known for their obligate aquatic niche, but their AD performance in wet environments remains unclear. Experiments were conducted to evaluate the adhesive strength of diving bell spider ADs under controlled wet and dry conditions. Results showed no significant difference in adhesive strength in wet conditions compared to dry conditions. This suggests that diving bell spider ADs overcome the challenge of wet adhesion. Further research is needed to understand the compositional and structural features that contribute to the adhesive performance in wet conditions. Such knowledge has implications for bioinspired adhesive technologies and the development of adhesives suitable for various environmental conditions.

Hydrodynamics of pelagic shark pectoral fins

Phillip Sternes, Sam Van-Wassenbergh, Tim Higham

The emergence and evolution of paired fins remains of high interest to organismal biologists, as their appearance triggered the rapid evolution and diversification of fishes in the Devonian. Despite their critical importance for fish evolution, the precise form-function relationships of the pectoral fins during various aspects of locomotion (e.g., steering, braking, stabilizing, lift generation) remain unresolved. Previous research on benthic shark species suggested that no lift was produced during steady swimming, based on the

vorticity patterns in the wake of the fin. However, no study has empirically examined the hydrodynamics of pelagic shark pectoral fins, which differ drastically in external morphology compared to benthic shark species. We used computational fluid dynamics to examine lift production from the pectoral fins of a pelagic shark, the shortfin mako (*Isurus oxyrinchus*). Unlike benthic shark species, we found that the pelagic mako shark produces lift when the pectoral fins are placed at an angle of attack (9°) corresponding to steady swimming. Additionally, we found the largest amount of lift was produced near known angles of attack for shark vertical movements (-13°, 20°). Overall, the pelagic mako shark has high efficiency (lift-to-drag ratio of > 6) over a wide range of angles of attack (7° to 17°), and maximum lift coefficient (1.4) was similar to, or greater than, other vertebrate aquatic appendages.

Element concentrations in *Silene* leaves under water stress

Janet Steven, Jenna Miladin, Katherine Ransone

The concentration of both essential and non-essential elements in plant leaves varies considerably among species. In crop plants, environmental stress can alter ion homeostasis in leaves, but the responsiveness of elemental concentrations in wild plants to environmental conditions is not well known. In this study, we investigate whether water stress alters elemental concentrations in *Silene latifolia* leaves, and how concentrations vary across North American *Silene* species. We grew *Silene latifolia* plants in the growth chamber under adequate water and water-limited conditions. We measured leaf elemental composition with a hand-held X-ray fluorometer calibrated for leaf tissue, which gives concentrations of elements heavier than sodium. We also measured herbarium specimens of North American *Silene*. We found that the *Silene latifolia* grown under water-limited conditions showed an increase the leaf concentration of several essential elements. The concentration of potassium, which is important for water balance in leaves, was doubled in the water-limited plants. We also found high potassium levels in our survey of the North American *Silene*, as well as greater concentrations of potassium in plants from warmer habitats. In addition, most species accumulated two non-essential elements, silicon and aluminum. Overall, it appears that *Silene latifolia* plants are capable of altering elemental concentrations under stress, and across *Silene* species high potassium levels may support survival in arid conditions. Flexibility in ion homeostasis could be contributing to the colonization of new habitats and adaptation to dry conditions in *Silene*. Our findings also have implications for adaptation to drought under climate change, and highlight the importance of soil composition in this process.

Tadpole and fairy shrimp responses to narrow spectrum ultraviolet light and broad spectrum light

Brian Stevens, Nicolas Lessios

Tadpole shrimp and fairy shrimp are branchiopod crustaceans that are thought to each have four spectral classes of photoreceptors in their compound eyes. A species of tadpole shrimp (*Triops longicaudatus*) and a species of fairy shrimp (*Streptocephalus mackini*) each have photoreceptors that are sensitive to ultraviolet, blue, green and red wavelengths of light. In previous studies, we have shown that tadpole shrimp tend to respond behaviorally by moving away from narrow wavelength light stimuli in the visible spectrum. Here, we show that they move towards light of narrow wavelength ultraviolet light of 365nm, an interesting finding that may underlie different neural circuits than those used to move away from light (photonegativity). It may be that the circuits used to move towards UV light of these wavelengths are similar to those of flying insects, which also tend to move towards ultraviolet narrow wavelength light. When exposed to broad spectrum light including ultraviolet light, we found tadpole shrimp move away from light with increasing intensity. In contrast to tadpole shrimp, both male and female fairy shrimp tended to be photonegative in response to ultraviolet light of 365nm. Male fairy shrimp were photopositive to light of 394nm. Male fairy shrimp could be using a UV- sensitive photoreceptor or blue-sensitive photoreceptor for this behavior. Female fairy shrimp tended to be photonegative to all wavelengths and intensities tested.

Mixed evidence of evolved plasticity in threespine stickleback following northern pike invasion

Dale Stevens, Anna Gilmartin, Isabella Reichel, Sydney Macedo, Matthew Wund, Kaitlyn Mathis

In the past seventy years, the northern pike (*Esox lucius*) has spread throughout southcentral Alaska, USA. This invasion has resulted in the extinction of numerous populations of competing piscivorous predators. Furthermore, threespine stickleback (*Gasterosteus aculeatus*) populations in the region have also gone extinct. Past work in this system has demonstrated mixed evolutionary responses of stickleback populations to northern pike. Chemical-cue-mediated anti-predator behavior and reproductive life history appears to have evolved in some populations following pike invasion, however other behavioral phenotypes show no sign of evolution. Evidence of evolution is found, it has been consistent with the risk allocation hypothesis, and stickleback populations appear to lose plasticity in their anti-predator behavior. In this experiment, we investigated whether morphological plasticity has evolved following pike invasion to determine further use this system to test the risk allocation hypothesis when studying prey evolutionary responses to invasive predators, and to determine if other plastic responses of threespine stickleback have evolved. To do this we used an artificial predator to regularly attack stickleback throughout their first year of development. We did this in six total stickleback populations, three with and three without northern pike. While the future data set will use geometric morphometrics to comprehensively quantify stickleback morphology, this talk will focus on comparing three phenotypes: body depth, eye diameter, and length of the second dorsal spine.

How vision guides proboscis movements for flower inspection in hawkmoths.

Anna Stöckl

Visually-guided reaching – to your coffee cup or a door handle – is a central feature of primate behaviour. In insects, however, limb movements often follow a stereotyped motor programme. Only a few instances of visual appendage guidance are known. These are the forelimb reaching movements of horse-headed grasshoppers and fruit flies during gap crossing and the antenna pointing of crickets and cockroaches. Here we describe the visual guidance of an unpaired insect appendage: the proboscis of the hummingbird hawk-moth (*Macroglossum stellatarum*). While hovering in front of flowers, these hawkmoths use visual features to guide their proboscis over their surface to the nectary.

Reconstructing proboscis and body movements during flower probing, we identify the contributions of flight control and active proboscis guidance to proboscis positioning. We demonstrate that proboscis guidance requires continuous visual feedback, which the animals actively seek out when a part of their visual field is occluded. We further characterise a prominent feature of visual proboscis control: lateralisation on the individual – but not population – level: the majority of individuals kept their proboscis preferably in the visual field of one eye. This ocular dominance was highly correlated with the probing position on bilaterally symmetric visual pattern. Thus, lateralisation could serve to improve the efficiency of this visuo-motor task, by rapidly selecting one of two options in a symmetric visual presentation.

Resource-based trade offs and phenotypic plasticity in butterfly melanism

Andrew Stoehr, Katelyn Gläenzer, Devin VanWanzele, Samantha Rumschlag

Adaptive phenotypic plasticity is one mechanism through which organisms cope with variable but predictable environments. However, phenotypic integration of multiple traits may sometimes result in maladaptive plastic responses to the environment. In the cabbage white butterfly (*Pieris rapae*) several wing pattern traits are heavily melanized in colder seasons which aids in thermoregulation through solar absorption. In contrast, some wing pattern traits are less melanized during colder seasons, resulting in negative correlations between these trait groups. Theory predicts that traits involved in resource-based trade-offs will be negatively correlated when variation in resource allocation is greater than variation in resource acquisition. However,

when variation among individuals in acquisition is greater than variation in allocation positive correlations are predicted. Through field studies and laboratory studies that vary resource allocation through temperature manipulation or vary resource (tyrosine, a melanin precursor) acquisition through dietary manipulation we find support for a trade-off hypothesis that could explain why these different melanin-based traits vary seasonally as they do. We also show that increased dietary tyrosine results in increased spot melanism under some conditions, supporting the more general idea that melanism may involve resource-based costs.

Preliminary Use of the Heterothermic Index in Large, Active Fishes

Ashley Stoehr

Temperature is a key determinant of fish movement patterns, as changes in temperature impact physiological rate processes and whole-body performance. Amongst large, active, pelagic, fishes, only the tunas, lamnid sharks, common thresher shark (*Alopias vulpinus*), and swordfish (*Xiphias gladius*) elevate the temperature of the red, swimming muscle above water temperature (i.e., RM endothermy). The capacity for RM endothermy, however, can vary between species, individuals within a species, muscle types within an individual, or with the environment. For these reasons, the historical, binary classification of “fishes with or without RM endothermy” oversimplifies thermoregulatory strategies and may obscure comparative studies. Here, the heterothermic index (HI), which was previously used to describe body temperature variability in small mammals and birds, was used to assess muscle temperature variability in fishes with and without RM endothermy. The HI was calculated using digitized temperature telemetry data obtained from captive and free-swimming fishes with and without RM endothermy. While limited results to date do not suggest an obvious relationship between the HI magnitude and “with or without RM endothermy” classifications, results do suggest that the HI could be useful in describing the impact of blood flow on muscle temperature and identifying outliers that could relate to tagging placement or physiological and behavioral adaptations related to thermoregulation.

Development of the central nervous system(s) of the solitary tunicate *Ciona*

Alberto Stolfi

Tunicates comprise the sister group to the vertebrates, and they have proven to be crucial model organisms for our understanding of chordate evolution. The majority of tunicates live a biphasic life cycle that alternates between swimming, benthic larvae and sessile, filter-feeding adults. During this metamorphosis, the larval central nervous system (CNS) is eliminated and replaced by an entirely new CNS in the adult. And yet, the neural progenitors that give rise to both larval and adult neurons are specified and pre-patterned in a highly stereotyped manner, resulting in interleaving cell lineages in the neural tube of the developing embryo. Here we show our latest findings on the development of this biphasic CNS in the model solitary tunicate, *Ciona*. More specifically, we will present a detailed investigation into a gene regulatory network that directs the unique transcriptional profile of a larval hindbrain neuron type, the ddNs, which have been proposed to be homologous to Mauthner Cells of fish and amphibians. We also show that a neighboring hindbrain cell population gives rise to neurons innervating the branchial basket of the adult, and that specific genetic perturbations can shift the balance between their survival and death during metamorphosis.

Rusty Crayfish use multiple sensory cues to assess predatory threats

Gaige Stopjik, Arthur Martin

The presence of predators can drastically influence the behaviors of prey organisms, such as altering how they use shelters, changing their foraging strategies, altering morphological characteristics, or influencing birth rates. Crayfish have been found to alter their behaviors as the size of a predator changes in relation to the focal animal's size. Recently, studies have shown that crayfish alter foraging and sheltering behavior based on the size of the

predator they smell. These recent studies have not investigated predation rates, or the influence of the predator being physically present. This study exposes rusty crayfish (*Faxonius rusticus*) to a control, the odor of a largemouth bass (*Micropterus salmoides*), or a physically present largemouth bass. In experimental trials, crayfish and bass were paired based on their gape ratio. Shelter usage and preference of crayfish was examined to show how they assessed risk in each of the situations. The goal of this study is to give a clear understanding of the ability of crayfish to assess the threat of the predator based on its size when exposed to its odor and/or physical presence.

Neuromuscular basis of woodpecker drilling behavior

Charles Stowers, Nicholas Antonson, Franz Goller, Matthew Fuxjager

Many species perform dynamic, high-speed movements to perform life-sustaining tasks, but the neuromuscular mechanisms of such behavior are poorly understood. In woodpeckers, for example, drilling is used to forage for food, with birds repeatedly striking their head against a substrate at ≈ 8 times sec⁻¹. This requires holistic movement that engages multiple muscles in tandem and across the body. However, temporal control of muscle recruitment patterns necessary for drilling are not known. We address this issue by collecting electromyographic (EMG) recordings from 6 muscles hypothesized to be active in drilling from downy woodpeckers (*Dryobates pubescens*). These muscles included the primary muscle that protracts (longus colli ventralis) and retracts the neck (longus colli dorsalis), as well as the occipitalis and rectus capitis lateralis (actuates ancillary head and neck movement), and finally the abdominal rectus sheath and main protracting tail muscle (lateralis caude). We recorded recruitment patterns from these muscles, while woodpeckers naturally engaged in drilling, tapping, hopping, head turning, and tail flexing to discern the neuromuscular bases of these behaviors. This study thus provides a fine-scale perspective on the neuromuscular coordination necessary to perform high-speed foraging behavior in woodpeckers.

Molecular characterization of the gastrotrich nervous system of *Lepidodermella squamata*

Katharina Stracke, Andreas Hejnal

Gastrotrichs are widely abundant aquatic animals whose precise phylogenetic position is still unresolved but suggested to be within the Spiralia. One such species is *Lepidodermella squamata* a freshwater gastrotrich containing less than 500 nuclei. Existing knowledge is heavily focussed on their reproduction and morphology, revealing a nervous system consisting of a commissural brain-like nerve condensation and two lateral longitudinal axon tracks. Because of their microscopic size of less than 200 microns, some traditional molecular techniques, such as in-situ hybridization, are prohibitive and few molecular investigations have been performed in this species. Therefore, we aimed to characterize gene expression patterns in adult *L. squamata* to define their nervous system structure on a molecular level. We did this via immunohistochemistry (5'-HT and FMRFamide staining) and hybridization chain reaction for sub-cellular gene expression in the nervous system. We found that gene expression is restricted to individual cells and located in the head region (foxA, foxB, prospero), around the lateral somata (engrailed, fez, lim1, pax6, syt1), dorsal commissure (lim1, syt1), lateroventral nerve cords (hox2, pax6, post2), pharyngeal plug (intermediate filament), adhesive tubules (fer3), and reproductive regions (elav, lamin, sFRP). Despite their size and simple reproduction and life cycle, *L. squamata* adults express genes commonly found in more complex brains.

Novel co-flowering in subalpine plant communities at Mt. Rainier National Park

Madeleine Strait, Janneke Hille-Ris-Lambers, Aji John, Manogya Chandar, Berry Brosi

Montane plant communities have short flowering seasons that are dependent on the melting of snowpack each spring, making them particularly vulnerable to the impacts of climate change. Previous research has highlighted that warmer temperatures and shifts in snowpack can have large effects on the timing of important life history events for plants in these systems, such as flowering and fruiting. Changes in phenology vary across species, due to

differences in sensitivity to environmental factors (e.g., temperature, soil moisture, snowmelt). Consequently, this range of responses can result in novel patterns of co-flowering within a community, arising from the loss or gain of overlap in phenological timing.

We analyzed data from the MeadoWatch program, a decade-long citizen science project monitoring the phenology of subalpine plants within Mt. Rainier National Park. We investigated whether shifts in environmental conditions have led to novel co-flowering events. Specifically, for species combinations that experienced novel co-flowering under environmental conditions that significantly differed from the average, we examined the individual flowering shifts for each species individually. We also assessed whether local (measured by microclimate data-loggers at each plot) or regional (at the scale of the entire National Park) environmental data were better predictors of these shifts. Understanding how abiotic factors shift patterns of co-flowering among plant species has important implications for plant-pollinator interactions, seed production, and long-term coexistence of species within this system.

Building an 'epigenetic clock': Utilizing DNA methylation patterns to predict age in fish

Emma Strand, Shelly Wanamaker, Richard McBride, Andrea Bodnar, Tim O'Donnell

Sustainable fisheries management relies on accurate age data to provide well-informed harvest recommendations by estimating population rates and stock sizes within age-based population models. Fish ages are estimated by removing and sectioning otoliths or other hard structures, but alternatives are needed because this method isn't applicable to all fish, labor and cost-intensive, potentially inaccurate, and lethal to the fish. Measuring DNA methylation as an epigenetic signature of age represents an alternate aging method to lethally sampling calcifying structures. In this study, we collected Haddock, *Melanogrammus aeglefinus* fin clips (n=140) from the NOAA Bottom Trawl Survey during Fall 2022 and Spring 2023 for use in whole-genome bisulfite sequencing (WGBS). From the WGBS data, we measured changes in DNA methylation, an epigenetic mark that results in a methyl group added to a cytosine, across CpG sites in all individuals. We used an elastic net regression model with otolith age as a fixed factor and sex as a random factor to identify CpG sites that were significantly correlated with age and return the minimum number of sites required to estimate age. Predictive capabilities of the model were tested by separating samples into training and test sets, resulting in an 'epigenetic clock' to provide accurate and non-lethal age estimates to improve age-based population models and promote sustainable fisheries management.

Inference of fossoriality and digging mode in fossil rodents using extant digging rodents as a guide

Sarah Saxton Strassberg, Kenneth Angielczyk

Fossoriality has evolved across various rodent clades (e.g., mole-rats, gophers, ground squirrels), and disparate digging modes (scratch, chisel-tooth, head-lift) are observed in extant rodents. Broad quantitative investigations of traits associated with fossorial specialization and digging mode are lacking, particularly in taxa that use multiple digging modes. Numerous extinct rodents show evidence of fossoriality, whether by being preserved in burrows or by exhibiting skeletal traits comparable to modern fossors. However, degree of fossorial specialization and digging mode in fossil rodents remain poorly understood. Developing indices of digging mode using extant species, then applying them to fossil taxa, may provide valuable insight into rodent fossoriality in deep time. We collected digging mode data (e.g., from behavioral/kinematic studies) and morphological data, including long bone lengths and widths; olecranon process, deltopectoral crest, metacarpal, and terminal phalanx dimensions; incisor procumbency angle; occiput height and angle; and nasofrontal suture complexity. Analysis of 389 specimens representing 151 species (79 extant, 72 fossil) suggests that nasofrontal suture complexity, occiput angle, and procumbency angle are effective metrics for inferring digging mode. Notable results of discriminant function analysis of extant and fossil taxa include that different *Palaeocastor* species used different digging

modes, that rhizomyids and a castorid converged on similar usage of both head-lift and chisel-tooth digging, and that *Epigaulus*, the "horned gopher," may have engaged in chisel-tooth digging.

Immune response to temperature and light-induced bleaching in the model anemone *Exaiptasia diaphana*

Micah Jay Strike, Erin Borbee, Sofia Diaz-de-Villegas, Lauren Fuess

Coral bleaching, or the breakdown of the coral-algal symbiosis, is one of the biggest threats facing coral reefs today. Bleaching events can be caused by one or more stressors including increased temperature and light exposure. As marine heat waves increase in severity and frequency, so too are bleaching events and coral disease outbreaks, which often occur in tandem. As such, it is crucial to understand how environmental stressors affect coral fitness and immunity. While the temporal association between bleaching and coral disease has been observed in nature, we currently lack studies that experimentally demonstrate these relationships. Here we leverage the model anemone *Exaiptasia diaphana*, a close relative of corals, to study the relationships between environmental stress, bleaching, and disease. Anemones from two genetically distinct clonal lines were exposed to a heat ramp coupled with increased light exposure to induce bleaching. Symbiont density and constitutive immunity were tracked throughout the experiment to determine how dynamic changes in symbiont density affect immune activity. We will conduct catalase, prophenoloxidase, melanin, and antimicrobial activity assays to quantify these relationships. The results from this experiment will provide insight on how anemones and corals respond to multiple stressors and will help inform conservation and restoration efforts on coral reefs following bleaching events and disease outbreaks.

Bony morphology dictates TMJ stress field positioning across common model species

Alyssa Stringer, Emily McParland, Peishu Li, Nicholas Gidmark

The mammalian jaw joint, the temporomandibular joint (TMJ), is a complex of hard and soft tissues that function for a nuanced range of motion. TMJ pathologies (temporomandibular disorders, TMD) have been linked with overloading of the soft tissue. To understand how TMJ loading differs between healthy and pathological populations, previous studies tracked movement of the TMJ stress field, the contact area between bone and articular soft tissues. Here we used XROMM to approximate the TMJ stress field of six model species used in TMD research (pigs, sheep, macaques, rabbits, rats, mice). We calculated the 3D position of the closest point from temporal fossa to mandibular condyle centroid for intact cadaveric heads in centric occlusion. Our single-point estimation of stress field in mice and rats lies on the anterior dorsolateral surface of the fossa, and its location is more variable than that of other species (presumably due to inherent noise of the XROMM workflow). Comparatively, pig and sheep stress fields are directly dorsal to the condyle centroid, while in humans and macaques, it tends posteriorly. Variation in stress field location across clinical model species suggests bony anatomy can significantly impact the loading regime of the articular soft tissues. Quantifying such variation could better our knowledge of TMD progression in these species and guide model selection in studies focusing on joint soft tissue stress and wear.

Developmental phenotypic plasticity varies between two distantly related anuran species

Sarah McKay Strobel, Eva Fischer, Molly Womack

Developmental environment influences later-life phenotype across taxa. However, environments can vary widely even within the same species. Anurans (frogs and toads) show extensive and predictable phenotypic plasticity in size at and timing of metamorphosis in response to environmental cues experienced during development, such as food availability, temperature, water volume, and tadpole density. This plasticity varies by severity for some cues, but studies investigating tadpole density often use small-severity treatments within species and/or highly disparate severities across species, which confounds our ability to assess how developmental environment affects phenotype in a comparative context. To address this data gap, we

reared two distantly related species, wood frogs (*Rana sylvatica*) and cane toads (*Rhinella marina*) at two larval densities and measured larval duration and mass at metamorphosis, as well as open-field behavior. In response to higher density for larval duration and for metamorphosis mass, wood frogs showed minimal to no differences while cane toads showed significant differences. The observed species-by-rearing-density interaction may be mediated by differences in activity level. Integrating our results into the existing literature suggests that families differ in their responses to the severity of density treatments; however, methodological differences in environmental severity limit interpretation. This research contributes to our understanding of how developmental environments influence species' capacity to respond to stressors and ecosystem changes.

Maternal Defense Strategies: Investigating Female Aggression in Dyeing Poison Frogs

Isabella Strohmeier, Sarah Westrick, Eva Fischer

Despite common bias toward thinking of mothers as caregivers, they often display increased aggressive behavior as an important part of parental care. Yet the mechanisms underlying aggression are much less studied in females than males. This is partially due to a historic bias of considering testosterone, a common driver of aggression, to be a 'male' hormone. In dyeing poison frogs (*Dendrobates tinctorius*), the male is typically the sole caregiver to the eggs and young tadpoles, while the female is the more aggressive sex and defends her territory from competing females. To understand the role of social and breeding context on female aggression, we conducted female intrusion experiments in home tanks of breeding pairs using within-individual comparisons in a two-by-two design (male partner present or absent, eggs present or absent). We measured testosterone levels before and after intrusion trials to investigate whether female testosterone correlated with aggression. We found that females were most aggressive to intruders when eggs were present and their male breeding partner was absent. We also found that females with eggs had higher testosterone levels than females without eggs, and the relationship between aggression and testosterone varied with condition. Using *D. tinctorius* as a model system, we can further understand the physiological and behavioral mediators of maternal aggression.

The amphi-Pacific disjunction in a mangrove gastropod clade explained

Ellen Strong, Tricia Goulding

Tropical amphi-Pacific disjunctions are amongst the most vexing biogeographic patterns and are notoriously challenging to explain. The gastropod family Potamididae (Cerithioidea) is circum-tropical in distribution and comprised almost exclusively of mangrove specialists, whose earliest undisputed members appeared during the Eocene and are of Tethyan origin. The genus *Cerithideopsis* is one of the oldest living genera of potamidids and is found in mangrove and salt marsh habitats of the New World tropics and the Indo-West Pacific. They are thought to have been widespread throughout Tethys and the Americas by the Middle Eocene and their trans-Pacific distribution the result of vicariance. Possible explanations include cooling at the end of the Eocene or early Miocene aridity which restricted the distribution of mangroves, or the closure of the Tethyan corridor at the end of the Early Miocene. Our serendipitous discovery of an unrecognized potamidid species presents an alternative scenario. *Tachyrhynchus erosus* is found in the North Atlantic, North Pacific, and Arctic Oceans primarily in sublittoral habitats from 13 - 457 m and is currently classified in the Turritellidae. Analyses of a three-gene dataset (nuclear 18S rRNA and 28S rRNA, mitochondrial COI) reveal the species to be an unrecognized member of *Cerithideopsis* and sister to all other Neotropical members of the genus. We use fossil calibrations to explore the timing of this remarkable invasion of marine habitats from mangrove ancestry.

Modulation of feeding mechanics in the red-footed tortoise, *Chelonoidis carbonarius*

Thomas Stroud, Kendall Steer, Chloe Edmonds, Khaled Adjerid, Johnson Gao, Kree Kerkvliet, Rebecca German, Christopher Mayerl

Animals feed on items of various sizes and textures, often modulating feeding kinematics to meet these demands. Generally, studies addressing the neurophysiologic responses to diverse food types during feeding focus on mammals. However, it is unclear how non-mammalian vertebrates modulate feeding performance in response to variation in texture or size. One lineage with a high dietary diversity within a single species are tortoises. We utilized X-ray Reconstruction of Moving Morphology (XROMM) and electromyography (EMG) to capture tongue, hyoid and jaw movements synchronized with muscle activation in the red-footed tortoise, *Chelonoidis carbonarius* feeding on food items of different sizes and textures. Feeding cycles per swallow varied with size and texture, with more cycles per swallow when feeding on harder, or larger food items. Conversely, the tongue, hyoid and jaw varied minimally in their movements depending on food texture or size. The tongue generally protracted and elevated during jaw opening and retracted and depressed during closing. The hyoid was typically retracted and depressed during jaw opening and protracted and elevated during closing. EMG patterns and kinematics differed drastically during feeding cycles compared to swallowing, highlighting the multifunctional nature of the neuromotor system powering feeding in vertebrates, suggesting that either turtles do not modulate their feeding function with food type, or that the range of food types we provided were not broad enough to elicit a motor response.

Sediment grain-size influences burial mechanics in *Xiphister mucosus*, a Pacific Northwest intertidal fish

Mikayla Struble, Cameron Hernandez, Sydney Vickers, Cassandra Donatelli, Emily Standen, Baxi Zhong, Alice Gibb

One strategy intertidal organisms use to survive low-tide periods is to bury in the sediment. *Xiphister mucosus* (Zoarcoidei), an elongate fish native to the Pacific Northwest (USA), uses this strategy in habitats dominated by coarse sand, gravel, and boulders. To investigate burial behavior, we filmed *X. mucosus* individuals burying head-first into an artificial sediment of damp clear acrylic beads (1, 2, or 3mm diameter). We measured their kinematics using DLTdv8 in MatLab and found they bury using a predictable sequence of behaviors. Rapid head oscillations aid the fish in penetrating the substrate during burial initiation. Then the fish produces c-shaped bends involving the head and anterior portion of the body. Next, large tailbeats produced by the posterior body push the fish into the substrate. Once the center of mass is buried, lateral undulations of the whole body propel the fish through the substrate with larger amplitudes at the anterior body relative to posterior body. Key aspects of burial behaviors in *X. mucosus* are similar to mechanisms described for other vertebrates. For example, skinks and amphisbaenians also use head oscillations during burial. When presented with larger grain sizes, *X. mucosus* produces longer-lasting and more frequent oscillations of the head, c-shaped anterior bends, and tailbeat "pushes". This suggests that *X. mucosus* can modify these burial behaviors to exploit habitats with larger grain sediments.

An Effect of Dominance on Filial Cannibalism in an African Cichlid: *Astatotilapia burtoni*

Josh Stueckle, Suzy Renn

Parental care is critical for offspring survival in many species, yet parenting is energetically costly and balanced against future reproductive potential. Mouthbrooding, an involved parenting method where an individual incubates and protects fry in their mouth, sometimes resulting in filial cannibalism, provides an interesting system to address this decision. We use an African cichlid, *Astatotilapia burtoni*, which both has a strong social hierarchy and maternally mouthbroods for around two weeks, protecting the fry for another two weeks post-release. We hypothesize that if dominance is positively correlated with ability to raise and defend fry, then less dominant fish are predicted to engage in filial cannibalism more often in order to recover energy. We used two assays to quantify dominance until the female consumed or consistently

ignored their fry. For baseline measures, each pair of females was housed in a divided tank, allowing visual but not physical interaction with another female of matched brooding stage and dominance behaviors were tallied. For a more acute measure, after both females released fry, they were periodically moved to an arena for staged interactions. We found that less dominant fish are more likely to consume their fry than more dominant fish, providing evidence that social dominance positively correlates with parental success in *A. burtoni*.

Wind gates search strategies in free flight

Stanley Stupski, Floris van-Breugel

Insects are unparalleled in their ability to locate the source of a volatile olfactory cue. For flying insects, searching for a mate, oviposition site, or a meal requires integrating information from many sensory modalities including not just olfaction but also flow sensing and vision. In laminar wind, many insects use a “cast and surge” behavior, surging into the direction of ambient wind upon encountering a chemical plume, and making crosswind counterturns after passing through the plume. In nature, however, the wind environment can be still or turbulent and it is unclear whether insects adjust their search strategies for different conditions. Here we present an optogenetic paradigm to independently control the wind, visual, and olfactory experience of freely flying *Drosophila melanogaster* with high temporal precision. We show that flies exhibit a search state in the absence of wind that is distinct from the highly stereotyped “cast and surge.” In still air flies make rhythmic saccades in a consistent direction while lowering their altitude. This “sink and circle” search is broadly a proximal search algorithm that is triggered specifically by the loss of an odor cue. Finally, we use our paradigm to provide insight into how flying insects might gauge properties of their wind environment without stationary or calibrated sensory systems.

Effects of Cold and Experimentally Elevated Glucocorticoids on Incubation Behavior in Tree Swallows

Audrey Su, Jennifer Uehling, Conor Taff, Maren Vitousek

Climate warming has caused many temperate songbirds to start breeding earlier in the year so that earlier stages of the breeding cycle, like incubation, are increasingly prone to inclement weather like cold snaps. Breeding successfully in these challenging conditions may depend on adaptive behavioral changes that can be mediated by glucocorticoids, hormones that are released in response to stressors. The degree to which glucocorticoids mediate changes in breeding behavior in response to inclement weather are not well understood. Here, we investigate the effects of cold exposure on incubation investment in Tree Swallows (*Tachycineta bicolor*), and whether glucocorticoids mediate these behavioral shifts. Working on a long-term study population of Tree Swallows in Tompkins County, New York, we simulated cold snap conditions by experimentally lowering internal nest box temperatures. We also experimentally elevated levels of glucocorticoids in a separate group of adult females to simulate the hormonal response to cold. To record incubation behavior, we used thermocouple sensors in each nest to measure incubation bout constancy and bout duration. We predicted that both cold and glucocorticoid treatment would reduce incubation behavior and that females with naturally weaker glucocorticoid responses will decrease incubation less in response to cold. The results from this study will shed light on the mechanisms behind the avian behavioral response to climate change during breeding and the changes in reproductive investment during inclement weather.

Tracing the evolutionary origins of chordate mesoderm in ambulacrarians

Yi-Hsien Su

Mesoderm develops into almost all organs between the ectoderm and endoderm, and has played a crucial role in the evolution of complex animal body plans. During chordate development, mesoderm is subdivided into several regions, such as the axial and paraxial mesoderm that give rise to notochord and somites, respectively. Both notochord and somites are novel characters of the chordate body plan and their evolutionary origins remain unclear. Ambulacrarians, including echinoderms and hemichordates, are the closest group to chordates, and together represent the three major phyla of

deuterostomes. To elucidate the evolutionary origin of chordate mesoderm, we investigate the deployment of mesodermal genes in the indirect-developing hemichordate *Ptychodera flava*. *P. flava* first develop into planktonic tornaria larvae, which are morphologically similar to echinoderm larvae, prior to metamorphosing into the adult body plan. Therefore, comparing gene usages between hemichordate and echinoderm larvae enable us to reconstruct ancestral condition of ambulacrarians, providing insights into chordate evolution. We employ both RNA-seq and candidate gene approaches to comprehensively screen for genes associated with mesodermal development in *P. flava* embryos. Both conserved and divergent toolkits for mesoderm development in deuterostomes are identified. We further look into cis-regulatory controls of brachyury; its expression in axial mesoderm is considered to contribute to the evolution of notochord. Our study thus set a foundation for understanding the evolutionary origins of chordate mesoderm-derived novelties.

Partial immunity after first infection is long lasting and reduces MG transmission

Madeline Sudnick, Erin Sauer, Ashley Love, Sarah DuRant

Physiological processes of hosts govern how pathogens can establish infection. Physiological differences between first infection and reinfection can influence disease patterns by altering transmission dynamics. The bacterium *Mycoplasma gallisepticum* (MG) causes severe conjunctivitis in birds and the likelihood of birds encountering the pathogen multiple times is high. Birds infected with the same MG strain maintain partial immunity to reinfection and have milder eye lesions for at least 14 months. We conducted two experiments to investigate questions related to MG immunity and transmission.

First, to determine if previous infection with MG alters the likelihood of transmission during a subsequent MG infection event we placed birds into flocks of four individuals either completely naïve to MG or previously exposed to MG once, then inoculated an index bird in each flock with MG. We found that MG only transmitted in flocks experiencing first infection ($n = 3$ of 8) and none of the flocks experiencing second infections ($n = 0$ of 9). Second, to investigate whether birds maintain partial immunity after an extended period without infection, we re-inoculated birds with MG three years after their original infection. We found differences in physiological response, antibody presence and bacterial load. Determining the strength of long term retention of immunity and differences in transmission will lead to a better understanding of disease dynamics in wild birds.

Fish armor - evidence of assaults and conflicting demands.

Adam Summers, Cassandra Donatelli, Matthew Kolmann, Megan Vandenberg

Armor certainly plays many roles in fishes, and in its defensive capacity current utility and selective pressures are revealed by a study of morphology. Current utility, whether armor is abraded, broken, cracked, or scarred can be assessed with the scanning electron microscope. The marks of failure and wear can be specifically attributed to particular insults through an ex vivo damage protocol with carefully selected stand ins for suspected damage dealers. Alternatively, cadaveric experiments using live predators can demonstrate the damage patterns left by very specific insults and methods of assault. There is information in a hierarchical analysis of the armor that can reveal potential sources and types of damage. At the level of the entire armor coat, the nature and extent of imbrication has something to say about the size of predicted penetrators. Armor with great gaps in coverage is ill-suited to narrow stabbing penetrators, but resists crushing or impact well. A single plate tells a story of what type of damage - bending, crushing, shear, penetration, spalling, or abrasion would be resisted well. Tradeoffs in resistance to these different insults offers some insight into selective pressures. Furthermore, for designs that do well against a particular damage type, there are tradeoffs in other realms, like locomotion or buoyancy, that may be important in sculpting morphology. The limits and canalization of moment is written in the fine scale detail of single plates. And surface sculpture could be stopping a narrow penetrator from skidding across a plate surface to a joint. Joints themselves can have microstructure that interlocks overlapping plates in response to an impact load. In short, we need more data on many levels of armor performance to understand its evolution.

Wake dynamics of revolving beetle wings

Kiruthika Sundararajan, Duvall Dickerson-Evans, Rory Miller, Gal Ribak, Roi Gurka

Due to the advent of micro aerial vehicles (MAVs), the study of low Reynolds number flyers such as insects and Bio inspired flyers has gained popularity among researchers. Fluid-structure interactions play a significant role in their enhanced aerodynamic performances. Thus, comprehending the intricate aerodynamics resulting from the motion of these flying objects involves understanding the near-wake field developed to the flapping and revolving movements. To investigate this, an experimental study was conducted to evaluate the flow structures in the wake field of revolving beetle wings (*Batocera rufomaculata*). A Particle image velocimetry (PIV) system was used to perform flow field measurements to quantify the wake dynamics and turbulent flow structures. The study involved conducting experiments on beetle wings at low Reynolds number ($Re \sim 104$) with varying angles of attack (AOA) to examine how these variations coupled with rotational motion using real wings impacts the flow dynamics. The study demonstrates the change in momentum and energy as function of angle of attack in a rotational frame of reference. In addition, proper orthogonal decomposition provides insight on the formation of flow structures and their relation to aerodynamic performance of the wing.

Ontogeny of physiological stress response in poison frog tadpoles

Ilsa surber, Eva Fischer

An essential challenge for all organisms is to detect and respond to environmental dangers, both behaviorally and physiologically. These responses are coordinated in part via the glucocorticoid stress response. In vertebrates, glucocorticoids (cortisol and corticosterone) are produced via the hypothalamic-pituitary-adrenal (HPA) axis. In larval anurans, there is behavioral and morphological evidence that stress response is not even across ontogeny, however changes in the HPA axis have not yet been examined. Our project sought to measure stress responses across stages of tadpole development in the Dyeing Poison Frog (*Dendrobates tinctorius*). We conducted an ACTH Challenge, a test where the HPA axis is stimulated following injection of Adrenocorticotrophic hormone, a signal to the pituitary gland to produce glucocorticoids. We injected tadpoles of different developmental stages with an ACTH treatment and a saline control, in a randomized order one week apart. We collected water-borne hormone samples pre-injection and post-injection and analyzed samples for corticosterone and cortisol using Enzyme-linked immunosorbent assays. We found that tadpoles produced both glucocorticoids, corticosterone is more abundant, and corticosterone increased in response to the ACTH challenge. We found no difference in stress response across developmental stages. This is likely because there was more variation in stress response among individuals than between developmental stages, thus stress response through the HPA axis is equally active across tadpole development.

Animal venom shapes interspecific interactions, physiology and reproduction

Joachim Surm, Sydney Birch, Jason Macrander, Adrian Jaimes-Becerra, Reuven Aharoni, Adam Reitzel, Yehu Moran

Venom is considered a complex trait as it is made of many toxic proteins shaping the pharmacology and hence the phenotype of the venomous organism. This characteristic together with the direct relationship of gene-toxin-phenotype make it an appealing system for studies at the functional level. In this work the sea anemone *Nematostella vectensis* as a venomous model organism provides us with the opportunity to test for the first time how toxin genotypes impact predator-prey interactions at the organismal level. Using newly developed genetic manipulation tools, we significantly reduced both RNA and protein levels of Nv1, the most prominent toxin in adult *Nematostella*. Strikingly, we documented a similar natural phenomenon in a native population of *Nematostella* which has undergone a genomic event resulting in loss of Nv1. We show that animals lacking Nv1 have reduced ability to defend themselves against grass shrimp, a native predator of *Nematostella*. Unexpectedly, mummichogs, fish which share the same ecological niche, are significantly

more attracted to *Nematostella* with wildtype levels of Nv1, hinting that Nv1 affects the fish's behavior. We also demonstrate that *Nematostella* lines lacking Nv1 grow faster and reproduce more often both sexually and asexually compared to Nv1-producing control lines. Thus, our results experimentally link an organism's venom to its physiology, reproduction and interspecific interactions and reveal an evolutionary tradeoff between defensive venom synthesis and reproduction.

Morphological adaptations in recurrent water-land transitions

Elizabeth Surovic, Terry Ord

Does entering a new environment encourage adaptive change, or do certain species' exaptations help them succeed in diversifying into new environments? Family Blenniidae is a group of small, cryptic, benthic tropical fish with species in all three stages of the water-to-land transition: aquatic, amphibious, and terrestrial. The intertidal regions of tropical islands in the Pacific and Indian Oceans are home to a large population of blennies. With tides rising and falling, temperatures, oxygen levels, and resources are constantly changing in these intertidal zones. This offers a rare chance to investigate whether these fish use facilitated adaptive change when they move to land or if they have exaptations that make the move easier. This study measured various morphological characteristics that were anticipated to be important for shifting from water to land in 72 species. It also includes a series of phylogenetic analyses, aiming to find exaptive traits and traits that have evolved due to land colonisation. This study not only looks at general adaptive habitat transitions but, more specifically, at how fish make such drastic transitions. This can relate back to the early Devonian transition and give scientists a new outlook on exaptations and adaptive change during that time.

Anatomical and behavioral correlates of grasping performance in the salt marsh harvest mouse

Diego Sustaita, Dulce Robles-Martinez, Mason Laurin

The endangered salt marsh harvest mouse (*Reithrodontomys raviventris*) occupies the brackish marshes of the San Francisco Bay estuary, where it experiences tidal and managed flooding on a regular basis. The mouse is thought to climb and cling onto taller vegetation to escape inundation, which should select for enhanced grasping performance. Thus, we compared their grasping forces to those of three coexisting terrestrial and habitat generalist rodent species found throughout the Suisun Marsh for context. We also measured the relative foot sizes of the mice to explore anatomical correlates of these forces. Finally, we relate differences in grasping forces to locomotor kinematics obtained from videos of mice moving across horizontal, angled, and vertical dowels of different diameters. Our preliminary data show that salt marsh harvest mice produce greater grasping forces (corrected for body mass) than the other species. Furthermore, manus surface area is positively correlated with grasping force, and grip force decreases with increasing object diameter. This allows us to predict that individuals with relatively larger manus surface areas or greater grasping forces will perform better across the more challenging smaller dowel diameters and inclined orientations. These results help pinpoint key aspects of morphology and performance that might be correlated to differences in climbing behavior and microhabitat use among species.

Mechanisms and function of active antennal mechanosensation in *Drosophila melanogaster*

Marie Suver, Olivia Nunn, Emily Kophs, Tobias McCabe

Animals use a variety of sensors to extract information to navigate the world and survive. Many of these sensors move during behavior, with some actively controlled by muscles for behaviors known as 'active sensing'. Active sensor movements tune sensation for particular behaviors, extending their function beyond an unactuated sensor. However, the behavioral function of active sensor movements, and the cellular and circuit bases for these behaviors, are not fully understood. In this talk, I will describe our recent work developing a new model for active sensing in the antennal mechanosensory and motor

center in the fruit fly *Drosophila melanogaster*. First, I will show how fruit flies actively move their antennae to alter sensory tuning of a mechanosensory feature. Next, I will describe our anatomical work mapping the antennal motor system. Lastly, I will describe our most recent work investigating the role of specific sensory and premotor neurons in controlling antennal movements during flight. Together, this work sheds new light on mechanisms enabling animals to modify how they acquire sensory information for stability during behavior.

Introduction to Chordate Origins: Evolution, Development and Regeneration

Billie Swalla

The evolution of the distinct chordate body plan has intrigued scientists for over a hundred and seventy years. Modern genomics and transcriptomics have allowed the elucidation of the Developmental Gene Regulatory Networks (GRNs) underlying developmental programs for particular tissues and body axes in invertebrates and vertebrates. The time is ripe to gather those working on deuterostome developmental GRNs to revisit the development and evolution of chordates and discuss the evolution of this unique body plan. It has been several years since the genomes of all of the major deuterostome phyla have been sequenced – echinoderms, hemichordates, tunicates, lancelets and vertebrates. Genomic analyses have shown that lancelets have a genome and body plan that closely resemble the vertebrates, although phylogenomic analyses suggest that the tunicates are the sister group of the vertebrates. We are gathering scientists from all over the world to gather at the SICB meetings in Seattle to discuss the current ideas of how chordates evolved. We look forward to a fantastic day!

Deuterostome Ancestors and Chordate Origins

Billie Swalla

The Deuterostomes consist of the Ambulacraria: hemichordates and echinoderms, and the Chordata: lancelets, tunicates and vertebrates. Hemichordates and echinoderms are sister groups and are critical for understanding the origin and evolution of the chordates within the deuterostomes. Enteropneusta hemichordates share many chordate features as adults, including a post-anal tail, gill slits, and a CNS, that deploy similar Genetic Regulatory Networks (GRNs). Genomic comparisons show that cephalochordates share synteny and a body plan similar to vertebrates, but phylogenomic analyses place tunicates as the sister group of vertebrates. Echinoderms and vertebrates have extensive fossil records, with fewer specimens found for Tunicata and Enteropneusta, or worm-like hemichordates. The results suggest that the deuterostome ancestor was a complex benthic worm, with gill slits, a cartilaginous skeleton, and a CNS. Notochord loss in echinoderms and hemichordates is as parsimonious as notochord gain in the chordates but has implications for the deuterostome ancestor. We review the current genomic and GRN data on the different groups of deuterostomes' characters to re-evaluate different hypotheses of chordate origins.

Ladies First: Sex Differences in Finches Influencing Cognitive and Behavioral Responses to Stress

Austin Swallow, Emily Brandow, Hunter Rogers, Jason Davis

Stress occurs when an organism's homeostatic balance is externally modulated. To compensate, organisms must alter their physiology and behavior in response to these changes in their environment. Due to variations in selection pressure and commensurate physiological differences, responses to stressors may differ between sexes in many species. Australian zebra finches, a highly social species, become stressed when they are even briefly socially isolated. The goal of this project has been to study interactions between social isolation and sex on finches' ability to adapt to cognitive challenge. To study this, zebra finches were socially isolated for 24 hours and then exposed to a cognitive memory challenge. We compared birds' behaviors, task performance, and corticosterone/testosterone ratios before testing, in both social situations and in socially isolated situations. Results suggest that sex may play a role in determining resistance to stress and overall cognitive performance.

Stable and fluctuating cold effects on muscle histology and oxidative balance in house sparrows

David Swanson, Chelsi Marolf, Ana Jimenez

Climate models predict increases in temperature variability which may promote phenotype-environment mismatches for birds wintering in cold climates. Typically, winter birds upregulate maximal shivering metabolic rates, which are supported by pectoralis muscle hypertrophy. We acclimated house sparrows for 6 weeks to warm (25°C), stable cold (3°C) and fluctuating cold (mean = 3°C, but range from -10 to 16°C) treatments to test the hypothesis that fluctuating cold induces additional flexibility in pectoralis muscle ultrastructure and oxidative balance across different tissues relative to stable cold. We found no significant differences in fiber diameter, number of nuclei per mm of fiber, or Myonuclear Domain across acclimation treatments. Fiber cross-sectional area vs. number of nuclei per mm and fiber diameter vs. MND were significantly positively correlated in control and fluctuating groups, respectively, but not in other treatments. Neither CAT nor GPx activity differed significantly among acclimation groups. Likewise, peroxyl scavenging capacity and LPO damage did not differ among acclimation groups. However, hydroxyl scavenging capacity was significantly higher in the control group than in either cold group. Acclimation to cold or fluctuating cold had little effect on muscle ultrastructure or oxidative balance in winter-collected house sparrows, as only hydroxyl scavenging capacity was lower than in warm-acclimated birds. This suggests that fluctuating cold temperatures do not impose additional phenotypic remodeling on winter house sparrows.

Amphibian Microbiome Inhibition by Chytrid Fungus

Morgan Swanson, Jacob Kerby

The pathogenic fungus *Batrachochytrium dendrobatidis* (Bd) has contributed to the global decline of various amphibian populations. A major component of the amphibian's defense against this pathogen is its cutaneous microbiome, acting within the innate immune response to inhibit the development of the pathogen. Microorganisms on the skin have been documented as capable of producing numerous antifungal compounds to combat the fungus. However, Bd produces extracellular metabolites that have been demonstrated to be immunomodulatory in nature toward members of the adaptive immune response. These include, but are not limited to, extracellular tryptophan, kynurenine, and the quorum sensing molecule tryptophol. The effects of these molecules on the microbiome of the amphibians are not well understood. Three different isolates of symbiotic cutaneous bacteria belonging to three separate genera (*Serratia*, *Bacillus*, *Staphylococcus*) taken from amphibians in Costa Rica were tested against the full metabolic production of Bd in culture as well as the principal metabolites listed above. Both the full production and the individual metabolites significantly inhibited the growth of all three bacterial isolates. This demonstrates that Bd metabolites can successfully inhibit the proliferation of symbiotic antifungal bacteria, and that the same immunomodulatory molecules also can aid in the successful evasion of the amphibian innate immune response. The effects of these molecules are vital to the understanding of the amphibian immune response and in any potential mitigation strategies.

South American tetras display interspecific variation in schooling behavior kinematics

Nathan Swanson, Ashley Peterson, Christopher Martinez, Matt McHenry

The study of social behavior in fishes, like schooling, is often limited to intra-specific assessment, likely due to the high degree of complexity associated with quantifying collective behaviors. We hypothesize that related species will exhibit diverse schooling behaviors and variation in their responses to stimuli based on differences in evolved schooling strategies. South American tetras in the family Characidae include many obligate schooling species, which provides a valuable opportunity to examine interspecific variation in the properties of fish schools. We assessed variation in schooling kinematics to determine how factors such as light level and school size may influence this collective behavior across three closely related species in the subfamily Stethaprioninae. We established a high-throughput method for recording collective behavior using a custom filming arena and machine learning tracking

software to independently examine each member of a school. We examined 5 schools per species under multiple light and school size treatments and measured several kinematic traits that included average speed, nearest neighbor distance, polarization, and rotation parameter. Preliminary results suggest a high degree of variation across species for each of the metrics. This interspecific diversity in schooling kinematics suggests that collective behavior is highly labile, and conclusions drawn from a single species may not be true for others. It also underscores the need for further efforts to understand the evolution of complex social behaviors.

The Genetic Diversity of *Aphis nerii*

Ikatri Swope, Brian Lomeli-Garcia, Abigail Cahill

Aphis nerii (milkweed aphids) are a successful invasive species known for being pests to cultivated plants. These insects reproduce asexually, leading to low genetic diversity. Previous studies have indicated that they are unable to overwinter because they do not produce overwintering eggs. This allows them to only successfully reproduce in warm temperatures. Here we used two experiments to firstly investigate the level of genetic diversity in populations of *Aphis nerii* and secondly their cold tolerance. Our results revealed that low genetic diversity was present despite geographical differences between the *Aphis nerii* in our study and that perhaps the aphids have difficulty withstanding cold temperatures for long periods of time. Despite this, some genetic diversity was present and further research is needed to understand how aphids may survive in colder climates within their non-native range.

Classification of Odor derived EAGs with Machine Learning

Joshua Swore, Marissa Dominguez, Melanie Anderson, Jeff Riffell, Malek Itani

Insects have a keen ability to detect numerous odors in their environment. These odors, known as volatile organic compounds (VOCs), provide the insect with information about food, predators and mates that may be in the area. Receptors are tuned specifically towards an array of chemicals each with unique sensitivity towards a given VOC. When a VOC binds with the receptor located on a neuron inside of the antennae of an insect, the electrical potential of that neuron changes, sending a signal to the brain where it is integrated and interpreted. We are able to intercept the relay by using an electroantennogram (EAG) to record the change in voltage when a VOC is detected by the insect. This change in voltage over time is observed as a “wave”. Some research has suggested that the shape, including intensity, slope, and duration are specific to the VOC that elicits the wave. Using machine learning we have begun to classify EAGs based on waveform shape. Using a panel of floral associated and disease related VOC and VOC mixtures, we collected the voltage response from the tobacco hornworm moth, *Manduca sexta*. This time series data was then used to train support vector machines and random forests to predict the VOC or mixture that elicited a given waveform. Classification results consistently performed better than a random guess.

Winter energy allocation and fitness shift in response to stochastic snow cover in a montane beetle

Andre Szejner-Sigal, Irja Ratikainen, Øystein Varpe, Caroline Williams

Climate change is increasing stochastic variation in snow cover, with implications for organisms that overwinter under snow. Snow cover can modulate winter energetic costs and thermal stress exposure. Deep snow cover buffers thermal fluctuations, reducing the risk of extreme cold, but it tends to increase the baseline energetic costs, due to lengthening of winter. Little or no snow cover exposes organisms to higher thermal variability and extreme cold, but winter is usually less costly due to low mean temperatures. However, we lack models that test how stochastic environments impact populations during winter, and their carry-over effects into the growing season. Here we use stochastic optimization modelling of energy allocation between maintenance and future reproduction using the beetle *Chrysomela aeneicollis* to assess the fitness consequences of changing snow cover patterns. We find that during dry winters, beetles starting winter with low energy stores have higher future reproduction compared to snowy winters, albeit dry winters having high mortality. Conversely, snowy winters are generally more benign,

but only beetles with high energy stores at the start of winter have higher future reproduction compared to dry winters. We conclude that dry winters may increase mortality, but may increase growing season reproduction due to low baseline energetic costs. Our predictions may broadly apply to organisms that use energy stores for both overwintering and future reproduction (i.e. capital breeding).

Corticosterone exposure causes long-term changes to methylation, physiology, and breeding decisions.

Conor Taff, Sabrina McNew, Leonardo Campagna, Maren Vitousek

When facing challenges, vertebrates activate an evolutionarily conserved hormonal response that can dramatically alter behavior and physiology. Although this response can be costly, conceptual models suggest that it can also prime more effective responses to future challenges. Little is known about whether this process occurs in wild animals, particularly in adulthood, or how information about prior experience is encoded. One potential mechanism is hormonally-mediated changes to DNA methylation. We simulated spikes in corticosterone and monitored the phenotypic effects one year later, and DNA methylation both shortly after treatment and a full year later. The year after treatment, experimental females had stronger negative feedback and initiated breeding earlier, traits associated with stress resilience and reproductive performance. We also found that natural variation in stress-induced corticosterone predicted patterns of DNA methylation, including methylation to the MC2R gene, which encodes the adrenocorticotrophic hormone receptor. Finally, corticosterone treatment causally influenced methylation on short (1-2 weeks) and long (1 year) timescales; however, many of these changes did not have clear links to functional regulation of the stress response. Taken together, our results are consistent with corticosterone-induced priming of future stress resilience, and support DNA methylation as a potential mechanism. Uncovering the mechanisms linking experience with the response to future challenges has implications for understanding the drivers of stress resilience.

Comparative Morphology of the Pectoral Free Rays Among Scorpaenoid Fishes

Natalia Taft, Jeremy Harris, Terry Grande

Pectoral free rays are some of the most striking adaptations of benthic fishes; they are used in a wide variety of behaviors such as walking, crawling, and digging on the bottom. Previous research on pectoral free rays emphasized the morphological and functional novelty of pectoral free rays in sea robins (Triglidae), that use them for distinctive walking behavior along the bottom. We hypothesize that these adaptations in sea robins are part of a broader suite of specializations among scorpaenoid fishes. We describe the intrinsic musculature and osteology of the pectoral free rays in three scorpaenoid families: Hoplichthyidae, Triglidae, and Synanceiidae. We have identified three specializations of the adducting musculature that we hypothesize are associated with substrate contact in these families. First, we describe adaptations of the adductor superficialis muscle found only in Triglidae, and propose revised functional hypotheses for this muscle. Second, we described the previously misidentified arrector radialis muscle, present in all families, and propose a hypothesis for its function. Third, we identify a new muscle, the adductor profundus ventralis (ADV), which is derived from the adductor profundus, and propose a different function for this structure than has previously been proposed. Our comparative analysis provides morphological and comparative context for understanding the function of pectoral free rays within Scorpaenoidei and in other groups.

Structural complexity in central multimodal processing in a nudibranch mollusc brain

Cheyenne Tait, Paul Katz

Nudibranch molluscs process multimodal sensory inputs during complex behaviors. In *Berghia stephanieae*, courtship lasts for hours and involves olfactory cues, tactile inputs, and close-range salivary exchange. Little is known about how these cues are integrated in a brain with approximately 10,000 neurons. In arthropods, sensory information is initially processed in brain

regions dedicated to one sensory modality with integration occurring elsewhere. It is unknown but suspected that mollusc processing is more diffuse or even occurs in the periphery. In Berghia, we used immunohistochemistry (IHC), in situ hybridization chain reaction (HCR), and axon tracing techniques to map the sensory neuropils of its brain. We revealed much of the overall structure of the nudibranch brain, including stereotyped axon tracts, three-dimensional glomeruli in certain locations, regions of bilateral sensory projections, and convergence of multiple sensory modalities to discrete neuropils. As in other taxa, these areas are compartmentalized by glia. Serotonin, histamine, and octopamine axon terminals were found upon the densest neuropil structure. This presence of neurotransmitters in specific fields is reminiscent of insect mushroom body neuroanatomy, suggesting function in higher order processing. Additionally, an antibody for PKA-Ca, a marker for arthropod mushroom bodies and learning and memory more generally also highlights the densest structure. In summary, structured neuropil regions within the central brain arising directly from peripheral neurons represent the substrate for multimodal processing in a nudibranch mollusc.

Body mass and growth rates predict protein intake across animals

Stav Talal, Jon Harrison, Ruth Farington, Jacob Youngblood, Hector Medina, Rick Overson, Arianne Cease

Organisms require dietary macronutrients in specific ratios to maximize performance, and variation in macronutrient requirements plays a central role in niche determination. Although it is well-recognized that development and body size can have strong and predictable effects on many aspects of organismal function, we lack a predictive understanding of ontogenetic or scaling effects on macronutrient intake. We determined protein and carbohydrate intake throughout development on lab populations of locusts and tested whether lab responses can predict results for field populations. Self-selected protein:carbohydrate targets declined dramatically through ontogeny, due primarily to declines in mass-specific protein consumption rates which were highly correlated with declines in specific growth rates. Importantly, lab results for protein consumption rates predicted results for field-collected locusts. However, field locusts consumed nearly double the carbohydrate, likely due to higher activity and metabolic rates. Combining our results with the available data for animals, both across species and during ontogeny, protein consumption scaled predictably and hypometrically, demonstrating a new scaling rule key for understanding nutritional ecology.

Daily consumption is not sufficient to fuel energy for repeated days of locust flight

Stav Talal, Geoffrey Osgood, Phoenix Pulver, Jon Harrison, Arianne Cease

To migrate successfully, self-locomoting animals must meet their increased energy needs. Much of this energy comes from lipid stores with a relatively small amount of carbohydrates in the body. Locusts are among the most well-known examples of migration, as their swarms travel vast distances to eat available vegetation, which often brings them into conflict with human agriculture. Despite their substantial impact on human populations and decades of research, we still have limited knowledge about nutritional and energy requirements of multi-day flights during swarms' displacement. To examine this phenomenon, and whether flying locusts can replenish their daily energy loss, we measured daily flight durations of tethered migratory locusts, *Locusta migratoria*, and their self-selected macronutrient consumption rates prior to, during, and after multi-day flights, and compared to a non-flying control group. Despite similar protein consumption, flying locusts increased carbohydrate consumption by ~30% during the three-day flight phase only. Flying locusts had ~30% less total body lipid than the control group, even though both groups had daily 19 h of food access during the flight phase. This suggests that daily flight demands required more energy than the locusts could replenish by daily consumption. These results highlight the importance of understanding migration energetics to better predict population movement dynamics.

Not Too Big a Stretch: Unsupervised behavior classification in bumblebees using dynamic time warping

Acacia Tang, August Easton-Calabria, Madalyn Laskowski, James Crall

Quantifying and categorizing the behavior of animals is an important but labor-intensive task central to many fields of biology. Recently, rapid advances in computer vision have facilitated a dramatic shift in animal behavior analysis from heavy reliance on human observation toward automation of both data capture and analysis. Unsupervised behavioral classification is a powerful approach that requires minimal human input, avoids potential observer bias, and can identify previously uncategorized behaviors. However, many of these techniques are inappropriate for applications in complex 3D environments or still require substantial human annotation. Dynamic time warping (DTW) is an algorithm used to align and characterize similarity between time series data that - while not widely applied in behavioral studies - is a strong candidate for unsupervised behavior classification, potentially requiring only relatively simple data (e.g., trajectories). Here, we explore using DTW combined with unsupervised clustering to classify behavior based only on movement of individual workers within bumblebee (*Bombus impatiens* and *bimaculatus*) microcolonies tagged with unique 2D barcodes. Specifically, we evaluate the utility of DTW for behavioral classification to study changes in worker behavior in response to environmental stressors (pesticides and temperature extremes), and how responses differ across species and populations. Finally, we highlight DTW as an effective approach for behavioral classification from time series data, with widespread potential applications in biology and animal behavior.

Migratory birds as an understudied reservoir of *Babesia microti*

Thomas Tao, Melissa Prusinski, Conor Taff, Corey Freeman-Gallant

The common yellowthroat (*Geothlypis trichas*), a migratory species of passerine bird, has not previously been studied as a potential reservoir of the tick-borne pathogen *Babesia microti*. We conducted a longitudinal study of a common yellowthroat population in Saratoga County, New York, where babesiosis is endemic, to examine the infection status and potential health consequences of *B. microti* infection in the avian host. We found the prevalence of infection increased over the study period from 56.5% in 2019 to 90.5% in 2021. Unlike other *Babesia* spp. detected in birds, we found *B. microti* infection does not significantly affect health or survivorship of the common yellowthroat. Migratory birds, including the common yellowthroat, may be underestimated reservoirs for *B. microti* and serve as the agent to spread the tick-borne diseases. The high prevalence, life-long persistence, and absence of major health consequences of *Babesia microti* infection characterize the potential role of the common yellowthroat as a reservoir for the tick-borne pathogen *Babesia microti*.

Intrinsic muscle properties of intact versus reinnervated guinea fowl LG

Rubi Tapia-Rayo, Marie Schwaner, Manny Azizi, Monica Daley

Surgical self-reinnervation can be a useful manipulation for investigating neuromechanical control mechanisms in locomotion. Self-reinnervation results in short-term muscle paralysis followed by motor recovery, with long-term loss of proprioception in the reinnervated muscle. We have found several changes in gait kinematics, motor control and in vivo muscle function following self-reinnervation of the lateral gastrocnemius (LG) in guinea fowl. However, the mechanisms underlying these changes remains unclear. Here we compare the intrinsic muscle properties of the guinea fowl LG between intact and reinnervated individuals. Based on in vivo data, we hypothesize that reinnervated individuals may have higher passive forces. We also anticipate that reinnervated individuals may exhibit a shift in fiber type distribution and number of motor units, which will be tested based on measurements of optimal velocity for peak power, maximum velocity and shifts in the force-frequency relationship in intact versus reinnervated LG. Preliminary analysis suggests that reinnervated LG has significantly higher passive forces compared to intact. This result is consistent observation that reinnervated birds have higher muscle-tendon force and ankle stiffness at the swing-stance transition in

locomotion, compared to intact birds. The findings suggest plasticity in the connective tissues contributes to the observed changes in in vivo muscle function.

Impact of competition level on knee ligament injuries in female alpine ski racers

Danielle Taylor, Catherine Bevier, Joshua Martin

Alpine ski racing is a high-risk sport with a high rate of knee injury. All athletes at the U16 level and above have similar training regimens and race conditions, and both genders compete and train under the same conditions, which makes ski racing a model sport for assessing intrinsic and extrinsic factors that influence injuries. The external risks that accompany alpine ski racing (terrain, conditions, training regimens) are virtually identical for males and females. Female competitors, however, are 2.3 times more likely to sustain a knee injury than their male counterparts. One factor that may influence knee injury is the level of competition: forces experienced and speeds reached increase with each competition level. In this study we used data gathered from female athletes across the top alpine ski racing programs in the U.S. and seven national teams to test the correlation between ski racing level and knee injuries in elite female alpine ski racers. While previous studies compared knee injury rates between males and females, the primary objective of this study is to determine a relationship between the frequency of ligament tears and the level of elite competition in alpine. We find that as the level of competition increases the frequency of knee ligament tears and re-tears both increase.

Metabolic rate allometry and collective behavior of needle ants

Kylie Taylor, Samantha Martin, Sara Filler, Ioulia Besspalova, James Waters

Social insect colonies exhibit emergent patterns across levels of organization including the division of labor, homeostasis, and metabolic allometry. We studied the metabolic rates of needle ants, *Brachyponera chinensis*, a relatively rare ponerine ant recently found in New England. We measured needle ant metabolic rates as individuals, in groups of varying sizes, and we also measured tissue specific metabolic rates. In the course of this work we observed pairs of ants engaged in short rapid antennal contact interactions. We observed these in the field as well as in laboratory enclosures. These interactions, tentatively being called vibe checks, do not appear to be aggressive in nature and they also do not appear to solicit trophallaxis. We used long-term recording and high-speed video to quantify the vibe check dynamics. These interactions are distinguishable from typical interactions due to prolonged contact and direct and rapid movements of the antennae. Ants were more likely to vibe check outside their nest enclosure, a pattern opposite that found for typical antennal contacts. Using social network analysis, we documented the connectivity of vibe checks between ants. Using Deep Lab Cut, we are quantifying the movements of the antennae as pairs of ants engage in this high-speed behavior. We thank the Rhode Island Natural History Survey and acknowledge funding from National Science Foundation awards IOS 1953451, OIA 1826689, and EF 2222418.

Discrete developmental phylogenetics and the evolution of plumage signals in young manakins

Liam Taylor, Richard Prum

Although biologists have studied the relationship between ontogeny and phylogeny for hundreds of years, most modern comparative perspectives frame development as if it were a continuous process. These continuous perspectives fail to describe discrete processes such as plumage development in birds. Manakins (Aves: Pipridae) are a neotropical clade of primarily polygynous, lekking birds. In some manakin species, young males pass through a discrete sequence of "predefinitive" plumage stages before molting into the "definitive" plumage associated with successful lekking displays. Here, we develop a graph-based description of plumage developmental sequences and analyze the evolution of those sequences across the manakin phylogeny. Our analyses uncover two distinct pathways for plumage sequence evolution: (1) plumage patch diversification and (2) discrete heterochronic shifts. We

also highlight repeated coevolution between colorful patches in predefinitive plumages and the sociosexual signalling patches in definitive plumages. This coevolution provides interspecific evidence that predefinitive plumages function as sociosexual signals for young males. Our discrete phylogenetic perspective not only allows us to investigate manakin plumage evolution, but also reveals new opportunities for studying other graph-structured characters such as gene trees, cell regulatory networks, and social networks.

It Takes a Village: Mentoring Villages for Biologists at Primarily Undergraduate Institutions

Kari Taylor-Burt, Eric Gangloff, Jerry Husak, Michele Johnson, Rachelle Belanger, Ariel Kahrl, Jason Macrander, Thomas Sanger, Lisa Whitenack

Faculty at primarily undergraduate institutions (PUIs) have professional responsibilities and priorities that are very different from those at large research institutions. There is often little to no training for these aspects of PUI faculty work, nor the intensive teaching obligations, for those seeking a career at a PUI. This interactive poster hosted by the SICB PUI Action Group will present our plan to organize mentoring villages to help bridge this gap for early-career PUI faculty as well as those interested in applying to PUI faculty positions. Unlike traditional mentoring dyads, mentoring villages include a group of individuals who can serve as potential mentors and mentees, providing mentees with expanded networks and diverse senior mentor and peer-mentor perspectives. We invite SICB members at all career levels to use the poster to sign up to be part of a mentoring village, give input on the specific composition of those villages, suggest topics for the program, and provide examples of effective (and ineffective) mentoring strategies. The poster will also serve as a space to build community at the meeting during the poster session and while on display at the PUI booth.

Colder temperatures augment bacterial pathogen persistence on bird feeders

Sara Teemer, Edan Tulman, Alicia Arneson, Steven Geary, Dana Hawley

Seasonal variation in temperature is associated with altered transmission rates of many infectious diseases. House finches (*Haemorhous mexicanus*) can experience fall and winter outbreaks of mycoplasmal conjunctivitis, caused by the bacterial pathogen *Mycoplasma gallisepticum* (MG). At colder temperatures, birds often rely on feeders to meet increased energy demands, potentially depositing MG onto surfaces as they feed. Despite the importance of feeder-based transmission in this system, it remains unknown how ambient temperature affects MG viability on feeders. We assessed such temperature effects using two experiments. We first pipetted equal MG doses onto replicate feeders held at one of two temperatures representing summer (27 °C) versus winter (4 °C). We then allowed MG to incubate for 0, 1, 2, 4, or 7 days at a given temperature and collected remaining inocula from individual feeders at each time point. In the first experiment, we implemented culture-based methods and found higher MG viability on feeders kept at colder versus warmer temperatures. In the second study, we used feeder swabs from each treatment to inoculate naïve birds and found that MG on feeder surfaces remained infectious to birds for significantly longer when incubated in colder versus warmer ambient temperatures. Further, MG on feeder surfaces remained infectious for longer than previously hypothesized. Overall, our results suggest a key role of ambient temperature in driving fomite transmission in this system and likely many others.

Do traffic noise regimes affect noise-induced cognitive impairment in zebra finches?

Chris Templeton, Michelle Winfield, Kyla Sandoval, May Murakami-Smith, Reece Rhinehart, Carrie Nunnenkamp

Anthropogenic noise pollution has a variety of negative impacts on animal ecology, morphology, and behavior. Recent experimental evidence demonstrated that noise can also impair cognitive processes in songbirds; simply hearing traffic noise can result in significantly decreased cognitive performance. Yet many birds seem to thrive in heavily noise-polluted environments, such as urban areas and near highways, suggesting that there is the potential

for overcoming the negative impacts of noise pollution on cognitive performance. We tested whether animals might be able to habituate to repeated traffic noise exposure by varying the noise playback regime and assessing cognitive performance in zebra finches. We used a battery of different foraging tasks designed to measure inhibitory control, motor learning, associative learning, and spatial memory. Results indicate that habituation could provide some release from noise-induced cognitive impairment, but the degree of relief depends on the specific type of cognitive task being performed.

Differential Innate Immune Component Contribution Against Two Bacteria in a Wild Freshwater Turtle

Jennifer Terry, Lorin Neuman-Lee

The innate immune system is comprised of multiple components that provide non-specific protection against potential pathogens. These components (e.g., non-specific leukocytes, complement, antimicrobial peptides, and natural antibodies) have different roles and mechanisms of action. Since the immune system is energetically costly and organisms are limited to a finite amount of energy, tradeoffs within the immune system may occur. We investigated innate immune component contribution in wild red-eared sliders (*Trachemys scripta*) in following a standardized stressor. We captured adult red-eared sliders during the active period in Arkansas, USA. We collected blood samples within 0-3 min, 120 min, and 240 min post-capture. We used fresh, frozen-thawed, and frozen thawed + heat-treated plasma in bacterial killing assays against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*. Our results suggest that different innate immune components contribute to observed bacterial killing against the two bacteria. This study provides evidence that nuanced tradeoffs occur within the innate immune system and assessing multiple metrics of immune function can reveal these complexities.

DNA methylation and pigmentation change following immune challenge in a small ectotherm

David Tevs, Justin Mukhalian, Emma Simpson-Wade, Christian Cox, Aaron Schrey, Lance McBrayer

Dependence on the external environment for thermoregulation suggests ectotherms may face additional challenges when mounting temperature based immune responses. Changes in pigmentation may assist ectotherms in responding to immune challenges by enabling more precise regulation of behavioral fever or hypothermia because of increased absorption of solar radiation. Variation in epigenetic characteristics may also assist in regulating immune induced pigmentation changes and in managing the body's energetic reserves following infection. Here, we tested how dorsal pigmentation and DNA methylation in the Florida scrub lizard (*Sceloporus woodi*) respond to two levels of immune challenge. We found changes in pigmentation that are suggestive of efforts to assist in behavioral fever and hypothermia depending on the intensity of immune challenge. We also found correlations between DNA methylation in liver tissue and pigmentation change along the dorsum, indicating color transitions may be part of a multifaceted immune response across tissue types. To our knowledge, these results present the first potential evidence of pigmentation change in ectotherms in association with immune response. The relationship between immune response, DNA methylation, and pigmentation change also highlights the importance of epigenetic mechanisms in organism physiology.

Temperature affects germ cell number which impacts sex determination in the red-eared slider turtle

Boris Tezak, Blanche Capel, Allison Montes

The red-eared slider turtle has a warm-female/cool-male temperature-sex determination (TSD) system, where sex is determined by incubation temperature during a critical period of embryonic development called the thermos-sensitive period (TSP). In species with genotypic sex determination, the supporting cell lineage of the gonad orchestrates gonadal differentiation. As a result, investigation of the molecular mechanisms behind TSD focus on understanding how gonadal supporting cells respond to temperature and regulate sex determination. However, somatic cells are not the only cells in

the gonad affected by temperature. We previously showed that high temperature leads to higher germ cell (GC) number regardless of sex. GC depletion in embryos incubated at intermediate temperatures results in a strong shift towards male-biased sex ratios, suggesting that GC number influences gonad fate. Here, we show that temperature affects GC numbers during their migration prior to the TSP. Additionally, we assessed the effects of high GC number on sex determination by comparing the sex ratio of embryos with low (control) versus high (experimental) GC number that incubate at a male-biasing temperature (28.5°C). While the sex ratio of control embryos was highly male biased, the gonads from embryos with high GC numbers displayed ovotestes. These experiments support the hypothesis that GC number can influence sex determination in amniotes and suggest a model in which temperature impacts sex determination in cumulative ways through multiple cell types.

Marine invertebrate microbiomes provide an immersive course-based undergraduate research experience

Robert Thacker, Raisa Rizzieri, Jose Moscoso, Anita George, Sowad Karim, Urmi Poddar, Aasma Shafiq, Ryan Silver

Marine invertebrates host unique communities of symbiotic microbes that vary across host lineages and environments. We facilitated an undergraduate laboratory course that brought students into an NSF-funded research project to investigate the ecology and evolution of marine invertebrate microbiomes. Students harvested marine invertebrates from settlement plates collected from multiple locations on Long Island, NY. After identifying their specimens, students extracted DNA to perform PCR and Sanger sequencing of CO1 and 18S marker genes. Amplification of bacterial 16S rDNA supported Illumina sequencing of invertebrate microbiomes. Homogenization of settlement plates facilitated CO1 metabarcoding of invertebrate communities using nanopore technology, followed by data analyses with MEGA, RAXML, MAFFT, R, and Galaxy software. In addition, students conducted their own research projects using published data obtained from GenBank and the scientific literature. The class found 144 species and generated DNA sequences for 35 of these taxa. Students analyzed microbiomes associated with 12 common host species (including bryozoans, tunicates, sponges, and gastropods), finding that microbiome species richness and community composition were more impacted by host identity than by location. Multiple bacterial lineages were uniquely associated with specific host taxa, yielding numerous hypotheses that can be explored by future undergraduate researchers. Our undergraduate laboratory course emerged as an immersive platform that instilled practical skills with broad applications such as computing proficiency, biochemistry, microscopy, data management, and statistics.

Variation in egg-laying patterns of the Pacific field cricket, *Teleogryllus oceanicus*

Aarcha Thadi

Females determine how and when to invest into reproduction during their lifetime. Acoustic sexual signals can aid in surveying potential mates in a population, so a female can decide between producing offspring with current or future mates. As females can modulate their reproductive behavior based on external cues, misinterpreting future mate availability may have consequences for investment into offspring production. Here, we investigate whether female Hawaiian Pacific field crickets, *T. oceanicus*, alter their egg-laying patterns in a novel acoustic environment wherein they are unsure of their next mating opportunity, as males in these populations have been rendered silent due to parasitoid-induced natural selection. Additionally, we look at the variability between individuals in their egg-laying patterns and investigate whether there are patterns. We reared female crickets in the presence and absence of cricket song and monitored their reproductive output over regular intervals. Females did not alter their egg-laying behavior across their lifetime based on whether they heard song. However, there was substantial unexplained variation in egg-laying patterns across individuals. Further, profile analysis revealed that females that laid more eggs initially had higher lifetime reproductive success. While females of these populations do not seem to be using acoustic cues to modulate their reproductive investment at the egg-laying stage, there is a need to study what else may drive individual variation in lifetime reproductive output.

Describing the Clitoris of Alpaca (*Vicugna pacos*) Using Comparative Anatomical Methods

Grace Thompson, Rachel Keeffe, Patricia Brennan

The clitoris, like many female reproductive organs, is a vastly understudied organ with a long history of misunderstanding. Despite poor representation in scientific literature, the clitoris plays an important role in pleasure, behavior, and reproduction among vertebrate animals. Clitoral stimulation has been linked to induced ovulation and increased rates of fertilization in domesticated agricultural animals. Alpaca (*Vicugna pacos*) clitoris anatomy has not been previously described despite the prevalence of alpaca farms across the world. Alpacas have unusually long copulation for an Artiodactyl (over 20 minutes on average), and a novel pattern of deep intromission with the penis causing abrasion and hematoma of the female mucosal layer along the upper reproductive tract. We hypothesize that the clitoris is a complex, functional organ in alpacas which may be stimulated during long copulation times, possibly to induce ovulation and/or prevent evolution of aversion despite copulatory wounding. Here, we determine gross anatomy and function of the alpaca clitoris through dissection, microCT scanning, histological preparations, and comparison to other domesticated Artiodactyls. In gross dissection we find the clitoris body is embedded in the vulvar commissure, but this structure only houses a relatively small part of the whole clitoris, as the erectile tissue extends cranially to the dorsal aspect of the cranial vagina, and seems to be extensive. Our work highlights the need for further investigation of clitoris anatomy.

Impact of Temperature Variation on Color Polymorphism and Behavior in Grasshoppers

Matthew Thompson, Michael Reichert

Color polymorphism is common in many species and it has many potential causes ranging from genetics to environmental factors. For example, *Syrbula admirabilis* grasshoppers have either green or brown color morphs, which could be influenced by environmental factors such as habitat choice and prey selection. Additionally, temperature could influence development, metabolism, and behavior, which may affect the resulting color morph. However, the relationship between color polymorphism, temperature, and behavior in *S. admirabilis* is poorly understood. Therefore, we investigated the effects of temperature on color polymorphism in these grasshoppers and assessed how color variation affects their behavior. We hypothesized that higher temperatures would increase the frequency of brown morphs, while lower temperatures would result in more green individuals. Additionally, we expected that color variations would correlate with differences in movement behavior, potentially due to temperature-induced metabolic changes. To test these hypotheses, juvenile grasshoppers were collected in the field and raised in a controlled laboratory setting with three different temperatures: 27, 34 and 40 °C. Their colors were quantified through spectroscopy and photography. We recorded videos of activity behavior, and quantified the total distance moved and latency to emerge from a shelter. Overall, our results contributed to the understanding of the effects of temperature on the mechanisms of color polymorphism and behavior.

The fecal metabolome in an endangered Killer Whale population reveals attributes of gut dysfunction

Sheila Thornton, Valentina Melica, Xiangjun Liao, Tanya Brown

Two populations of fish eating “type Killer Whales are found in Canadian Pacific waters. Over the last four decades, the Northern Resident Killer Whale (NRKW) has experienced a consistent annual population growth of 2-3.5%, while the endangered Southern Resident Killer Whale (SRKW) has languished and consists of just 73 individuals. SRKWs exhibit lower fecundity, higher mortality, and lower body condition index scores when compared to NRKWs. Primary threats to recovery include acoustic and physical disturbance, reductions in prey availability, and contaminants. Fecal samples were collected from both populations and samples were assessed using a metabolomics panel comprised of 254 metabolites and 23 steroid and thyroid hormones. As there are no reference values for Killer Whale fecal metabolome, the SRKW values were compared to those from the NRKW population. SRKW exhibit elevated concentrations of fecal amino acids, energy

metabolites and inflammatory markers, and lower levels of total bile acids, free fatty acids, and conjugated bile acids when compared to NRKW. Aspects of the SRKW metabolome resemble the human metabolomic phenotype associated with gastrointestinal disorders such as Crohn’s Disease. Combined with complementary studies on the Killer Whale microbiome, these data provide evidence of gastrointestinal dysfunction in this population of endangered whales and open the door for functional analyses to identify the physiological impacts of threats, which will ultimately support advice on threat mitigation for SRKW recovery.

The Influence of Group Size on the Startle Responses of Giant Danios in Light and Darkness

Ben Tidswell, Eric Tytell

Swimming in a school helps fish avoid predators by distributing the risk between an individual and the other fish around it. It can also provide confusion effects, making it harder for a predator to catch any fish. Our previous work has shown that giant danios (*Devario aequipinnatus*) sense one another but do not form cohesive schools in darkness, and our current experiments investigate how groups of fish in light and darkness change their startle responses as the size of the school increases. We initiated startle responses in the giant danios by dropping weights into water and the number of fish that startled, as well as their locations and latency to startle were all recorded. We believe that these fish in darkness may feel safer, as they cannot be seen by a visual predator, and therefore do not form schools. But when a predator attack is stimulated, they may feel much more at risk. Our hypothesis is that fish make changes to their startle behavior based on the number and location of other fish around them, and their ability to sense those other fish. We predict that fish are more likely to startle in darkness when they have less information about the fish around them and more likely to startle as the number of fish in the group decreases, with fewer fish to distribute risk between.

Trans-generational proteomic plasticity in coral response to thermal bleaching

Emma Timmins-Schiffman, Tanya Brown, Brook Nunn, Rayhan Khanna, Lisa Rodrigues, Elizabeth Duseles, Miranda Mudge, Callum Backstrom, Mike Riffle, Jeremy Axworthy, Brendan MacLean, Jacqueline Padilla-Gamino

Temperature-induced bleaching in corals is increasing worldwide as sea temperatures rise. Bleaching, the loss of endosymbiotic algae, leads to breakdown of stored lipids across species. *Montipora capitata* is a bleaching-resilient species since it can quickly transition to heterotrophic feeding when symbiont metabolites are unavailable. However, the ability to survive thermal bleaching on short timescales may obscure physiological trade-offs that could impact long-term fitness. In this study, *M. capitata* colonies underwent experimental thermal bleaching and were followed for 9 months to determine impacts of symbiont loss and reacquisition during gametogenesis. Immediately post-bleaching, proteomic biomarkers in bleached colonies indicated symbiont rejection and loss and a likely up-regulation of metabolic enzymes, suggesting heterotrophic feeding. There was also a decrease in abundance of carbonic anhydrase, suggesting a down-regulation of somatic growth, and in proteins involved in sex hormone production. Oocyte lipid deposition and reproduction proteins remained dysregulated in bleached colonies throughout gametogenesis. All colonies produced mature eggs, but eggs were smaller from bleached colonies. Gamete proteomes from bleached parents revealed a likely metabolic down-regulation, suggesting an adaptive and/or plastic response in gametes that contained less lipid. This detailed map of cellular and macro-physiology throughout gametogenesis reveals that *M. capitata* energetically prioritizes gametogenesis over somatic growth and that even though gametogenic output may be altered, gametes may regulate metabolic rate to make limited resources last throughout their planktonic phase.

An in vivo examination of snake muscle shortening and vertebral column curvature using X-ray video

Jessica Tingle, Kelsey Garner, Henry Astley

Multiarticular muscle systems are widespread across vertebrates, including in necks, digits, tails, and trunks. In secondarily limbless tetrapods, the multiarticular trunk muscles power nearly all behaviors. Using snakes as a study system, we previously used anatomical measurements and a mathematical modelling approach to derive an equation relating trunk muscle shortening to postural change. However, some of trunk muscles have substantial tendinous connections, raising the possibility of elastic energy storage, which could lead to a decoupling of muscle length change from joint angle change. As the next step towards understanding multiarticular muscle function, we must determine whether in vivo muscle shortening produces the postural changes predicted by mathematical modelling. A departure from predictions would implicate elastic energy storage. To test the relationship between muscle strain and posture, we implanted radio-opaque metal beads in three muscles of interest in four corn snakes (*Pantherophis guttatus*), then recorded X-ray videos to directly measure muscle shortening and vertebral column curvature during locomotion. Comparison of preliminary in vivo data with our equations has not yielded evidence for any appreciable departure from the predictions, suggesting that tendons do not store appreciable elastic energy during the locomotor modes we elicited. The ability to predict muscle shortening directly from observed posture will facilitate planned future studies examining how snakes manage the potentially competing demands of mechanical output and control.

Immune-pineal-retinal axis in amphibians: unveiling a novel connection?

Stefanny C Tison, Patricio Garcia-Neto, Braz Tison-Jr, Aymam Cobo-de-Figueiredo, Regina Markus, Fernando Gomes, Vania R Assis

Melatonin is a hormone known as an endogenous temporal marker signaling the dark phase of the day. Although the retina is the main site of melatonin production in amphibians, little information is available about the natural variation in the ocular melatonin levels and its modulation following immune stimulation. We investigated the daily variation of plasma and ocular melatonin levels in bullfrogs (*Lithobates catesbeianus*) and their modulation following an immune stimulation with lipopolysaccharide in yellow cururu toads (*Rhinella icterica*). For the daily variation, bullfrogs were bled and euthanized for eye collection every 3h over 24h to determine plasma and ocular melatonin levels. We found a positive correlation between ocular and plasma melatonin levels, with maximum values at night (10 pm) for both plasma and eyes. For immune modulation, toads received an intraperitoneal injection of lipopolysaccharide or saline solution during day (10 am) or night (10 pm). Two hours after injection, toads were bled and euthanized for eye collection to obtain plasma and ocular melatonin levels. Also, liver and bone marrow were collected to investigate local melatonin modulation. Interestingly, the lipopolysaccharide injection decreased only ocular melatonin levels, reinforcing the eyes (retina) pivotal role as a melatonin-production organ in amphibians. However, in the liver and bone marrow, melatonin levels increased after lipopolysaccharide stimulation in toads. These findings suggest a potential immune-pineal-ocular axis in amphibians.

How a worm knots and why

Ishant Tiwari, Vishal Patil, Saad Bhamla

Limbless organisms such as worms, eels, and snakes need to grip, feed, groom etc. by using their bodies as emergent limbs or grippers. One way they achieve this is by knotting their flexible bodies to exert force, grip objects, etc., to compensate for their lack of limbs. How they form these 'living knotoids' and un-knot effectively to avoid getting tangled has not been systematically studied. We investigate this peculiar behavior of knot formation in one such organism called the California Blackworm (*Lumbriculus variegatus*). We find that the blackworm can sometimes spontaneously contort its body into a knotoid when observed in lab conditions. The knots observed varied in their level of complexity, ranging from the simple overhand knot with 3 crossings, to knots with as many as 6 crossings. We further observe that the knot forms

because of the active motion of the head, and the knot eventually diffuses out of its tail end. We discuss how the worms use these knots to achieve biological functions such as cleaning debris from their bodies. Through a comparative biology lens, we also discuss the universality of this knotting behavior in slender organisms, from microscopic bacteria to massive pythons, across six orders of length scales. Using analytics and numerical simulations, we exhibit a correspondence between worm kinematics and the observed knotting spectrum.

Food availability and sirtuins as regulators of the cellular stress responses in intertidal mussels.

Lars Tomanek, Melissa May, Maria Vasquez

A key unknown for predicting the impacts of climate change is the role of food availability on the ability of marine organisms to respond to greater levels of stress. While greater food availability increases tolerance to acute heat stress in mussels, a key regulator of energy metabolism and stress responses, sirtuins (or NAD-dependent deacetylases), show higher activity levels in food-restricted mussels. Exposing mussels simultaneously to acute heat and salinity stress and sirtuin inhibitors suggests that sirtuins are involved in regulating key elements of the stress response, creating a possible contradiction. We approached this contradiction by comparing the proteomic responses of mussel (*Mytilidae*) species from different biogeographic regions, with known differences in thermal tolerance, to heat and salinity stress, compared the effect of food availability on feeding rates, and on redox balances and anti-oxidative stress responses under different time courses. Here we are trying to sort through these findings to possibly reconcile this contradiction and propose a model for the role of food availability to respond to greater environmental stress in mussels.

Decoding social communication in a eusocial ant species, *Harpegnathos saltator*

Rooskva Torhalsdottir, Aiden Masters, Benjamin Morris, Lydia Naughton, Gregory Pask, Amu Tawawalla, Lulu Wright

Insects use olfaction to drive a variety of fundamental behaviors such as finding mates, foraging for food, finding egg-laying sites, and communicating with conspecifics. *Harpegnathos saltator* ants are a primitively eusocial species and success in colony life relies on highly-sensitive olfactory systems. This species has highly expansive odorant receptor (OR) families that have been attributed to pheromone communication using cuticular hydrocarbons (CHCs) which serve to signal fertility, caste, and colony identity. Here we use in vivo heterologous expression to functionally characterize individual ant ORs (HsOrs) in *Drosophila melanogaster*. We performed single sensillum recordings (SSR) against a panel of relevant hydrocarbons in order to assess CHC sensitivity of individual HsOrs. A receptor-to-ligand map was made based on neuronal responses to specific hydrocarbons and the strongest ligands were further examined with subsequent dose response assays. With the addition of these characterized HsOrs, this represents the most ORs decoded in any insect species, and enables further investigation on the molecular evolutionary signatures necessary for successful eusocial communication.

Evolution Underground: Sensory Compensation Parallels Eye Loss in Neotenic Salamanders (*Eurycea*)

Ruben Tovar, Brittany Dobbins, Dana García, Tom Devitt, Paul Gignac, David Hillis

Understanding how some divergent lineages evolve morphological similarities remains a central question in evolutionary and developmental biology. Groundwater salamanders from central Texas genus *Eurycea* (*Paedomolge*) offer exceptional potential to address this question because of past independent invasions of subterranean environments. Members of this clade exhibit variation along a continuum of morphological adaptations to the environmental pressures of life underground. Organisms that occupy subterranean habitats converge on similar phenotypes thought to be the outcome of similar selective pressures. We hypothesize that subterranean salamanders will have reduced eyes relative to surface species, and compensate for lack of

vision with alternative sensory modalities. Herein we test differences in adult optic volumes using diceCT scanning method, track ocular development, and quantify the anterior lateral line system between multiple surface and subterranean salamander species. We identified parallels in soft tissue development including the lens and retina, indicating similar early developmental progression. However, total ocular volume decreases through development in the subterranean *E. rathbuni* relative to *E. nana*. Moreover, the greatest amount of total ocular (95.4%) and neuromast (82.4%) variation is explained between the two ecotypes among different species. These results suggest parallel eye loss and compensatory sensory modalities leading to subterranean evolution among Eurycea, while illustrating the utility of a rising tetrapod system for studying homoplasy and phenotypic evolution.

Variation in thermal sensitivity of diapause drives life history timing in an insect pest

Jantina Toxopeus, Eddy Dowle, Lalitya Andaloori, Greg Ragland

Physiological time is important for understanding the development and seasonal timing of ectothermic animals, but has largely been applied to developmental processes that occur during spring and summer such as morphogenesis. There is a substantial knowledge gap in the relationship between temperature and development during winter, a season that is increasingly impacted by climate change. Most temperate insects overwinter in diapause, a developmental process with little obvious morphological change. We used principles from the physiological time literature to measure and model the thermal sensitivity of diapause development rate in the apple maggot fly *Rhagoletis pomonella*, a univoltine fly whose diapause duration varies substantially within and among populations. We showed that diapause duration could be modelled with simple linear relationships between diapause development rate and temperature. Low temperatures were necessary for most individuals to complete diapause, similar to many insects. However, we also found evidence for an ontogenetic shift in the thermal sensitivity of diapause: diapause development proceeded more quickly at high temperatures later in diapause and in the absence of quiescence, a phenomenon not previously reported. Increasingly warmer temperatures during and after winter may impact the phenology of this and other insects with temperature-sensitive diapause, impacting their viability.

DEVELOPMENTAL DIVERGENCE OF TRAP-JAW AND NON-TRAP-JAW STRUMIGENYS ANTS DURING METAMORPHOSIS

Leonardo Tozetto, Julian Katzke, Gaurav Agavekar, Evan Economo

Strumigenys ants are a diverse group of small leaf-litter predators with many lineages exhibiting power-amplified trap-jaw mandibles. The rearrangement of the mouthparts into ultrafast trap-jaws has been reinvented several times around the world, a remarkable case of convergent evolution of form and function. Thus far, no one has explored the development pathway of this convergent trait. In this study, we compare the mouthparts development of three species of Strumigenys, one non-trap-jaw species and two convergently evolved trap-jaw species. We use micro-CT reconstructions focusing on the late-larval, prepupal and late-pupal stages to highlight the metamorphosis and developmental trajectories of different parts. We sought to understand when trap-jaw and non-trap jaw forms diverged in their development, and whether sequences are similar across independent evolution. We found that development of relevant traits is similar until the pre-pupal stage, when body parts get rearranged during metamorphosis. Moreover, our findings reveal a curious feature of trap-jaw development: the mandibles develop folded during prepupa and open at pupation assuming an open position close to locked, while non-trap jaws develop in the closed position. We propose this could be a developmental failsafe mechanism that supports the complex morphological interaction of mouthparts in the latch. Our results shed light on similarities and differences across forms and clades in development of a complex biomechanical innovation, and sets stage for further investigation of developmental mechanisms.

Usage of game trails vs. human and cow paths by wildlife

Kaitlyn Tracy, Sarah Foltz

Appalachia contains large amounts of undeveloped agricultural and wildlands crisscrossed by trails. Some are made and used by humans, while others are made and used by wildlife. This research examines whether there is a difference in wildlife usage of game trails (those created by wildlife movement) versus paths created by humans or domestic livestock for movement through these spaces. Based on previously published analyses, we expect large wildlife species to be less likely to use active human and livestock paths than smaller wildlife. We went to three different locations, used twelve game cameras, and placed them along trails in the Appalachian region of Virginia. Each location assessed had four cameras, two on game trails near where they intersected a human path and two on the human paths themselves. The cameras were left on the trail for nearly a month. Some locations were accessible to the public, while others were on private property. The analysis of this data is ongoing; preliminary results suggest that fewer deer use the active cow paths. This may be because humans are perceived as predators by many large prey animals such as deer and have a high chance of encountering humans on those trails made by humans or domestic livestock.

Analysis of frenzy swimming in three morphologically-distinct species of sea turtle hatchlings

Samantha Trail, Jeanette Wyneken

As hatchling sea turtles emerge from subsurface nests on the beach, they exhibit an innate and highly-conserved hyperactive state: first rapidly crawling to the water, followed by almost continuous swimming for 24-48 hours as they escape coastal, predator-rich waters. This rapid swimming (the “frenzy”) is dominated by powerstroking, which is characterized by bilateral, elaborate flapping of both forelimbs (flippers). In all species, flippers have a hydrofoil shape and generate the powerstroke’s thrust. Despite the well-conserved nature of frenzy swimming and wing-like flipper shape across all sea turtle species, these hatchlings are morphologically distinct. We compared the frenzy swimming in leatherback, loggerhead, and green turtle hatchlings from nests in Southeast Florida. We hypothesize that species-specific morphological differences reflect in kinematic differences in the powerstroke. To test this hypothesis, hatchlings were collected on the day of emergence from the nest, measured, and placed in a sea water-filled tank, and frenzy swimming was recorded (120 fps, 4K resolution). Hatchlings were attached via tethers to a force transducer to synchronously record thrust with the video. We found (1) leatherback flippers are proportionately the largest, green turtles’ are intermediate, and loggerheads’ the smallest. (2) Green turtles produce the greatest maximum thrust/g body mass of the three species. (3) The thrust (force) x time plots are distinct among the three species reflecting their kinematic differences.

Keeping it in the Family: Discerning Patterns of Scale Shape diversity in Reef Fishes

Sean Trainor, Kory Evans

Fish scales and integuments represent an understudied system that can yield insights into form-function relationships that may govern how these organisms interact with their environments. Fish scales also offer a diverse and varied source of bioinspiration in their shape or internal structure. Some structures on the external surface of fish scales are thought to help fish navigate their environment. Other internal structures may allow for a higher degree of protection from mechanical stresses. In this study, we have examined the scale shapes of 45 reef fish species using micro-computed tomography scanning and three-dimensional geometric morphometrics to examine patterns of scale shape diversity within an assemblage of coral reef fishes. Preliminary data shows some taxa, i.e., Acanthuridae, show a broad diversity in scale width and depth. Other taxa, like Labridae, show stark homogeneity in scale shape. These results suggest that phylogeny has a strong effect on scale shape, and further study into other taxa can help determine how the trait is conserved across broader clades of fishes.

The cellular dynamics of medusa development reveals distinct developmental trajectories in Medusozoa

Matthew Traver, Paulyn Cartwright, Kent Winata

Medusozoans are characterized by the presence of a medusa (jellyfish) stage as part of the life cycle. Despite being a prominent trait in medusozoan evolution, the cellular mechanisms underlying the development of this life cycle stage are poorly understood. Characterizing cell proliferation, cell migration and programmed cell death in several hydrozoan and scyphozoan species, we found that the development of the medusa displays distinct cellular mechanisms between lineages. Using Edu labeling, hydroxyurea treatments and Dil cell tracing we found that in the colonial hydrozoans *Podocoryna carnea*, *Podocoryna exigua* and *Craspedacusta sowerbii*, the formation of the medusa relies on cell migration and subsequent local cell proliferation as it buds from the polyp. Whereas the benthic hydromedusae *Staurocladia*, does not require cell proliferation as it buds new hydromedusae from its bell. Using TUNEL assays and pan-caspase inhibitor treatments, we found that in all surveyed hydrozoans apoptosis does not play a role in the development or release of medusa. By contrast, the scyphozoan medusae *Aurelia coerulea* and *Sanderia malayensis* undergo extensive apoptosis and subsequent cell proliferation is required for the formation of the medusa and its organogenesis. The difference in medusa developmental trajectories between scyphozoans and hydrozoans could challenge the traditional view on the homology of the medusa between these two lineages.

Coral eco-immunity in a disease landscape of unknowns

Nikki Traylor-Knowles

Since the 1970s, Florida Coral Reefs (FCR) has lost over 90% of coral cover due to anthropogenic stressors and disease outbreaks. Currently, FCR is experiencing one of the most dramatic and extreme heat stress episodes ever recorded in human history. I hypothesize that these successive disruption events have created dysbiosis of the coral holobiome which has led to an overactive coral immune system, further causing coral death and disease. To test this, we have examined the gene expression changes associated with Stony Coral Tissue Loss Disease (SCTLD) in the coral *Orbicella faveolata*. We will present how the timing within the infection affects immune gene expression, and how individuals differ in their response to SCTLD. Lastly, I will present evidence that overactive immune function is found in other corals that have experienced stress including bleaching and other anthropogenic induced stressors.

Substrate deposition and tunnel remodeling in fire ants *S. invicta*

Laura Treers, Daniel Soto, Michael Goodman, Daniel Goldman

Many animals rely on construction of excavated nests for survival. Fire ants (*S. invicta*) create in diverse soils nests composed of complex arrays of tunnels, above which sits a porous "mound." Principles by which ants effectively excavate in confined conditions have been elucidated; less is known about where ants deposit excavated material, and thus how the nest environment emerges. Here we use a quasi-2D "ant farm" setup using small groups (~300) of fire ants. To monitor material flow, we use 6 layers of colored sand filling a container 7.9±1.6 cm deep, with particle size 75-400 µm, saturated with water 10% by mass. Ants formed 4±1 tunnel structures spanning >50% of the depth of the container over 60 hrs. Ants brought material up tunnels to the surface, leading to proto-mounds whose structures were layered inversely to the original substrate. Additionally, on 92% of major tunnels (3 trials), material from deeper layers accumulated on walls of excavated tunnels. For example, after 45 hours one tunnel diameter at its widest location was 3.3 mm and shrunk to 1.8 mm after 60 hours. We posit this represents intentional reshaping of tunnel walls, possibly to ease locomotion and decrease energy expenditure, thus providing safety advantages in early nest construction. These results can reveal principles by which organisms and multi-agent systems intentionally modify their environment to improve collective performance.

Evolution, Physiology, and Biomechanics of Insect Flight: An Introduction to the Symposium

Lisa Treidel, Jon Harrison, Caroline Williams

The evolution of flight is recognized as a key adaptation explaining the unparalleled success and diversification of insects. While there was a strong tradition of integrative studies in insect flight during the 1970s-2000s, the field has recently fragmented leaving the ecological and evolutionary aspects of flight poorly integrated with mechanistic and biomechanical perspectives. This symposium will bring together researchers working on the evolution, ecology, physiology and biomechanics of flight to stimulate integration of these fields to answer questions about how and why flight arose and is maintained in insects. By bringing together researchers from different disciplines who share an interest in insect flight, an integrative and interdisciplinary community will be built that will enhance our ability to overcome obstacles and generate new research directions in integrative biology.

How do dispersal strategies evolve? Uncovering the genetic basis of flight polymorphisms

Lisa Treidel, Caroline Williams, Colin Meiklejohn, Kristi Montooth

The genetic basis of complex multivariate traits, such as dispersal strategies, remains largely unknown, hindering our ability to predict their evolution. In wing dimorphic *Gryllus* field crickets, flight-capable long-winged (LW) and flightless short-winged (SW) morphs express alternate dispersal and life history strategies, making them a powerful model system for studying complex multivariate traits. Morph determination is hypothesized to be controlled by an underlying quantitative trait, liability, and a threshold. When liability exceeds a threshold, wing and flight muscle development are inhibited, resulting in flightless SW morphs. The control of multiple flight related traits by liability could explain their coordinated evolution. We tested this hypothesis by setting up parental crosses (LWxLW; SWxSW; LWxSW; SWxLW) of *Gryllus lineaticeps* and assessing offspring morph frequencies and flight muscle maintenance. As predicted for a heritable trait, the frequency of LW offspring was significantly less in SWxSW relative to LWxLW families, and we observed a positive correlation between the frequency of LW offspring and the maintenance of flight muscles across families. Morph frequencies among females were more LW-biased compared to males, but males were more likely to maintain their flight muscles in early adulthood than were females. This suggests multiple and different thresholds for the traits between sexes. Together, our results are consistent with the threshold trait model and provide novel support for a common genetic basis of flight related traits.

Are Neuroendocrine Mechanisms Related with Migratory Preparation in Red-winged Blackbirds?

Alexis Trester, Page Klug, Jeffery Kittilson, Timothy Greives

Long-distance bird migration is observed across the globe. This migration requires extensive lipid storage to fuel such an energy-intensive event. However, little is known about the specific neuroendocrine mechanisms inducing hyperphagic behaviors in preparation for migratory flight. Expression of orexigenic and anorexigenic genes within the hypothalamus prior to this major life event are likely to play a key role in regulating these changes. Here we will examine changes in two orexigenic peptides, Ghrelin receptor (GHS-R) and neuropeptide Y (NPY), and two anorexigenic peptides, leptin receptor (LEPR) and cocaine-and amphetamine-regulated transcript (CART). We collected brains from red-winged blackbirds (*Agelaius phoeniceus*) from the late summer (September) to the end of flocking in the autumn (early November). Stable hydrogen isotopes (dH) of feathers were analyzed to estimate latitude of origin, indicating if the bird is native or stopping-over. RNA was extracted from hypothalamic tissue punches and expression of the four aforementioned genes were quantified using qPCR. We hypothesize that expression of orexigenic factors will be significantly higher and expression of anorexigenic factors will be significantly lower in individuals caught in late fall and during stop-over to aid in increased lipid storage prior to long-distance flight. Our findings will contribute to current knowledge on the physiological changes that birds undergo to dramatically change feeding behavior prior to long-distance migration.

Non-Consumptive Effects of *Panulirus argus* and *Panulirus guttatus* on Coral Reef Grazers

Alexis Trevillian, Jason Spadaro

Caribbean king crabs, *Maguimithrax spinosissimus*, has been proposed as a solution for combating algae overgrowth on coral reefs along the Florida Keys. This species is a known grazer which consumes large quantities of algae. *Panulirus argus*, the Caribbean spiny lobster and *Panulirus guttatus*, the Caribbean spotted spiny lobster are both known predators to coral reef grazers including *Diadema antillarum* and *Maguimithrax spinosissimus*. In this study, we aimed to observe the relationship between these predator species and coral reef grazers. Using Y mazes where two chemical cues (control vs control, control vs *P. argus*, control vs *P. guttatus* and *P. argus* vs *P. guttatus*) were randomly pumped in we were able to test the various experimental treatments. Each Y maze was divided into three sections: A header, B header and neutral zone. Grazer movements were recorded over a span of twenty-four hours for analysis. Videos were used to calculate time spent in each section. Our results showed that *P. argus* was preferred over *P. guttatus* in the two predator treatments. We also saw that *D. antillarum* showed a much stronger response against *P. guttatus* compared to *M. spinosissimus*. Additional trials need to be completed to produce a larger sample size. If similar results are seen, this research could lead to considering *P. guttatus* a negative keystone species and require new management solutions for these predators.

Spontaneous Magnetic Orientation in Poison Frogs (*Dendrobates auratus*)

Abigail Trocinski, Alayna Mackiewicz, Kenneth Lohmann, Sabrina Burmeister

Diverse animals, including amphibians, are capable of orienting to Earth's magnetic field. Green and black poison frogs (*Dendrobates auratus*) exhibit intensive parental care that depends on a strong cognitive map and excellent short-distance navigational abilities. We investigated whether *D. auratus* possess magnetic sensitivity using a spontaneous orientation assay. Frogs were tested in an arena surrounded by a magnetic coil system that could be used to control the ambient magnetic field. Prior to experiments, frogs were randomly assigned to one of four groups. Each frog was placed singly into the arena where it experienced a magnetic field in which north was positioned either in geographic north, east, south, or west ($n=8$ frogs/group). Each frog experienced just one of these conditions and was tested 10 times, after which a mean orientation angle for the frog was calculated. Analyses indicated that, as a group, frogs exhibited spontaneous bimodal orientation along the magnetic northwest-southeast axis, implying that *D. auratus* can sense Earth's magnetic field and use it as a directional cue. These results indicate that *D. auratus* may provide a useful model system for future studies of magnetoreception, particularly for unraveling the neural basis of the magnetic sense.

Developmental scaling of furca morphology affects ultrafast jump kinematics in springtails

Thu Truong

Springtails (Collembola) are microscopic, non-insect hexapods with a remarkable ability to jump up to twenty times their body height in the span of milliseconds. They do this by utilizing their furca, a jumping appendage attached ventrally to their abdomen, to launch themselves off of a substrate to evade potential dangers. The furca is actuated using a latch-mediated spring actuated (LaMSA) mechanism that rapidly releases stored elastic energy to instantaneously propel the "mass" - the furca. Though LaMSA mechanisms have been studied in other organisms, little is known about the development of LaMSA in springtails throughout its lifespan and how factors such as age and body size affect their ultrafast jumps. In this study, we focus our analyses on a springtail's stages of life and aspects of their jump mobility such as height and angular velocity at takeoff. We utilize microscopic imaging and high-speed video to investigate a terrestrial springtail, *Homidia sauteri*. We observe how developmental differences in body and furca size affect jump dynamics. Results show that adult *H. sauteri* springtails with more considerable furca lengths tend to jump more parallel to the substrate compared to juveniles, and have increasingly higher velocities and takeoff accelerations.

These findings offer valuable insights on unknown facets of LaMSA mechanisms, and can help further applied research in the fields of physics, robotics, and other biotechnologies.

Developmental scaling of furca morphology affects ultrafast jump kinematics in springtails

Thu Truong, Jacob Harrison, Payton Bush, Saad Bhamla

Springtails (Collembola) are microscopic, non-insect hexapods with a remarkable ability to jump up to twenty times their body height in the span of milliseconds. They do this by utilizing their furca, a jumping appendage attached ventrally to their abdomen, to launch themselves off of a substrate to evade potential dangers. The furca is actuated using a latch-mediated spring actuated (LaMSA) mechanism that rapidly releases stored elastic energy to instantaneously propel the "mass" - the furca. Though LaMSA mechanisms have been studied in other organisms, little is known about the development of LaMSA in springtails throughout its lifespan and how factors such as age and body size affect their ultrafast jumps. In this study, we focus our analyses on a springtail's stages of life and aspects of their jump mobility such as height and angular velocity at takeoff. We utilize microscopic imaging and high-speed video to investigate a terrestrial springtail, *Homidia sauteri*. We observe how developmental differences in body and furca size affect jump dynamics. Results show that adult *H. sauteri* springtails with more considerable furca lengths tend to jump more parallel to the substrate compared to juveniles, and have increasingly higher velocities and takeoff accelerations. These findings offer valuable insights on unknown facets of LaMSA mechanisms, and can help further applied research in the fields of physics, robotics, and other biotechnologies.

Mammal Mandible Madness: A switch in jaw form–function coupling during the evolution of mammals

Jack Tseng, Sergio Garcia-Lara, John Flynn, Emily Holmes, Timothy Rowe, Blake Dickson

The evolutionary shift from a single-element ear, multi-element jaw to a multi-element ear, single-element jaw during the transition to crown mammals marks one of the most dramatic structural transformations in vertebrates. Research on this transformation has focused on mammalian middle-ear evolution, but a mandible comprising only the dentary is equally

emblematic of this evolutionary radiation. We show that the remarkably diverse jaw shapes of crown mammals are coupled with surprisingly stereotyped jaw stiffness. This strength-based morphofunctional regime allowed mammalian jaws to effectively resist deformation as they radiated into highly disparate forms with markedly distinct diets. The main functional consequences for the mandible of decoupling hearing and mastication were a trade-off between higher jaw stiffness versus decreased mechanical efficiency and speed compared with non-mammals. This fundamental and consequential shift in jaw form–function underpins the ecological and taxonomic diversification of crown mammals.

Reproduction in the three-banded panther worm *Hofstenia miamia*

Libby Tseng, Vikram Chandra, Marcela Bolaños, Mansi Srivastava

Phylum Xenacoelomorpha is a deep-diverging bilaterian lineage of aquatic worms. Although reproductive anatomy has been described in several species, we know little about the physiological and behavioral mechanisms of reproduction in these animals. Here, we study reproduction in the three-banded panther worm *Hofstenia miamia*, a lab-tractable hermaphroditic acoel worm. We determined the anatomy and developmental timeline of *Hofstenia*'s reproductive systems. We found that fertilized eggs are located in a single cluster along the ventral midline, immediately posterior to the male testis, and anterior to two lateral ovaries containing follicle-like structures that run down the posterior half of the worm. To capture egg-laying and mating, we then developed methods for behavioral capture in high resolution. We found that worms load individual eggs into their pharynx and then lay them through their mouths, demonstrating that some acoels perform oral oviposition. Moreover,

Hofstena appear to make active decisions in selecting suitable oviposition sites. Next, we found that Hofstena mate through bouts of 'penis fencing' involving hypodermic insemination. Although mating bouts have variable structure and dynamics, they typically begin with two worms making physical contact, circling around each other, and they terminate in an immobilizing bite during which one worm deposits sperm within the body of the other. We are currently studying the quantitative dynamics of mating to understand the behavioral rules worms employ during this complex behavior.

Hanging on Duckweed Roots: Substrate Use and Cooperative Behavior in California Blackworms

Harry Tuazon, Saad Bhamla

California blackworms (*Lumbriculus variegatus*) are freshwater aquatic worms that thrive in low-oxygen benthic zones. When granular substrates are absent, these worms collectively converge and adhere, forming cohesive aggregates known as "blobs." Consequently, these aggregates trigger the onset of anoxia due to high population density. In natural settings with water surfaces covered by algae, duckweed, or debris, blackworms adopt a unique strategy. They climb the roots of floating duckweed and entangle underneath the rhizosphere, using the floating plants for mobility and nourishment. Our research shows that the worms skillfully manipulate these floating roots to move, taking advantage of their vertical hanging position. We hypothesized that this position allows for more efficient foraging and speeds up collective locomotion due to the reduced friction. This behavior not only emphasizes their complex social interactions but also creates protective floating structures. We also show that this strategy provides defense against predators, especially carnivorous freshwater leeches in the mud. The worms' use of roots for adaptive locomotion and cooperative behavior expands the range of tactics organisms use to navigate and migrate in their ecosystems. This inventive root use demonstrates that even simpler creatures can adapt and benefit from their environment in novel ways.

Latch and Float: Unveiling Collective Respiration Survival Strategies of California Blackworms

Harry Tuazon, Emily Kaufman, Vishal Patil, Tuhin Chakraborty, Saad Bhamla

California blackworms (*L. variegatus*) are aquatic worms found in freshwater benthic zones which can form floating collectives on the underside of the water interface. Due to thigmotaxis, they collaboratively form highly dense protective entangled collectives, which consequently induces anoxia (dissolved oxygen, DO, < 1 mg/L). While they respire through their skin, blackworms bolster respiration by elevating and waving their tails to increase DO intake. In shallow waters, tails bend at the air-water interface, enabling direct air oxygen absorption. In large collectives, worms craft floating entangled "buoys" by pinning their tails on the interface for support, attributed to exposed geometry which facilitates individual-scale interfacial latching. We hypothesize that the emergence of worm buoys fosters collective survival from predators such as carnivorous leeches by allowing worms to remain strongly entangled with one another without being oxygen limited. We use transfer entropy analysis to correlate DO and tail formation in respiration experiments to determine that DO concentration plays a significant role in initial buoy formation and during a complete buoy cycle in a bidirectional manner. Furthermore, we hypothesize that the mechanics of exposed tails, investigated through interfacial fluid dynamics modeling, can support more than individual worm body weight through its external morphological geometry. In our internal topological analysis of the buoy dynamics, we employ 3D simulations to reveal the intricate relationship between entanglement and vertical movement. Using Kirchhoff elastic filament modeling, we found that pinned worms within the interface core rise due to increasing tangle complexity, effectively showcasing a topological lift mechanism.

Developing an open-source workflow for analyzing morphological traits in bones

Mallory Tucker, Sofia Piggott, Matthew Fuxjager

Analyzing variable, non-circular osteological shape can be difficult or even inaccurate when using analytical programs based on perfect circles. Additionally, measuring hollow bones can pose issues when trying to quantify bone solidness because programs need to accurately differentiate between what is or is not bone. Many capable software programs currently available are proprietary, which can limit access to these analyses and sometimes hinder experimental repeatability. We developed a workflow using only free, open-source software programs that directly address these issues and reduce human error during multi-person data collection. We focused on building a workflow that prepares and helps carry out morphological and biomechanical bone analyses, using a combination of μ CT data, SegmentGeometry in 3DSlicer, and BoneJ in Fiji. Our main improvements were done in Fiji, where we focused on bettering the shape recognition and thresholding of the bone samples. We reworked the ROI procedure to provide a more exact outline of the bones and improve noise reduction. We also integrated an additional tool that allowed us to measure the total bone solidness and calculate ossified bone solidness, which works well with hollow bones. Overall, this workflow provides an open-source, accessible way to analyze bones of varying shapes, while simultaneously decreasing human error through the integration of more automated methods.

Thermal refugia and persistence of Texas horned lizards (*Phrynosoma cornutum*) in small towns

Mary Tucker, Daniella Biffi, Dean Williams

Texas horned lizards (*Phrynosoma cornutum*) have disappeared from many areas in Texas, especially from urbanized areas, probably in large part due to loss of suitable habitat. Our previous studies have found that horned lizards persist and occur at high densities in some small towns in southern Texas. Nevertheless, this species has continued to decline and disappear from these towns. Long-term data from Kenedy and Karnes City indicate that when study sites experienced significant shrub and vegetation removal horned lizards declined by 79%. We hypothesize this may in part be due to the degradation of the thermal landscape for these lizards. We determined the preferred temperature range (Tset25 – Tset75) of lizards at our study sites and took field measurements of body temperature (Tb). Temperature loggers were also placed in three microhabitats across our study sites. Shrubs and vegetation provided the highest quality thermal environment, especially for about 5 hours midday when temperatures in the open and buried under the surface exceeded the lizards' critical maximum temperature (CTmax) or were above their preferred temperature range. Horned lizard density was positively related to the thermal quality of the habitat across our sites. Texas horned lizards in these towns require a heterogeneous mix of closely spaced microhabitats and especially thermal refugia, such as shrubs and vegetation along fence lines and in open fields. Maintaining thermal refugia is one of the most important and practical conservation actions that can be taken to help small ectotherms persist in human modified landscapes and cope with increasing temperatures due to climate change.

Extreme Astrocytic Turnover during Transition into non-breeding conditions in the Songbird

Will Tucker, Susanna Shepard, John Boyd, Elizabeth Scalzi, Kathryn Chung, Tracy Larson

Reactive proliferation – or the birth of new cells following cellular death – was discovered to occur in the region of the brain controlling singing behavior, called HVC (proper name), as HVC of Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelli*) degenerated upon transition into physiological non-breeding conditions. The surprising discovery of reactive proliferation has prompted questions on the relative contribution of types of cell death, death of differing cell types (neurons versus glia) towards the phenomenon, and the function of newly born cells. To address these questions, we characterized the astrocyte number in HVC of breeding and nonbreeding sparrows. We find that astrocytes change in number between seasons, although this

difference is temporary during transitions and return to similar homeostatic numbers during stable breeding and nonbreeding conditions. We also deployed BrdU pulse-chase methods along with lineage specific markers to assess cell identity of seasonally-induced reactive proliferation, and find high levels of new astrocytes in the adult brain. Many, but not all, of these newly-born astrocytes incorporate into HVC, suggesting that astrocytes exhibit a large turnover event following seasonal neuronal loss. These new astrocytes might participate in the return to short day HVC cellular homeostasis following withdrawal of breeding stimuli changes to the song circuit and behavior. Characterization of astrocyte dynamics will allow for inquiry into the broader role of astrocytes in maintaining homeostasis in neural and behavioral plasticity.

Assessing the health of an impaired urban stream: Collaboration by a college and local government

Karen Tuerk, Erika Iyengar

Assessing and remediating the health of streams, especially ones that traverse multiple municipalities, is a complicated process. This process is rendered more complicated by the variability of different reaches of the stream as well as of the interests and capabilities among the human parties involved. Little Cedar Creek, in Allentown, Pennsylvania, is designated by the Department of Environmental Protection (DEP) as impaired by excessive sediment loads. This small waterway travels through at least three municipalities, becoming subterranean for at least one section that borders a golf course, before re-emerging and joining Cedar Creek, classified by the DEP as a trout stream. We report the first quantitative study of the macroinvertebrate diversity, sediment, velocity, and chemical parameters of Little Cedar Creek. The intention is for a long-term monitoring project to assist in suggesting remediation efforts and assessing their efficacy. Our baseline-sampling results will be reported here, as well as proposed next steps. Challenges will be discussed, including extremely high variability within even proximate sections of the stream, impacts of extreme flooding, sections dominated by taxa more typical of lentic ecosystems, and externally-determined sampling methodology that might be unwieldy or statistically problematic in urban reaches of streams.

Quantifying sexual size dimorphism in a common marsh treader, *Hydrometra martini*

Jacob Tupper, Sam Miess, Victoria Roper, Andy Dzialowski

Sexual dimorphism in body size and morphological features is common among insect species. For hemipterans, females are usually bigger than males; size is frequently associated with reproductive success. Other morphological features, such as leg shape, eye size, and antennal characteristics, additionally vary between insects of different sexes. Quantifying sexual dimorphism has been done in a variety of common species; however, this is not the case for the common marsh treader (*Hydrometra martini*). To investigate dimorphism in *H. martini*, 60 specimens of (35 females, 25 males) were obtained at Sanborn Lake (Stillwater, Oklahoma) in July 2023. 22 morphological features were measured for each individual, and an additional 14 morphological characteristics were calculated from these measurements. Morphological measurements were performed using a Linitron® Z850 dissection microscope, with a resolution of 0.01mm (10 μ m). Welch's t-tests were run to compare measurements between the two sexes. Of the 36 morphological features that were measured or calculated, 12 were significantly different between males and females ($p < 0.05$), including abdomen length ($t = -6.49$, $p < 0.001$), eye size corrected for head width ($t = 4.44$, $p < 0.001$), and anal spine ($t = 10.70$, $p < 0.001$). Additionally, permutational multivariate analysis of variance (PERMANOVA) based on the 22 measured features revealed a significant difference between the males' and females' morphology ($nperm = 999$, $F = 10.7$, $p = 0.002$).

Assessing fertility rates of leatherback sea turtle (*Dermochelys coriacea*) eggs

Emily Turla, Gabriella Carvajal, Samantha Kuschke, Jeanette Wyneken

Leatherback sea turtle (*Dermochelys coriacea*) nests have highly variable hatching success that trends lower than that of other sea turtle species. Many

eggs fail and show no signs of embryonic development when necropsied. To understand this egg failure, it is fundamental to identify whether eggs with no signs of development are infertile or have very early-stage embryos that die before signs of development become apparent during necropsy. To investigate the rates of infertility versus early-stage death, 300 freshly oviposited leatherback eggs were collected from 10 nests and incubated ex situ in the lab. These eggs were closely monitored throughout incubation, and if egg chalking (a sign of development resuming after diapause) did not occur, eggs were necropsied. Perivitelline membranes of necropsied eggs were collected and analyzed for the presence of sperm or embryonic cells using fluorescent microscopy protocols that have been used successfully in avian and other reptile fertility studies. These techniques previously were not confirmed to work in sea turtles. This study positively identified the presence of sperm or embryonic cells in the perivitelline membranes of all 8 unchalked eggs, with the other 292 eggs either resulting in successful hatching or in dead embryos. Our results are the first to demonstrate high fertility in leatherback sea turtle nests of Southeastern Florida.

Protamine-like Molecules Compact Sperm DNA in the Annelids, *Lumbriculus* and *Lumbricus terrestris*

Kay Tweeten

The annelids, *Lumbriculus* and *Lumbricus terrestris* were analyzed for basic proteins to gain insights into the biochemical properties of sperm DNA-binding proteins from these invertebrates. Basic proteins were extracted from sperm and from reproductive segments of sexually reproducing worms by hydrochloric acid extraction. Solubilized proteins were analyzed by acetic acid-urea gel electrophoresis in comparison to vertebrate proteins including histones from calf thymus and protamine from herring. Highly basic, low molecular weight proteins were present in the reproductive segments and sperm of sexually reproducing *Lumbriculus* and *L. terrestris*. These proteins, which were not observed in segments from worms that were not sexually reproducing, were more basic than histones, suggesting they were protamine-like. Further evidence for protamine-like properties for these proteins was obtained by staining of cells in various stages of spermatogenesis with chromomycin A3. The ability of this fluorescent probe to bind to DNA decreases as histones are displaced by protamines during spermatogenesis. In comparison to spermatogonia and primary spermatocytes, mature sperm from *Lumbriculus* and *L. terrestris* showed little staining by CMA3. Decondensation of sperm DNA by treatment with heparin and dithiothreitol resulted in binding of CMA3 to the sperm DNA. These results suggest that the compaction of DNA during sperm formation in *Lumbriculus* and *L. terrestris* is similar to that of many vertebrates, with histones being replaced by very basic, protamine-like molecules.

Thermal Acclimation as an Emergent Trait in Eusocial Bumblebees

Tamara Tyner, Eric Riddell

Global bumblebee declines are causing detrimental effects on ecological and economical scales from the loss of pollinators. There are several factors that could underlie these declines, including changing environmental conditions that challenge the ability of bumblebees to regulate nest temperature and reproductive output. A typical response to environmental variation is acclimation, which allows organisms to reversibly adjust traits in response to changing conditions. Current studies have demonstrated that bumblebees lack that ability to acclimate, but these studies have only investigated acclimation in individuals. To gain a greater understanding of these eusocial organisms, we studied thermal acclimation in commercially reared *Bombus impatiens* nests. We measured metabolic rate, water loss rate, nest temperature, and behavior in an initial temperature ramping experiment (acute temperatures of 10°, 20°, 25°, 30°, and 35°C). After twenty-one days in one of three acclimation treatments (10°, 20°, and 30°C), we measured these same traits in a final ramping experiment. We found evidence for acclimation in nest temperature, metabolic rate, and water loss rate where the degree of acclimation depended on the acclimation treatment and the acute temperature treatment. Individuals also consistently participated in incubating behavior in colder temperatures and fanning behavior in warmer treatments during ramping. Despite the lack of

acclimation in individual bumblebees, our research highlights the importance of incorporating emergent traits of eusocial organisms when predicting the effects of environmental variation.

Artificial Light at Night and Parasite Loads in the Dark-eyed Junco (*Junco hyemalis*)

Ariel Tysver, Samantha Diedrich, Sarah Wanamaker, Ellen Ketterson

As the human population continues to grow and urbanization increases, exposure to artificial light at night (ALAN) will continue to increase for many wildlife species. This poses a large challenge to many species, including songbirds, that mediate various physiological processes and behaviors based on the light cycle. Disruption of these body processes can have consequences for the health, behavior, and fitness of an organism. Research has established that exposure to ALAN decreases melatonin production in songbirds, which has broader implications for immune function and regulation, and may also alter the gut microbiome of these birds. Given these significant effects, it is likely that prolonged ALAN exposure may also alter other aspects, such as the gastrointestinal parasite community in these birds. We exposed a subset of our birds to constant dim ALAN for 7 weeks, while others experienced a natural photoperiod, and quantified the amount of coccidia, a common gastrointestinal parasite found in these birds, throughout the experiment to test whether exposure to ALAN alters the population of these parasites within their host. Results will point toward the relationship between coccidia load in the gastrointestinal tract and exposure to ALAN, with potential implications for the health of songbirds with increasing urbanization.

Comparing the role of body wavelength in anguilliform and carangiform swimming performance

Eric Tytell, Erik Anderson, Martha Sutter, Yordano Jimenez, Alexandros Anastasiadis, Auke Ijspeert, Karen Mulleners

Although swimming fish are often thought to maintain a constant body wavelength, the wavelength can vary substantially. In an eel-like robotic swimmer, body wavelength has a strong effect on swimming speed and energy consumption, with maximal swimming speeds occurring at higher wavelengths and minimum energy consumption occurring at shorter wavelengths. Swimming eels display a similar pattern of performance, but it is not known whether carangiform swimmers show the same relationship between body wavelength and swimming speed, particularly since the wave amplitude on the anterior body is very low. Here, we present data on the swimming kinematics of 10 scup, using an automated system that digitized very large numbers of tailbeats at a large number of discrete speeds (60 per fish, on average). We examine the correlation between body wavelength, the body amplitude envelope, and swimming speed.

Experimental glucocorticoid elevation alters activity and dietary choices in a wild songbird

Jennifer Uehling, Conor Taff, Jennifer Houtz, Monique Pipkin, Paige Becker, Allison Injaian, David Winkler, Richard Gabrielson, Maren Vitousek

Foraging behavior can be drastically altered by challenges. Challenges such as inclement weather can change foraging directly by affecting what diet items are available. However, challenges can also have indirect effects on foraging behavior via glucocorticoids, hormones that rise in response to challenges. Although captive studies confirm that glucocorticoids causally affect activity, findings in free-living animals do not show a clear consensus on how these hormones drive foraging activity, and whether differences in glucocorticoid levels are related to dietary content. Here, in wild adult tree swallows (*Tachycineta bicolor*), we investigated whether glucocorticoids predict two metrics of activity (duration of time active and offspring feeding rate); and, whether shifts in activity also corresponded with shifts in bird diet. To do so, we experimentally elevated glucocorticoid secretion in incubating females, measured females' activity patterns using solar-powered radio tags ("LifeTags") and PIT tags, and measured bird diets with DNA metabarcoding. By mid-provisioning, birds that were induced to upregulate glucocorticoid secretion had higher durations of time active and higher provisioning rates than

control birds. During this period, adult females with experimentally elevated glucocorticoids consumed lower percentages of macronutrient-rich aquatic insects, which are important for tree swallow health. These results suggest the endocrine mediators of the stress response – even in the absence of an actual challenge – have the potential to change activity and foraging patterns, with possible fitness consequences.

Effects of cultural eutrophication on the mating biology of amphipods

Rachel Uhlig, Joseph Alcuatas, Rickey Cothran

We are assessing patterns of sexual selection in amphipods in the genus *Hyalella* across a gradient of land use in NW Pennsylvania lakes. These natural lakes have varying nutrient levels that are likely due to differences in agricultural land use in each watershed. We will present current results of patterns of sexual selection across the nutrient gradient. Sexually selected traits are expensive to build and maintain so changes in nutrient availability is expected to strongly affect the development of these traits. We hypothesized that increased nutrient loading would make it easier for poor quality males to build sexually selected traits compromising their use as measures of male quality. Moreover, one of the focal traits is a weapon that is used both in competition with other males and to force females to mate. If low quality males are able to build large weapons, the total number of well-armed males faced by females will increase forcing females to devote more time and energy in deflecting mating attempts. Ultimately both of these mechanisms, eroding the information quality of traits and increasing weapon size, are expected to result in decreased female fitness and, as a result, poor population health.

Putative roles for sensory hairs on dragonfly wings

Myriam Uhrhan, Richard Bompfrey, Huai-Ti Lin

Insect wings undergo continuous deformations caused by the interplay of aerodynamic, inertial, and elastic forces in flight. We have previously shown that dragonflies possess a high number and diversity of wing sensors suitable for proprioceptive, strain, and localized airflow sensing. Here, we focus on the sensory hairs across the wing blades and explore how their position and morphology may relate to their roles in measuring angle of attack, flow stagnation, or local flow direction. Based on detailed anatomy from several imaging techniques, we modelled the flow around the sensors using three-dimensional computational fluid dynamic simulations to identify perceptible environmental stimuli and small-scale fluid-structure interactions. Firstly, the wing angle of attack can be measured from a class of sensory hairs on the ridges of the corrugated wing. Our simulations show that the combinatorial activation of these hairs can represent an angle of attack range critical for gliding flight. Secondly, two rows of sensory hairs at the leading edge of the wing experience very drastic changes of flow direction and can provide an indication for the stagnation point. Finally, there is a class of sensor, termed the bristle bump complex (BBC), found, uniquely to date, on dragonfly wings. Our simulations reveal how the BBC's biomechanical properties confer directional selectivity favouring chordwise flow pattern that may be an essential parameter to monitor in dragonfly flight.

Sugar feeding by invasive mosquito species on ornamental and wild plants

Irving Upshur, Mikhyle Fehlman, Vansh Parikh, Clement Vinauger, Chloe Lahondere

Feeding on plant-derived sugars is an essential component of mosquito biology that affects key aspects of their lives such as survival, metabolism, and reproduction. Mosquitoes locate plants to feed on using olfactory and visual cues. *Aedes aegypti* and *Aedes albopictus* are two invasive mosquito species to the US, and are vectors of diseases such as dengue fever, chikungunya, and Zika. These species live in heavily populated, urban areas, where they have a high accessibility to human hosts as well as to plants in backyards and town landscapes. Therefore, it is important to understand what plants may attract or repel mosquitoes to inform citizens and municipal authorities accordingly. Here, we analyzed *Ae. aegypti* and *Ae. albopictus* sugar-feeding behavior with eleven different commonly planted ornamental plant species.

We then assessed feeding activity using the anthrone method and identified volatile composition of plant headspace using gas-chromatography mass-spectroscopy. Finally, we determined the sugar-feeding activity of field caught mosquitoes using the plant DNA barcoding technique and compared these results with the eleven ornamental species tested in the laboratory. The potential for application to disease vector control is also discussed.

The collisional geometry of economical walking predicts human leg and foot segment proportions

Jim Usherwood

In human walking, the body rises and falls over a stance leg before a smooth step-to-step transition into the next vaulting stance. The transition results from joints effectively locking and unlocking in rapid sequence due to simple geometric changes loading and unloading a series of muscles. Remarkably, the geometry that makes the transition smoothest – and so theoretically most economical – predicts human leg and foot proportions. This may account for why modern humans have a knee half-way down their legs, short heel and toes, and a stiff, longer midfoot. And why a comfortable step is 2-3 feet long.

Human walking appears complicated, with many muscles and joints performing rapidly varying roles over the stride. However, the function of walking is simple: to support body weight as it translates economically. Here, a scenario is proposed for the sequence of joint and muscle actions that achieves this function, with the timing of muscle loading and unloading driven by simple changes in geometry over stance. In the scenario, joints of the legs and feet are sequentially locked, resulting in a vaulting stance phase and three or five rapid 'mini-vaults' over a series of 'virtual legs' during the step-to-step transition. Collision mechanics indicate that the mechanical work demand is minimised if the changes in the centre of mass trajectory over the step-to-step transition are evenly spaced, predicting an even spacing of the virtual legs. The scenario provides a simple account for the work-minimising mechanisms of joints and muscles in walking, and collision geometry allows leg and foot proportions to be predicted, accounting for the location of the knee halfway down the leg, and the relatively stiff, plantigrade, asymmetric, short-toed human foot.

Legs as linkages and lollipop sticks

Jim Usherwood

Animal legs appear very complicated, with many muscles and joints performing rapidly varying roles over the stride. However, the function of walking, running, trotting and galloping is simple: to support body weight as it translates economically. While extensive attention has been paid to the elastic role of tissues – particularly tendons, but also muscles – in helping by returning energy lost with a spring-like recoil, recent work focusses on the mechanisms by which mechanical work is avoided in the first place. Much of the complexity and function of animal legs can be understood by considering the work-avoiding mechanisms of a range of simple linkages.

This poster presents a suite of mechanical demonstrators of the principles at play in animal and human legs. Designs include those that can be constructed from easily sourced materials (lollipop sticks, card, split pins) and others with laser-cut parts of negligible unit cost. Hands-on examples will be available. Give-away models will relate to human walking, and the talk (and paper) 'The collisional geometry of economical walking predicts human leg and foot segment proportions'. Current designs are available at: jimusherwoodresearch.com/toys

The tripedal gaits of tripawds

Jim Usherwood, Zoë Self-Davies

The gaits of bipedal and quadrupedal animals are largely well-characterised while, with no true, naturally occurring, tripedal gaits, very little is understood regarding locomotion on three legs. There is a population of domestic tripedal mammals in the form of canine amputees which provides a unique perturbation into the study of locomotion. This study assesses whether these individuals use distinct limb sequences, with clearly defined kinematics and kinetics, which could be determined as discrete 'gaits'. Here we present kinematic and

kinetic data for a group of hind- (N=6) and fore-limb (N=6) canine amputees across a range of speeds. At higher speeds, three-legged dogs use a three-beat gallop-like gait. Forelimb amputees use a rotary-like gallop whereby the hindlimb ipsilateral to the remaining fore is always the 'leading' limb. Hindlimb amputees use both rotary- and transverse-like sequences, varying the lead within the forelimb pair. At low speeds, some individuals show a walk-like gait where the remaining pair of limbs has an approximately 50% phasing, and paired-limb stride period increases with decreased speed. However, the single limb maintains a consistent period across speeds because a long unsupported period would demand excessively high-amplitude 'bounces'. This difference in cycle period for single and double limbs results in no consistent phasing between fore- and hind-limbs, breaking some definitions of a 'gait'. Further, there are no discernible discontinuities in the force-speed relationship that would indicate discrete gait transitions. However, the study reveals the mechanisms underlying the step timing of tripedal dogs at low speeds, and why their gaits have not been simply classified with classical methods.

A multiomic approach to understand tradeoffs between symbiosis and immunity in a reef-building coral

Maria Valadez-Ingersoll, Hanny Rivera, JK Da-Anoy, Thomas Gilmore, Sarah Davies

Understanding molecular mechanisms governing the coral-algal symbiosis will likely be key to successful coral protection and restoration efforts. While ongoing research has highlighted potential tradeoffs between symbiotic state and whole-cnidarian immunity, differential regulation of immune pathways among different cell types and symbiotic states is largely unknown. Here, we leverage single-cell RNA-seq to develop an atlas of cell-type composition and gene expression across symbiotic states in the facultative coral *Oculina arbuscula*. Our atlas reveals 20 cell clusters with representation across both symbiotic states. While a putative immune cell cluster displayed no strong differences in regulation of immune pathways between symbiotic states, gastrodermal cell clusters displayed signatures of immunosuppression in symbiotic as compared to aposymbiotic samples. This compartmentalization of immune system regulation may enable the coral to host algal symbionts within gastrodermal cells while still maintaining general organismal immunity via normal immune cell function. Paired with physiological assays and proteomic analysis to further elucidate functional differences between symbiotic states, this work provides a comprehensive analysis of the cellular and molecular characteristics of the coral-algal symbiosis, laying the groundwork for future studies to investigate how these mechanisms are modulated in corals under stress.

A Canary in a Coal Mine - How Symbiotic Bacteria Serve as a Proxy of Host Fitness Under Heat Stress

Brent Zeyus Valdez, Malcolm Thieme, Andres Hobbs, Michele Nishiguchi

Symbiotic associations are found in all organisms on Earth, where microbes contribute to the overall fitness of the relationship. These associations are stable, yet recent artificial warming has jeopardized the survival of these organisms. The beneficial association between bobtail squids (Cephalopoda: Sepiolidae) and their *Vibrio* bacterial symbionts is an ideal model that can be used as a yardstick to understand how abiotic factors influence host-microbe associations. Here, we examined the impacts of temperature on symbiotic competency. *Vibrio* isolates from bobtail squids harvested from Hawaii (ES114), Australia (ETBB1-C), the Philippines (EAS005), and Japan (EM17) were adapted under three test conditions: heat stress (32°C), cold stress (18°C), and a neutral temperature (25°C). All strains were experimentally evolved over the course of 1000 generations. Symbiotic characteristics such as growth rate, bioluminescence, motility, and biofilm formation were measured throughout the study. We observed that bioluminescence under heat stress decreases during selection, whereas, cold stress enhances bioluminescence across all strains. Faster growth rates were also characteristic at 32°C compared to their ancestral strains. Our results indicate that strains evolved at higher temperatures are able to combat severe stress, but convey less fitness to their host's ability

to counterilluminate. Thus, disentangling the trade-offs due to increasing temperature will give a broader perspective on the ability of symbioses to adapt to changing climate conditions.

Investigating the Regulation of Life-cycle Metamorphosis in the Crystal Jelly, *Aequorea victoria*

Vannessa Valdez, Brian Tsukimura

Hydrozoans exhibit a wide diversity of developmental strategies where the classic medusa life cycle can be divided into three stages: planula larva, hydroid, and hydromedusa. The free-swimming planula larva settles and metamorphoses into a sessile and typically colonial polyp called a hydroid. The hydroid undergoes morphogenesis to develop asexual buds that are released as jellyfish termed hydromedusae. When sexually mature, hydromedusae broadcast spawn their gametes into the ocean to be fertilized and begin the life cycle once more. There is an extensive list of genes and biological pathways hypothesized to be responsible for the regulation of life-cycle metamorphoses in the Medusozoa clade of Cnidarians (Scyphozoa, Cubozoa, Staurozoa, & Hydrozoa). Ongoing research has yet to discover what is driving metamorphosis and whether it is universal across Medusozoa or species-specific. This project investigates which RNA transcripts are associated with life-cycle metamorphosis from a hydroid to a hydromedusa in *Aequorea victoria* using differential expression analysis. The goal is to stray from traditional approaches that seek to find novel homologs of known developmental genes. Overall findings point to rare transcripts that are significantly overexpressed in the hydroid stage being involved in the metamorphosis into a hydromedusa. These findings indicate that hydromedusa metamorphosis could involve a combination of both universal and species-specific transcripts. These findings also contribute to the genomic, transcriptomic, and developmental data available for Medusozoa.

Application of a genotoxic stressor to primary cell cultures derived from a marine tunicate.

Celeste Valdivia, Alison Gardell, Baruch Rinkevich, Dietmar Kueltz

Botryllus schlosseri is a common sessile, colonial ascidian species found globally in shallow, temperate, coastal zones. *B. schlosseri* undergoes weekly stem cell-mediated asexual reproduction, termed blastogenesis, and is a powerful model system in the fields of cell biology, immunology, and developmental biology. Previous research exploring *B. schlosseri* cell line development has focused on optimization of primary in vitro methods for hemocytes and bud-derived epithelial cells. However, despite decades of work, efforts have not yet yielded a continuous cell line for this species. Here we attempt to promote cellular immortalization through exposure of epithelial tissue of *B. schlosseri* to the clastogenic compound, nickel chloride, which generates oxidative damage and DNA strand breakage in aquatic invertebrates. Quantitative polymerase chain reaction (qPCR) will be used to investigate the effects of acute sublethal nickel chloride exposure on the expression of stress-, senescence-, and stemness-related genes in primary epithelial cell cultures from mature, field-sourced *B. schlosseri*. Specifically, we will measure transcription of mortalin (HSPA9), caspase-9 (CASP9), SRY-Box transcription factor (SOXB1), MYC proto-oncogene (MYC), and POU Class 3 (POU3). Cell viability will be evaluated post-exposure using propidium iodide staining. Additionally, a single cell gel electrophoresis DNA breakage assay will be used to determine the extent of clastogenic damage. Proliferative effects will be assessed through micrograph analysis of the epithelial monolayer area and doubling time. Funding provided by NSF MCB-2127517.

Regional variation in tendon fascicle properties

Miles Valencia, Emily Yamauchi, Viktor Gevirtzman, Manny Azizi

A tissue's ability to adapt to changing environmental conditions is partly dependent on its remodeling rate. Skeletal muscles completely remodel almost every few weeks, which is reflected in their ability to generate increasingly more force. However, tendinous tissue take a significantly longer time to remodel. Previous work has shown that the core region of the human Achilles tendons undergoes little remodeling, likely maintaining the same mechanical properties reached at skeletal maturity. These results predicts that remodeling

due to aging, exercise or pathology will result in regional variation in the mechanical properties of tendons. To investigate regional variation in tendons, we dissected fascicles from the core and peripheral regions of guinea fowl (*Numida meleagris* L.) flexor digitorum tendons. Tendon morphology was measured followed by mechanical tests including cyclic loading, stress-relaxation, and ramp-to-failure tests. Preliminary results suggest that core fascicles have lower stiffness but higher failure forces compared to peripheral fascicles. In addition, peripheral fascicles lost more energy during cyclic loading tests compared to core fascicles. Overall, preliminary results suggest that viscoelastic properties vary regionally within a tendon, and implies that such regional variation may alter the mechanical relationships between skeletal muscles and in series tendons.

How big were these old, dead frogs? Inferring mass and SVL for a Late Cretaceous community of frogs.

Maria Vallejo-Pareja, Maya Victor, Jonathan Bloch, David DeMar, Gregory Wilson-Mantilla, David Blackburn

The fossil record of frogs (crown-group Anura) extends for more than 150 million years. Yet, little is known about how anuran species and communities responded to past climatic or extinction events. Further, inferring biology based on fragmentary anuran fossils makes it challenging to evaluate traits that might respond to these events. Here, we focus on body size, a trait that can be inferred from incomplete fossils, because it is constrained by physiology and has implications for dispersal, diversification, and extinction. We investigate methods for estimating body length (snout-vent length, SVL) and mass from partial anuran skeletons and provide an example of applicability in the fossil record. We used measurements of ilia and SVL derived from CT-scans of >100 living species (in 16 families, ranging from 11–152 mm SVL) as well as field-collected mass and SVL data of >18K frogs worldwide. Using ordinary least square regression, we show that the ilium measurements provide useful estimates of SVL. The strong allometric relationship between mass and SVL allows us to then infer mass from the SVL estimated from measurements of the ilia. We apply this method to Late Cretaceous frog communities from the Hell Creek Formation, Montana, to characterize the distribution of body sizes immediately before the KPg Extinction.

Investigating quadrupedal gaits in the horse (*Equus ferus caballus*) using musculoskeletal models

Pasha van-Bijlert, Ineke Smit, Thomas Geijtenbeek, Anne Schulp, Karl Bates

Quadrupedalism is the most common form of terrestrial locomotion amongst vertebrates. Theoretically, infinite phase relationships are possible between fore- and hindlimbs. Nevertheless, gaits and muscle activation patterns fall into relatively few distinct categories, irrespective of body size. This is surprising, given the disparate morphologies seen in extant vertebrates, and size-dependent effects on skeletal stress. To deepen our understanding of equine gait selection, we have developed a musculoskeletal model of the horse (*Equus ferus caballus*) for use in predictive gait simulations. We constructed our model using 3D surface scans of a horse skeleton, combined with inertial and muscle properties from published literature. Simulated gaits were acquired using direct collocation (feedforward control).

The simplest version of the model (two joints per limb, one muscle per joint) is capable of a variety of gaits (walk, trot, pace), with ground reaction forces closely matching those observed in real horses. A more complex version of the model including scapulohoracic motion, three joints per limb (17 degrees of freedom total), and up to fifty muscles can also adopt walking gaits, although the gaits are more sensitive to the relative tunings of the multiarticular muscles. We are currently incorporating feedback-based control to our workflow. Ultimately, our model may serve as a template for a larger comparison on the effects of size on gait selection, and could potentially inform veterinary practice.

Modulation of the stretch feedback pathway in the heart of the American lobster, *Homarus americanus*

Karin van-Hassel, Madison Thies, Michelle Padilla-Soto, Grant Griesman, Eryn Dickinson, Xuan Qu, Patsy Dickinson, Daniel Powell

The cardiac ganglion (CG) is a central pattern generating neural network that produces the heartbeat in the American lobster, *Homarus americanus*. The CG has nine electrochemically coupled neurons: four premotor neurons that send signals to five motor neurons, causing bursts of action potentials from the motor neurons. Driver potentials (DPs) are slow and sustained calcium-based depolarizations that trigger these bursts of action potentials which in turn drive cardiac muscle contractions. Muscle contractions vary in strength based on the burst duration and burst frequency. We have established a generally excitatory feedback response to cardiac muscle stretch, mediated by stretch-sensitive dendrites. To investigate this feedback, we induce the stretch response by isolating the CG with the surrounding muscle and applying ramp-shaped stretches with three phases: the rising phase which causes a phase delay in the DP onset, the hold phase which increases DP frequency while decreasing DP duration, and the release phase which increases the DP duration. This process is modulated by neuropeptides that are known to act directly on both the CG and peripheral sites such as the neuromuscular junction and cardiac muscle. Here we show that the neuropeptides GYS and SGRN suppress the stretch response. In contrast, the neuropeptide myosuppressin decreases the DP frequency and modulates each phase of the stretch feedback pathway differently.

Quantification of three-dimensional architecture of axial muscle fibres in larval fish

Noraly van-Meer, Martin Lankheet, Johan van-Leeuwen

The axial muscle fibres of adult bony fish are arranged in complex helical trajectories. Anteriorly, fibres make relatively large angles to the medial and horizontal planes (azimuth and elevation, respectively). This may enable approximately uniform strains across the transverse plane, supporting similar specific power contributions from all fibres. In the tail, fibres run approximately parallel, stiffening the tail to transmit power towards the tail fin, rather than generating considerable power. In the anal region of larval fish, the adult helical pattern is initially absent and develops over several weeks. How muscle architecture develops along the body, however, remains unknown. To fill this gap, we made high-resolution 3D scans of 2-12 days post fertilisation (dpf) zebrafish larvae with fluorescent muscles. Variations in fluorescence level allowed segmentation and quantification of individual muscle fibres. Results of five 4-dpf larvae show that the helical arrangement of muscle fibers is already clearly present anteriorly, with elevation and azimuth angles ranging between -30 to 30 degrees. Similar to adult fish, the angles relative to the central axis decline posteriorly, to run nearly in parallel in the tail region. A comparison across different larval ages shows how fiber sizes and orientations vary with age, as well as over the body. This study lays the groundwork for understanding how larval fish rearrange their musculature during development, and how this influences their swimming performance.

Spectral and bi-modal learning in the yellow-fever mosquito, *Aedes aegypti*

Grace Van-Susteren, Jeff Riffell

Insects encounter, integrate, and process a variety of sensory stimuli in their environments. Multisensory cues are often more attractive to mosquitoes than single-modal cues, including during the localization of hosts and floral nectar. In addition to olfactory stimuli, spectral stimuli can play important roles in insect lives, including those of mosquitoes – food sources, hosts, and oviposition sites can be differentially colored, and mosquitoes display unlearned preferences for specific spectra. Mosquitoes exhibit learned avoidance towards host-associated olfactory cues after conditioning, but their ability to learn spectral and multi-modal cues has not been examined. Here, we use a two-choice T-maze and a small cage free-flight behavioral assay to examine mosquito's learned responses towards spectral and bi-modal cues, including host-related spectral cues, after an aversive classical conditioning paradigm. The conditioning paradigm consists of ten training trials where a sensory stimulus is

presented for 60 seconds, the second half of which is paired with an aversive, unconditioned stimulus consisting of a shaking motion mimicking host defensive behavior. Mosquitoes are then tested 24 hours later. Preliminary results demonstrate that *A. aegypti* can associatively learn 527 nm (green) stimuli but not 450 nm (blue) stimuli via aversive conditioning in contrast with the naïve controls that did not exhibit a preference between the spectra. These are the first results to show spectral learning in mosquitoes - subsequent experiments will include additional spectral stimuli and their combination with olfactory stimuli.

How different is suction kinematics in Malawi cichlid species that mainly feed on algae?

Sam Van-Wassenbergh, Jana De-Ridder, Vincent Dujardin, Julia Camacho-Garcia, Peter Aerts, Hannes Svardal

The evolution of cichlids includes the appearance of a wide range of specialized feeding methods stemming from a suction feeding strategy. Species eating large, mobile prey have found to display higher head expansion amplitudes, and are hypothesized to be more kinematically efficient. However, suction generation to efficiently transport detached algae calls for different optimisation criteria compared to capturing large, evasive prey. Knowledge of the three-dimensional kinematics and volumetrics of the head during algae suction can help us derive such criteria. We analysed three species of Malawi cichlids using simultaneous lateral and ventral-view videos of feeding on attached algae tablets, and on sinking pieces of shrimp: (1) *Rhamphochromis* sp. 'Chillingali', a piscivore with an elongated body and jaws, (2) *Chindongo saulosi*, an algae picker/nibbler with a small mouth, and (3) *Labeotropheus trewavasae*, an algae scraper with a broad, straight mouth and fleshy nose. We found a notable difference in the way the expansion sequence of the buccopharyngeal cavity was manifested, with the algae specialists not displaying the typical suction feeder's sequential anterior-to-posterior expansion and compression waves. Consequently, not only jaw action is modified in algae eaters, but also the kinematic pattern of head expansion. This suggests that trade-offs between powerful suction and efficient feeding on algae are important, and may explain why algae scraper's opportunistic switching to suction feeding can only be successful on easy prey.

From Tip to Tail: A Story of Armor Diversity

Megan Vandenberg, Olivia Hawkins, Eric Chier, Julia Cervone, Cassandra Donatelli, Adam Summers

Armor is a morphologically diverse multifunctional feature found across vertebrate taxa, evolving a score of times in both marine and freshwater fishes. Here we investigate how different armor morphologies impact drag reduction using poachers (*Agonidae*) as our model group. The family includes 47 species that sport full body armor from tip to tail, none have a swim bladder and the class is nested in the sculpins. Poacher armor morphology ranges from completely smooth flat plates, to grand protruding spines, to imbricate bumpy notches. In this work, we used micro-computed tomography (μ CT) scanning to describe and categorize the diversity of poacher armor. Using a character tree, we sorted each species into an armor morphotype based on the presence of a bump or a spine and the intricacy of the plate. We measured length, width, and height for individual plates as well as overlap percentage and armor-to-skeleton ratio and compared these measurements across species and morphotypes. From the μ CT scans we 3D printed solid models of 10 species, and used digital particle image velocimetry (DPIV) to visualize flow patterns around the models to investigate the interactions between plate morphology and flow. We show that spinier armor reduces drag by generating vortices that keep the boundary layer attached further along the length of the fish.

Transcriptomics inform immune cell type evolution in diverse metazoans

Lauren Vandepas, Kevin Wong, Frederick Goetz, Nikki Traylor-Knowles, Adam Lacy-Hulbert, William Browne

Metazoan immune cells deploy diverse defensive behaviors against microbial challenges, including phagocytosis, secretion of inflammatory cytokines,

and the casting of extracellular DNA “traps” (e.g. ETosis). Invertebrate immune cells (hemocytes, coelomocytes, amoebocytes) in bilaterians such as insects and molluscs phenotypically exhibit a variety of morphologies and cellular behaviors. However, behavioral and transcriptional signatures driving invertebrate immune cell behaviors are broadly undescribed, particularly among non-bilaterians, and pathogen defense mechanisms associated with the evolution of metazoan innate immunity remain understudied. Using RNAseq, we have analyzed gene expression from hemocytes isolated from the oyster *Crassostrea gigas* and phagocytic cells isolated from the model ctenophore *Mnemiopsis leidyi*. We have also performed cell behavioral assays to identify potential immune behaviors of specific hemocyte and amoebocyte subtypes. These comparative data sets for both functional immune cell behavior and immune cell gene expression provide an opportunity to explore not only conserved mechanisms of pathogen defense but also novel aspects of metazoan innate immunity evolution.

Discovering the Baseline Biodiversity of Cumaceans in the circum-Antarctic

Victoria Vandersommen

Peracarids are the dominant crustaceans in the stenothermic waters of Antarctica, and are an ecologically important food source for whales, birds, and fish. Cumaceans (Crustacea: Peracarida) are understudied despite their high abundance (up to 31,548/m² in McMurdo Sound) and diversity in Antarctic marine sediment, with 89-91% of species endemic to the region. Antarctica has experienced rapid warming for the last 50 years, prompting concern about how this warming will impact marine communities. To determine baseline cumacean diversity in Antarctica, samples from around the Antarctic will be used. We will use DNA barcoding to delimit species based on sequence divergence and determine species distributions. Due to cumaceans' poor dispersal capabilities, it is expected that each site will have genetically distinct lineages. However, if cumaceans in the Antarctic region are dispersing with the Antarctic Circumpolar Current, we would expect to see homogeneity across sites. While molecular analysis is in the beginning stages, sampling in the West Antarctic Peninsula resulted in 44 cumacean morphospecies, and 37 morphospecies in the East Antarctic. Results will benefit future monitoring efforts in the Antarctic, as well as illuminating the uncategorized diversity of an overlooked order of crustaceans.

First report of cave-adapted mite harvester arachnids from New Zealand

Unitas Vang, Rachel Christensen, Elsa Viereg, Sarah Henderson, Phil Sirvid, Anna Stewart, Sarah Boyer

Mite harvesters (suborder Cyphophthalmi) are a type of tiny arachnid known to be extremely poor dispersers with species ranges typically no larger than 50km in any dimension. These animals inhabit leaf litter on forest floors throughout New Zealand, one of the world's biodiversity hotspots. Recently, a population of Cyphophthalmi in the genus *Aoraki* was discovered in a cave in the northern South Island, representing the first record of subterranean mite harvesters from New Zealand. A comparison of ratios taken from appendage measurements of the cave animals and nine other *Aoraki* species showcases trends characteristic of troglobitic arthropods. Specifically, the elongated appendages of the cave specimens suggest adaptation to subterranean environments. We sequenced the mitochondrial locus COI from the cave population and populations of the closely related species *A. westlandica* in order to better define the cave population's phylogenetic position and assess the possibility that it represents a new species.

Effects of urbanization on morphology in Western Fence Lizards (*Sceloporus occidentalis*)

Victoria Vang, Ramiro Barajas, Sofia Hernandez-Corona, Daisy Xiong, Emily Spain, Bree Putman, Angela Horner

Urbanization has led to considerable habitat loss and change, presenting survival challenges for numerous species. Understanding these challenges can provide valuable insights for the fields of wildlife conservation and

evolutionary biology. In this context, we studied the morphology of Western fence lizards (*Sceloporus occidentalis*) from urbanized and natural locations to detect if urbanized lizards are experiencing differential selection. Our research spanned three distinct sites in San Bernardino and Los Angeles counties, California, USA, each with urbanized and natural habitats available for sampling. We measured lizard body size, limb, toe, and tail proportions, as well as locomotory performance. We hypothesized that urbanization may present different locomotory and behavioral challenges compared to natural settings and predicted that urbanized lizards would have shorter/smaller limb and body proportions. Preliminary data suggest that there are significant differences among our chosen sites, but relatively few direct effects of natural vs. urban habitats. Tail length was significantly shorter from urbanized populations across locations, whereas limb proportions differed significantly among locations. Intriguingly, our findings diverge from previous studies on lizard adaptations to urban settings, emphasizing the need to explore diverse species to gain a comprehensive understanding of adaptations to urban landscapes.

A quantitative comparison of six meiofaunal extraction methods across phyla at Friday Harbor Labs

Rebecca Varney, Rhiannon Nolan, Kemi Ashing-Giwa, Jody Bourgeois, Alyssa Biggs, Ashley Cockram, Erin Fitzgerald, Adriana Halvonik-Sanchez, Nicholas Liou, Alexandria Marquardt, Julian Quinones, Katherine Rogers, Lachan Roth, Lucia Roth, Dario Russo, Ariel Shatsky, Kayli Stowe, Megan Schwartz

The meiofauna are functionally defined as animals between 40 and 500µm that live between sand grains in aquatic environments. Meiofauna are generally understudied, so appreciating the biases introduced by sampling techniques is critical to our general understanding of this diverse group. However, to date few quantitative studies have explored this bias. Here, using samples taken from the same location, we systematically apply six common meiofaunal sampling techniques and compare the relative abundance of common meiofaunal taxa. Though a few phyla were targeted by specific methods, it seems that shaking and decanting is by far the best method for capturing diversity and abundance. Additionally, the dramatic improvement of taxonomists across only a few samplings demonstrates a need for taxonomic training prior to sorting efforts. Together, our data reveal that despite established wisdom for specific phyla, the simplest method still captures the most diversity from a given sample of sediment.

Examining the basis of performance in high wind conditions in a lizard, *Anolis sagrei*

Princeton Vaughn, Claire Middleton, Alex Heine, Ishani Sinha, Andrea Rummel, Shane Campbell-Staton

As climate change progresses, the frequency and intensity of high wind weather events will increase. Hurricanes are devastatingly powerful storms and have long-lasting effects on ecological and evolutionary processes, including impacting community structure and population-level morphology. These impacts have been widely studied, however, little attention has been given to how tropical storms shape ecological and evolutionary dynamics. Tropical storms are less powerful, but more frequent, than hurricanes. These storms are still destructive and can have wind speeds up to 32 m/s (71 mph). Using *Anolis sagrei*, a lizard widespread throughout the Caribbean, we examined clinging performance by exposing them to tropical storm wind speeds via a custom-built fan. We then utilized X-ray imaging to extract detailed morphological measurements and genome-wide association studies (GWAS) to understand which aspects of the genome are influencing performance-relevant morphology. This approach allows us to gain a deeper understanding of how variation in an ecologically-relevant performance measure is underpinned by morphological and genetic variation within a population. Taken together, these results broaden our understanding of how animals will respond to extreme weather events.

Consequences of chronic consumption of thickened milk on infants in an animal model

Alex-Ann Velasco, Elska Kaczmarek, Max Sarmet, Kendall Steer, Thomas Stroud, Alexane Fauveau, Maressa Kennedy, Ani Smith, Hannah Shideler, Skyler Wallace, Morgan Billie, Javier Ceja-Navarro, Christopher Mayerl

Infant feeding relies on complex sensorimotor integration in order to be successful. One of the most common ways that oral sensation is altered when feeding infants is by varying milk texture, or viscosity. However, there is limited research on the long-term consequences of thickened formula on infant physiology. To test the potential consequences of the chronic use of thickened formula on infant feeding function, we raised three infant pigs on thickened formula and five infant pigs (from the same litter) on standard formula. We collected ontogenetic data on growth, feeding efficiency, intraoral pressure generation during suckling, and the fecal microbiome. We compared these data between control piglets and piglets raised on viscous formula. Suckling frequency remained consistent throughout ontogeny and between groups. In contrast, by the end of infancy, control pigs generated greater intraoral suction, fed more efficiently, and were larger than pigs raised on thickened milk. We also found differences in the fecal microbiome between piglets raised on a thickened formula and piglets raised on standard formula. Together, these data show that infants respond behaviorally to being raised on thickened milk throughout infancy, as well as physiologically. This work demonstrates the importance of understanding the chronic impacts of thickened milk on infant physiology and how it impacts feeding performance.

Gait and Kinematics of a Tropical Arboreal Ant (*Cephalotes atratus*) on Wet Substrates

Kaylee Velasquez, Keegan Lutek, Stephen Yanoviak, Alyssa Stark

Given their inability to fly when faced with suboptimal conditions, wingless cursorial insects (i.e., ants) are largely dependent on surface conditions when foraging. Despite high precipitation levels (and subsequent wet surfaces) in the tropics, investigating how tropical arboreal ant species perform and behave in these conditions has only begun to be quantified. Although locomotor and adhesive performance decreases with increased surface wetness, field observations suggest that some tropical ant species, like *Cephalotes atratus*, are willing to traverse wet and soaked surfaces after a rain event. As hexapodal animals, ants primarily utilize a tripod gait to locomote in their environments. Alterations to the typical tripod gait and locomotor kinematics associated with this (i.e., stride length, stride frequency) may develop as a response to maintaining stability over challenging surface conditions like when wet. This study aims to quantify if and how *C. atratus* alters its gait and kinematics on wet and soaked surfaces. The results of this study are important for assessing possible compensation strategies available to the ant locomotor system on biologically relevant conditions.

Thermal Stress Reduces the Effects of Methyl Farnesoate in the Porcelain Crab *Petrolisthes cinctipes*

Abraham Velazquez, Brian Tsukimura

Organisms that reside in the rocky intertidal experience seasonal thermal fluctuations between winter and summer periods. Thermal stress can decrease an organism's reproduction. *Petrolisthes cinctipes*, is an anomuran decapod that inhabits the upper rocky intertidal and regularly experiences thermal stress. Methyl farnesoate (MF) is involved in the development and reproduction of crustaceans. This study investigates how MF might mitigate the negative effects of thermal stress on reproduction in *P. cinctipes*. Crabs were collected during both the summer and winter months. *P. cinctipes* were subjected to a control temperature (12°C), or an elevated temperature (20°C) for four hr/day. Four MF concentrations (1 ng/ml, 3 ng/ml, 10 ng/ml & 30 ng/ml) were injected daily for 5 days in each temperature group. Gonadosomatic index (GSI) was analyzed as the reproductive parameter for the MF treatments. Thermal stress (20° C) tended to lower GSI regardless of MF treatments, relative to controls. At 12°C, MF increased GSI levels only during the winter months, the normal reproductive season of these crabs. In the generally low reproductive summer

period, MF had no effect on GSI. These results show that MF can upregulate GSI during the reproductive season (winter) but is not involved in mitigating the negative effects of thermal stress.

All lines lead to neuromasts- evolution and development of the vertebrate lateral line

Vishruth Venkataraman, Michael Coates, Victoria Prince, Marco Lopez-G, Theresa Christiansen

The lateral line is a placode-derived sensory system of all major vertebrate lineages, crucial for behaviors like schooling and predation. It consists of hair and support cells, embedded in dermal canals or grooves. While broad comparative anatomy of this system has been widely studied, molecular analyses have largely been limited to the posterior (i.e., flank) lateral line of the zebrafish. We used mitochondrial labelling, alkaline phosphatase staining and wholemount immunohistochemistry to analyze developmental morphology of the lateral line in the skate *Leucoraja*, the catshark *Scyliorhinus*, the anuran *Xenopus* and the catfish *Ancistrus*. Preliminary results show conserved expression of the pro-neural transcription factor *Sox2* across species, albeit with varying spatiotemporal patterns. In elasmobranchs, *Sox2* is initially expressed as a continuous stripe along the developing lateral line, and later restricted to neuromast support cells. *Xenopus Sox2* parallels this expression pattern cranially, but its posterior line matches zebrafish development, with *Sox2* expressed in a discrete migrating primordium. Catfish present yet another pattern among studied teleosts, with lateral line primordia elongating cranially. Our data suggest that a further 'pleural line' – unique to batoids – may develop from an independent primordium not connected to other cranial lines. We conclude that neuromasts develop via diverse mechanisms, with zebrafish not fully representing even the teleosts. Future analysis of adult canals and innervation will clarify evolutionary disparity of this sensory system.

Investigating genomic and plastic contributions to thermal tolerance in a non-indigenous crab

Yaamini Venkataraman, Sara Shapiro, Sarah Zuidema, Julia Kelso, Mikayla Newbrey, Lauren Stephenson, Carolyn Tepolt

European green crabs (*Carcinus maenas*) are a prolific non-indigenous species (NIS) with high thermal tolerance that negatively impact species important for tribal food sovereignty and commercial shellfish production in North America. Recent examination of a putative inversion found a high proportion of amino acid-changing mutations in genes strongly linked with thermal tolerance, suggesting a genetic basis for thermal tolerance. The low overall genetic diversity due to serial bottlenecks and high thermal plasticity exhibited by crabs presents a genetic paradox: how is thermal tolerance maintained with seemingly low adaptive potential? This talk will explore genetic and environmental contributions to thermal tolerance of *C. maenas* populations in Massachusetts and Washington, USA. Crabs were exposed to cold (5°C), ambient (15°C), or warm (25°C or 30°C) temperatures for at least one month, and monitored weekly using time-to-right and respirometry. In both populations, crab righting response and rate of oxygen consumption slowed at 5°C compared to 15°C, but there was no change in physiology between 15°C and 25°C or 30°C treatments. Metabolome and lipidome analysis elucidated biochemical pathways used to maintain homeostasis. Thermal tolerance phenotype was also compared between supergene genotypes and temperatures to determine how genotype and environment shaped individual performance. This work illustrates the importance of understanding factors that contribute to within-population stress tolerance, as individual variation may improve overall population fitness and influence spread of NIS.

Functionally stratified encoding on *Drosophila* halteres

Anna Verbe, Bradley Dickerson

Dipterans' impressive aerial capabilities can be in part attributed to the halteres, specialized mechanosensory organs known as the only biological gyroscopes. Halteres also provide crucial timing information to the flight circuit via modulation of a set of tiny muscles that insert at their base. Halteres are covered in arrays of strain-sensitive mechanosensors (campaniform sensilla) that are arranged in distinct groups, which are hypothesized to have distinct

directional sensitivities. However, studying the haltere—a tiny oscillating structure—during flight remains an open challenge. Using a genetically encoded calcium indicator expressed in the haltere afferents of *Drosophila*, we asked how sensory information is encoded by these arrays during visually mediated flight maneuvers. We developed an experimental setup to record the activity changes in the dorsal haltere campaniforms through the cuticle with a standard epifluorescent microscope during tethered flight.

We found that during wide-field visual motion, haltere campaniform sensilla activity is modulated throughout flight. Additionally, halteres seem to be implicated in triggering spontaneous turning events as well as modulating their strength in a nonlinear manner. These patterns of activity appear to be related to the functional segregation of the haltere motor system. Our results demonstrate the crucial role of biomechanics in determining the dynamic range of sensors so that they can mediate maneuvers for both stabilization and active maneuvers.

Long-term forecast of thermal mortality with climate warming in riverine amphipods

Wilco Verberk, K. Hoefnagel, Ignacio Peralta-Maraver, Mathieu Floury, Enrico Rezende

Here we describe a flexible analytical framework to forecast mortality risks by combining laboratory measurements on tolerance and field temperature records. Our framework incorporates physiological acclimation effects, temporal scale differences and the ecological reality of fluctuations in temperature, and other factors such as oxygen. We investigated the heat tolerance of riverine amphipods acclimated to different temperatures and oxygen levels. By integrating experimental data with high-resolution field data, we derived the daily heat mortality probabilities under different oxygen levels, considering current temperatures as well as 1 °C and 2 °C warming scenarios. By expressing heat stress as a mortality probability rather than an upper critical temperature, these can be used to calculate cumulative annual mortality, allowing the scaling up from individuals to populations. Our findings indicate a substantial increase in annual mortality over the coming decades, driven by projected increases in summer temperatures. Thermal acclimation and adequate oxygenation improved heat tolerance and, importantly, their beneficial effects were magnified on longer timescales. Consequently, acclimation effects appear to be more effective than previously recognized and crucial for persistence under current temperatures. However, even in the best-case scenario, mortality is expected to approach 100% by 2100 for some species. Similarly, mortality risks vary spatially: In southern, warmer rivers, riverine animals will need to shift from the main channel towards the cooler head waters to avoid thermal mortality. Overall, this framework generates high-resolution forecasts on how rising temperatures, in combination with other environmental stressors such as hypoxia, impact ecological communities.

The repeated evolution of animal venoms, from stinging cells to toxin structures

Aida Verdes

The repeated independent origin of venom provides one of the most fascinating examples of convergent evolutionary novelty across phenotypic levels. The building blocks of animal venom systems—from venom secreting glands and stinging cells, to toxin structures and molecular targets—have convergently evolved in more than 100 lineages across the animal kingdom offering a unique opportunity to investigate the origins of novel convergent traits. However, how animals have repeatedly evolved this key adaptive trait is still unknown. Recent advances in single-cell RNA-sequencing technologies, are transforming our understanding of the origins of novelty, dissecting the molecular mechanisms underlying the development of many traits. However, the specific position of cells within a tissue is often critical for its function and therefore, linking molecularly defined cell types with morphological, histological, and physiological data is fundamental to understand how novel traits arise. Spatially resolved transcriptomic methods can fill this gap by determining the specific location of molecularly defined cell types within tissues. We are leveraging these two technologies to analyze spatial gene expression patterns at single cell resolution in several lineages of venomous marine invertebrates

including annelids, nemerteans and molluscs, allowing us to better understand the molecular mechanisms underlying the repeated evolution of one of the most successful adaptive traits in the animal kingdom.

Using eDNA Metabarcoding to Track Changes in Tropical and Temperate Amphibian Communities

Rachel Verdi, Brandon Hoenig, Myah Madril, Sydney Dawson, Corinne Richards-Zawacki

Identifying the species that comprise an ecological community is critical for characterizing biological diversity and developing effective conservation strategies. However, traditional sampling methods, which often require intensive fieldwork and direct observations, may be ineffective due to low-density populations, species with cryptic behaviors, or even physical limitations that prohibit sufficient sampling. For these reasons, researchers frequently supplement their traditional approaches with indirect techniques to passively identify and monitor community members. One such indirect method is the use of environmental DNA (eDNA) – or the genetic material that is shed by organisms within their environments – to detect and classify organisms. In this study, we sought to compare the effectiveness of eDNA metabarcoding to traditional amphibian visual encounter surveys at multiple sites in North, Central, and South America. Using PCR primers targeting the 16S gene of the amphibian mitochondrial genome, we amplified and sequenced DNA collected from water samples from tropical and temperate sites and analyzed the resulting high-throughput sequencing data with QIIME2. Preliminary results indicate that, in addition to detecting target amphibian taxa, these primers also allow for the classification of non-amphibian taxa, thus potentially providing an avenue for a more comprehensive characterization of community composition across space and time than traditional surveys. We intend to use these methods and their resulting data to improve our knowledge of amphibian communities and their resilience trajectories in response to chytridiomycosis.

Intrinsic and extrinsic influences on glucocorticoids in red crossbills (*Loxia curvirostra*)

Ben Vernasco, Jamie Cornelius, Amalia Moore, Heather Watts

Glucocorticoids play a fundamental role in coordinating animal responses to environmental challenges. Individuals exhibit consistent variation in both baseline glucocorticoids and the glucocorticoid response to environmental perturbations. However, the sources of individual variation in glucocorticoid physiology are much less well understood. Here, we use established experimental paradigms to explore environmental and physiological sources of variable glucocorticoid physiology in a captive population of wild-caught red crossbills. Red crossbills are nomadic songbirds that exhibit adaptive responses to social information associated with food shortage, including heightened neural sensitivity to glucocorticoids and better body mass preservation. First-year and adult (2nd year or older) red crossbills were paired and maintained in individual cages within an isolation chamber. Baseline and stress-induced glucocorticoids were measured before and after a standardized, 3-day food restriction. We first tested for age- and sex-specific glucocorticoid responses to food restriction and then examined the extent to which a suite of individually repeatable traits (telomere length, mass, food intake, activity) explain variation in baseline and stress-induced glucocorticoids within age classes. We examined social influences on circulating glucocorticoid levels by examining the extent to which the same traits of an individual's partner relate to circulating glucocorticoids. This experimental design provides novel insight into environmental, social, and physiological sources of variable glucocorticoids in an ecologically relevant context.

Does experimentally increasing *Felis catus* exposure impact nestling growth in violet-green swallows?

Manon Vezinet, Valerie Brewer, Dorothy Zahor, Jamie Cornelius, Suzanne Austin

The presence of non-native predators, such as domestic cats (*Felis catus*), poses a significant threat to songbird populations. In addition to causing direct mortality, cats may alter how parents evaluate risk and allocate parental

investment. As perceived predation risk increases, parents may invest less in their current brood to reduce risk to their own survival. This reduced investment may affect nestling growth, which can directly impact both nestling success and parental fecundity. This study investigates how a simulated predator exposure by a non-native predator affects nestling growth rates in violet-green swallows (*Tachycineta thalassina*). We hypothesized that nestlings of parents exposed to experimentally increased perceived predation risk would have lower growth rates than nestlings from unmanipulated control nests. We increased perceived predation risk by placing a synthetic cat mount near experimental nests during the early nestling period. We then measured nestlings, calculated nestling growth rates, and compared those rates between experimentally increased perceived predation risk and unmanipulated controls. This study will advance our understanding of how the presence of a prevalent non-native predator may alter nestling growth and condition.

Neural correlates of seed husking in songbirds

Clara Vicera, Cristian Andres Gutierrez-Ibanez, Andrew Iwaniuk, Douglas Wylie

Birds possess a wide range of behaviours which are reflected in their neural system. Behaviours that require increased sensory input result in an increase in the relative size of the neural tissues processing this action, a phenomenon known as Jerison's Principle of Proper Mass. Previous studies have shown that in avian species with feeding behaviours that rely heavily on tactile information from the beak, like waterfowls and beak-probing shorebirds, there is an enlargement of the principal sensory nucleus of the trigeminal nerve (PrV), which receives inputs from touch receptors in the orofacial region (i.e., the beak and tongue). A notable feeding behaviour that also requires increased tactile input from the orofacial region is seed husking, in which birds use their beak and tongue to remove the outer husk of seeds. Seed-husking behaviour has evolved repeatedly among songbirds (Order: Passeriformes), particularly in the superfamily, Passeroidea. In this study, we measured the volume of PrV, as well as the trigeminal (MV), and the facial motor nuclei (MVII) in 50 species of songbirds, including several families of granivores from the superfamily Passeroidea. Our results show that PrV, but not the trigeminal or facial motor nuclei, is enlarged in songbirds that husk seeds. This suggests that seed husking requires enhanced sensory inputs, but not specialised motor control.

Social Status Scuffle: Aggressive Behavior Used to Establish Dominance Between Male *Lythrypnus dalli*

Beverly Victoria, Makenzie Reed, Anna Jirik, Devaleena Pradhan

Species that live in a linear social hierarchy use a combination of visual and violent aggressive behaviors to gain and emphasize status within a group. In the sexually plastic fish, the bluebanded goby, *Lythrypnus dalli*, three aggressive intensities are displayed by all fish in groups of two males and one female. Low and medium intensity aggression may include the use of sexually dimorphic traits such as dorsal fins and size to emphasize dominance, whereas high intensity aggression may include physical contact interactions. We investigated the impact of each aggressive intensity in groups of two dominant males and one female, all size mis-matched, on Days 1, 3, and 5 of the Dominance Phase during hierarchy resolution. We used the software KINOVEA to analyze 10-minute videos to reduce speed of movement and zoom in up to 600%. Fish of each status performed different proportions of each aggression type. There was a positive relationship between duration and rate of dorsal fin raise. Medium intensity aggression was a good indicator of status, as in previous studies. Males that won the dominant position in their groups displayed higher rates of high intensity aggression on Days 1, 3, and 5 compared to males predicted to change sex into females. This is an indication that physical contact aggression drives hierarchy resolution in this social context.

Is there a tradeoff between male weapon size and testis size in the common daddy long-legs?

Elsa Vieregg, Rachel Christensen, Unitas Vang, Haley Heine, Zade Alafanji, Prashant Sharma, Sarah Boyer, Sophie Neu, Ethan Laumer, Charlotte Wood

Phalangium opilio, also known as the common daddy long-legs, is a globally-distributed arachnid species. Despite its ubiquity, little is known about the basic biology of *P. opilio*, including fundamental aspects of its reproductive ecology and evolution. *P. opilio* is sexually dimorphic with the males possessing cheliceral horns, weapons which have been found to be important in male-male contests. In other arthropod systems, researchers have documented a tradeoff between male weapon size and testis size (e.g. in *Onthophagus* horned beetles). We measured horn size, testis size, body length, and body mass in male *Phalangium opilio* from Minnesota and Wisconsin (USA) in order to explore the possibility that similar tradeoffs operate in this familiar yet understudied arachnid.

The Role of Osmotic Stress Transcription Factor 1 in the Seawater Acclimation of Atlantic Salmon

Jocelyn Villacreses, Michelle Monette, Stephen McCormick

During migration of anadromous from freshwater to seawater, ion transport effectors in osmoregulatory tissues function to regulate internal ion concentrations, however, much remains unknown regarding upstream osmosensing and signal transduction pathways. We examined gene expression of osmotic stress transcription factor 1 (OSTF1) in Atlantic salmon smolts in response to seawater transfer to examine whether OSTF1 is playing a role in seawater acclimation. We first investigated the time-course of OSTF1 regulation in the gill in response to seawater transfer, then the impact of handling/transfer, and tissue-specific differences in regulation. To do this, smolts were transferred from freshwater to seawater and sampled after 0h, 1h, 4h, 8h, 24h, and 72h. OSTF1 was upregulated in the gill 1h after seawater transfer and returned to baseline levels after 4h. Next, smolts were acclimated to freshwater and transferred to either freshwater (handling/transfer control) or seawater and sampled after 1h. OSTF1 expression was then measured in gill, intestine, kidney, and liver. Our results show that OSTF1 is upregulated in the gill in response to both handling/transfer alone and seawater transfer, with a similar pattern of regulation in the intestine and kidney, but not in the liver. Together, our data support the conclusion that OSTF1 is an early response gene that may be playing a role in the cellular stress response in smolts, particularly in tissues with a direct role in osmoregulation.

Functional correlates of molar shape and the trophic diversification of akodontine rodents

David Villalobos-Chaves, Sharlene Santana, Rafaela Missagia

Dental morphology (shape and size) is highly informative about what an animal is adapted to eat. In vertebrates, this dietary signal is particularly strong in extant and extinct animals that have molars, as the main function of these teeth is to finely comminute food particles for further digestion. This ecomorphological relationship has been key to understanding the diversification of highly diverse clades, including many groups of mammals. To test the relationship between molar morphological diversity and trophic diversification, we quantified the three-dimensional molar topography of a speciose clade of Neotropical Sigmodontinae rodents (tribe Akodontine) and tested its relationship with multiple dietary metrics. When analyzing the influence of diet on molar shape, we found that the molar topography of herbivorous species is significantly different from faunivores and omnivores. Specifically, herbivorous species have complex and sharp molars that may allow them to grind plant material more effectively. Moreover, the molars of specialist insectivorous species show a greater number of shearing surfaces than non-strict insectivorous and omnivorous species, possibly reflecting specialization to process insect cuticles. Overall, our data support morphological and functional adaptations that have allowed this speciose group of rodents to diversify and specialize in resources ranging from plant material to insects.

Spotlight on cephalopods: How early evolutionary steps constrain photophore morphology

Bridget Vincent, Emily Lau, Sriram Ramamurthy, Clara Bourguignon, Todd Oakley

The early changes that often lead to the evolution of complex traits can have disproportionate effects on the trajectory of the trait. Early, pivotal steps may be reinforced by subsequent steps and thus become entrenched (difficult to both lose and change) in a lineage. The processes that lead to patterns of entrenchment, i.e. constraints, maladaptive ratchets, or adaptations, remain largely unexplored, however this is necessary to predict evolutionary outcomes. Here, we explore how the mode of light production in cephalopod light organs, or photophores, may heavily influence later evolutionary steps in the organ's morphological disparity across origins. Using a maximum-likelihood coalescent phylogeny and ancestral state reconstruction, we find that the morphologies of bacteriogenic photophores (those that use light produced by symbionts) are less morphologically diverse than autogenic photophores (those that create their own light). This suggests the bacteriogenic morphologies are more entrenched and less likely to change. The mode of light production may serve as a pivotal step in constraining evolutionary routes to diversity. We propose bacteriogenic photophores are more entrenched due to the mechanistic constraints (colonization, housing, and culturing needs) imposed by light-producing symbionts. Our results suggest early steps in an evolutionary trajectory can lead to similar constraints across origins, thus leading to convergent, entrenched morphologies.

Removing mechanosensation in the parrot tongue influences limb-loading during tripedal locomotion

James Virga, Stratos Kantounis, Reuben Jacobson, Melody Young, Edwin Dickinson, Callum Ross, Michael Granatosky

Parrots have developed a distinct way of moving, using their head as a third limb while climbing vertically. However, currently it is unknown how parrots gather sensory information to control their head movements when using this three-limbed climbing technique. Observations of climbing trials reveal that parrots consistently touch substrates with their tongue while grasping with their beak. The parrot's tongue contains many mechanoreceptors, suggesting that the tongue touch might help them assess the properties of the substrate. It is possible that parrots use this information to adjust their head placement and control how much weight they put on their limbs while climbing. Here, we present the results of experimental trials using temporary nerve blocks and placebo treatments to understand whether mechanosensation in the tongue influences locomotor performance. Our results showed that when the tongue's sensory function was blocked, the forces that propel the head forward during climbing were significantly reduced. Additionally, the tensile normal forces that are typically seen in the head while climbing were also decreased. Further, when the tongue's nerves were blocked, the amount of weight the parrots put on their hindlimbs increased significantly compared to the control and placebo conditions. Our findings show that the parrot tongue plays a crucial role during climbing: the only known example of tongue-integrated locomotion.

Host preference and host availability for an introduced intestinal parasitic copepod

Nitara Vishwanath, Joshua Riddle, Pauline Yu, Dorothy Coleman

Generalist parasites can engage with available hosts in a multitude of ways given local habitat variation and host density variation. A copepod intestinal parasite of intertidal bivalves, *Mytilicola orientalis*, is an obligate direct parasite introduced to Europe and North America through the aquaculture trade. Native and introduced bivalve diversity of the Salish Sea is high, and in this study, the copepod exhibited a significant infection prevalence ($X^2 = 8.0673$, $P < 0.02$) in the infaunal species *Mya arenaria*, even in habitats where other available epibenthic host species (*Mytilus trossolus* complex, *Magellana gigas*) were present. In *Mya arenaria*, the high infection rate (40%) by the parasite also resulted in an average infection intensity of 0.89, and a non-significant host-size relationship, but infection intensities and rates were inconsistent in the *Mytilus trossolus* complex, and lower in *Magellana gigas* hosts. The parasite

was consistently observed in the other two host species in habitats where they were the locally dominant epibenthic species, superceding the presence of a diverse infauna. Previous reports from the South Puget Sound region focused on the parasitism on bivalves of economic importance, but to our knowledge, this is the first report of *Mytilicola orientalis* utilizing *Mya arenaria* as a host species in this region. The parasite preference for an infaunal host over an epibenthic host occurred even where the preferred host was not the highest abundance species.

Small changes in nest temperature have lasting effects on the sensitivity to stressors

Maren Vitousek, Conor Taff, David Chang-van-Oordt, Jennifer Houtz, Jennifer Uehling, Daniel Ardia, Thomas Ryan, Monique Pipkin

Many organisms are exposed to increasingly variable environments as a result of climate change. Tree swallows (*Tachycineta bicolor*) breeding in our long-term study population now face nearly double the risk of experiencing a cold snap during breeding – which can have devastating impacts on nestling survival. To test whether these cold snaps also have long-term effects on the individuals that survive them, we combined data on the effects of naturally occurring cold snaps with experiments in which nest temperature was manipulated. We found that small changes in developmental temperature, during both the incubation and nestling period, can have profound effects on the development of the HPA axis, increasing the response to future challenges. These effects seem to be particularly strong, and long-lasting, when cold exposure occurs before hatching. Recent experiments suggest that this increased sensitivity to stressors may help birds cope more effectively with future cold challenges; however, developmental cold exposure also triggers a variety of phenotypic costs. These findings illuminate how inclement weather can have lifelong impacts on the individuals that experience it, altering the sensitivity to future challenges. Depending on the future environment this mechanism could reduce the impact of climate variability on populations, or impose an additional, hidden cost of climate change.

Phenotypic Plasticity Associated with the Adoption of Social Positions in Clown Anemonefish

Lili Vizer, Douglas Alvarado, Colleen Bove, Annabel Hughes, Sarah Davies, Peter Buston

Central questions of animal behavior include how individual phenotypes influence the formation of dominance hierarchies and how dominance hierarchies influence the development of individual phenotypes. Here, we investigate how individuals modify their phenotype as they assume social positions within clownfish dominance hierarchies. We hypothesize that an individual's social context (paired or solitary) and social position (dominant or subordinate) will impact multiple aspects of its phenotype: size, appetite, aggression, and gene expression. We tested this idea by creating 30 replicates of juvenile clownfish — each replicate consisting of size-matched paired individuals in one tank and a size-matched solitary individual in another tank — and monitored these fish over five weeks. We show that, within pairs, individuals adopting the dominant position grew twice as much as individuals adopting the subordinate position, and individuals in subordinate position grew the same amount as solitary individuals. We show that paired individuals initially consumed more food per capita than solitary individuals, but this difference diminished over time as size differences emerged. Similarly, we show that within paired individuals, the aggression levels declined over time as size differences became pronounced. Finally, we show functional variation in gene expression between individuals adopting dominant and subordinate positions, while few differences were observed between subordinate and solitary individuals. This study highlights how social position can influence the development of individual phenotypes within dominance hierarchies.

Don't 'neglectum': DNA barcodes for an endemic Hawaiian nerite snail species

Naomi Vliet, Norine Yeung, Kenneth Hayes, Peter Marko

The Hawaiian Islands are a biodiversity hotspot, with a high rate of endemism due to their geographically isolated location. Hawai'i is home to six endemic nerites from four different genera. Pipipi is the inoa Hawai'i for two morphologically similar endemic marine snails, *Nerita picea* and *Neripteron neglectum*. These two species inhabit distinct regions on rocky shores, with *N. picea* found from the splash zone to the high water mark and *N. neglectum* reported only in tide pools and other locations on the shore that remain immersed. Molecular phylogenetic analyses place *N. picea* in a clade (Neritinae) consisting exclusively of species in the marine genus *Nerita*, but there are currently no DNA sequences for *N. neglectum*. For this project, pipipi morphologically identified as *N. neglectum* were collected from Hale'iwa 'Ali'i Beach Park as part of a biodiversity survey, barcoded with mitochondrial cytochrome oxidase subunit I (COI), and combined with previously published sequences retrieved from GenBank in a phylogenetic analysis. The new sequences formed a monophyletic clade closely related to COI sequences from *Neripteron* and other genera (Neritinae) found in freshwater and estuarine habitats. Future studies could expand to include sequences from other *Neripteron* species in Hawai'i, as well as the other lesser-studied endemic Hawaiian nerites.

Transgenerational effects of stress on the plasma proteome of a marine mammal

Emily Voelkner, Dan Crocker, Jane Khudyakov

The physiological stress response enables animals to respond to changes in their environment, and if experienced during breeding, to communicate that information to their offspring by hormone signals. In mammals, these signals may be transferred via the placenta prenatally, or via milk postnatally. Activation of the stress axis repeatedly and at critical life history stages such as lactation, especially in capital-breeding mammals such as phocid seals, has the potential to affect maternal investment in milk quality and postnatal development and stress reactivity in the pup, and consequently, fitness. We examined the effects of maternal stress axis activation on lactating female and offspring physiology in northern elephant seals using a proteomic approach. We collected blood from females treated with adrenocorticotrophic hormone (ACTH) during lactation and their pups before and after weaning and profiled their plasma proteomes using LC-MS/MS. Treatment with ACTH, which elevated cortisol levels in females, their milk, and their suckling pups, substantially altered the plasma proteome of the latter. Suckling pups of ACTH-treated females exhibited changes in abundance of apolipoproteins involved in lipid uptake and storage, proteins associated with immune function, adipokines that regulate insulin sensitivity, and lipophilic hormone transport proteins when compared to controls. Our data suggest that maternal transfer of stress hormone signals in milk significantly alters postnatal development in offspring.

Losing tails and preferred space: autotomy alters perch height in male day geckos, but not females

Marina Vollin, Amber Wright, Tim Higham

Caudal autotomy, the voluntary severance of a tail, can have a wide variety of consequences for the organism, including decreased locomotor performance, loss of energy stores, and altered social dynamics with conspecifics. Among vertebrates, tail autotomy has been studied extensively in lizards. Given that locomotor and clinging performance are often correlated with habitat use in arboreal lizards, we predicted the negative effect of autotomy on locomotor performance and stability would lead to a shift in habitat use in the arboreal day gecko *Phelsuma laticauda*. To test this prediction, we captured wild day geckos from the introduced population on O'ahu, Hawai'i and housed them in large outdoor experimental enclosures. After one month of acclimation we autotomized half of the recaptured individuals and recorded habitat use in the enclosures over the course of two weeks using a combination of standardized scans and focal animal observations. Although we found a significant difference in perch height between the autotomized and control groups, the difference was highly correlated with sex. Autotomized females occupied the same average perch height as intact females, but autotomized males were

found at significantly lower height in the environment than intact males. Since day geckos prefer to occupy higher, smoother perches, we theorize male day geckos may experience a loss in social standing as a result of autotomy, relegating them to marginal habitat after tail loss.

Bowfin bite: XROMM analysis of prey processing in *Amia calva*

Emily Volpe, Katrina Whitlow

Food transport, how an organism manipulates prey items and moves them to the stomach during feeding, is crucial to animals' survival. This poses unique challenges in taxa where predators swallow large prey items whole, like fishes. So how exactly do fishes transport these large food items to the stomach once consumed? Food transport can help us answer this question, but the mechanisms involved are poorly understood. Sparse studies have examined chewing and repositioning behaviors, prey transport, and swallowing in carp, tilapia, catfish, and stingrays, with variable conclusions across taxa. This study used X-ray Reconstruction Of Moving Morphology to examine the kinematics of food processing in *Amia calva*, a generalist predator in the Holostean clade. Preliminary analysis shows extensive prey repositioning over a series of chews before a swallow. Fish chewed 7-76 times before swallowing, many of which were associated with anterior-posterior prey motion, including both front-to-back and back-to-front repositions. Comparison with strikes shows lower but still surprisingly high-magnitude bone rotations during most chew cycles. Future analyses will quantify the relationship between prey motion and cranial kinematics, including tongue motion, to understand the drivers of prey repositioning across cycles. Ultimately these data will be compared with studies of processing across species, giving us a better understanding of the kinematics and evolution of processing in fishes.

Royal Scholars: Enhancing Science Self-Efficacy for Low-Income Students in STEM

Janice Voltzow, Christie Karpiak, Michelle Maldonado, Stacey Muir, Declan Mulhall

The Royal Scholars Program, sponsored by an NSF S-STEM grant, assists low-income students to develop their identities as STEM professionals, with a focus on increasing their science self-efficacy, to explore careers in STEM, and to take steps along the appropriate pathways to these careers to improve the STEM workforce. The program includes a weekly seminar for all scholars that provides them with a strong sense of community and the opportunity to discuss their progress, concerns, academic and career plans, and develop common STEM skills. The program incorporates support for independent research or internships and opportunities for outreach with the local community. The program has supported a total of 25 students across all STEM disciplines over the last five years. Retention to the second year was 100% (25/25), compared to a typical rate of less than 80% for low-income first-year students in STEM fields. With four cohorts now graduated, retention through graduation has been 84% (21/25). This compares favorably to the institutional rate of around 65% for low-income students in STEM fields. This program has produced substantially higher rates of retention of economically marginalized students in STEM fields than is typical.

Evolutionary Decoupling of Dimorphic Scaling Patterns in Ants

Erica Vong, Hélène Orfali, Shannon Parisien, Chloe Forrest, Rajendhran Rajakumar

Describing evolutionary patterns and adaptive significance of morphological variation provide the foundation for understanding the evolution of form. A morphological pattern that has long fascinated biologists includes scaling relationships, termed allometry. Traits grow proportionally or disproportionately to body size. The study of allometry in the context of development and evolution is prevalent in the insect literature. In particular, developmental plasticity has facilitated the repeated evolution of a worker-soldier caste system across the ants. Soldiers differ from workers in several ways including task specialization and exaggerated allometric scaling. A key morphological innovation of

ants contributing to their ecological success is their elongated scape, the first antennal segment. Elongation of the scape provides biomechanical flexibility, and enhances interindividual communication and environmental perception. Surprisingly, preliminary data collected using a *Pheidole* species, a hyperdiverse genus with >1200 species that has dimorphic soldier-worker castes, reveals a unexpected pattern that does not conform to common scaling patterns. We found that differences in scape size between the small worker and the big soldier is ambiguous, suggesting there is no inter-caste scaling, yet there remains intra-caste scaling of scape-to-body size. We next sampled across the ant phylogeny and found the origin of this decoupling of scaling between castes. This finding challenges the previous understandings of how traits scale to body size throughout development and will contribute to understanding the evolution of form.

Visualizing the Activity of *Photinus pyralis* CecX Against Bacterial Strains

Michelle Vovsha, Moria Chambers, Sarah Smith

Antibiotic resistance continues to be a worldwide concern with more than 2.8 million antibiotic-resistant infections occurring in the United States each year. Natural antimicrobials, specifically antimicrobial peptides (AMPs) are promising for human therapeutics due to their anti-inflammatory activity and low toxicity against mammalian cells. A novel cecropin CecX was recently identified in the common eastern firefly, *Photinus pyralis*. By quantitatively and qualitatively assessing the antimicrobial effects of CecX, we can learn more about the activity of CecX against pathogens relevant to human health. To assess the potency of CecX, we determined the minimum concentration of peptide needed to inhibit the growth of multiple bacterial species in liquid culture. These species included ESKAPE pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*), *Escherichia coli* and various *Providencia* species. We found that CecX is able to inhibit multiple bacterial species including some of the ESKAPE pathogens. To assess whether CecX is inhibiting the bacteria through pore formation in the membrane, we are imaging bacterial cells treated with peptides using scanning electron microscopy (SEM). Our findings so far suggest that CecX is potent against certain gram negative species and the SEM images suggest that the mode of action is pore formation. Future work will determine whether truncations of CecX maintain antimicrobial activity as full length peptides can be challenging to therapeutically deliver due to their size.

Modeling population persistence through increasing temperature and food restriction in zebra finches

Haruka Wada, Wonil Choi, Victoria Coutts, Alex Hoffman, Todd Steury

Organisms in the wild face a number of stressors, such as inclement weather and food shortage, throughout their lives. Some can tolerate the stressor, some can acclimatize through physiological and behavioral responses, while others cannot. A question remains as to how responses and consequences of increasing temperature and food shortage at an individual level influence population resilience to an environmental change. Using captive zebra finches as a model system, we ran several studies to determine how nutritional stress or heat conditioning during the nestling/juvenile periods, and high incubation temperature and reduced eggshell pore availability during the embryonic period, alter reproductive output and survival at life history transitions. Using these data, supplemented with data from the published literature, we ran population dynamics models to estimate relative population growth. Under nutritional stress, we observed a λ of 1.042 compared to 1.050 under ad libitum condition, resulting in 47% decline in population size after 2 years. When eggs were incubated at high or low temperatures, we observed a λ of 1.038 (high temp) and 1.030 (low temp), resulting in ~88% decline in population size under both treatments after 2 years, compared to eggs incubated at an optimal temperature (λ of 1.089). Population declines after undergoing high incubation temperature were exacerbated when fewer eggshell pores were available. The models suggested a key parameter affecting population growth is hatching success.

Thoracic muscle mass increases with age and flight in the adult stalk-eyed fly, *Teleopsis dalmanni*

Catherine Waggoner, Kayla Pehl, John Swallow, Jason Vance

The stalk-eyed fly, *Teleopsis dalmanni*, reaches sexual maturity at 4 weeks post-eclosion, which contributes to an increase in abdominal mass with age, presumably due to investment into gonads. However, thoracic mass also increases during this period, improving thorax-to-body mass ratio and maximal flight capacity even as flies become heavier. The purpose of this study was to investigate the basis of this growth in the thorax, and determine whether flight restriction impacts this growth. We investigated 1-to-42 day old male ($n = 126$) and female ($n = 114$) flies, which either were restricted from flight by removing their wings on day 1 ($n = 97$), or were allowed to fly, having intact wings ($n = 143$). Flies were anesthetized with CO₂, photographed, and dissected, and wet and dry mass of body segments were obtained. Thoraces were soaked in petroleum ether, then desiccated to determine lipid dry mass; then, thoraces were soaked in KOH, then desiccated to determine muscle dry mass. Increased thorax mass was comprised of investment into flight muscle, as muscle dry mass increased logarithmically with age in both treatment groups; thorax lipid mass did not change with age. However, flies that were restricted from flight had less muscle mass than flies with intact wings which were allowed to fly. Thus, investment into flight muscle coincides with -and offsets- the ontogeny of sexual maturation, but “training” contributes to the trajectory of this growth.

The importance of predatory chemical cues in shaping snail growth and shell strength

Madison Wagner, Paul Moore

Non-consumptive effects are widely thought to be more powerful than consumptive effects in eliciting prey behavioral responses. Although predator-prey dynamics have been largely studied in ecology, there is missing information on the physiological responses of small, aquatic invertebrates exposed to predatory cues. To examine the role that non-consumptive effects have on aquatic invertebrate physiology, we presented prey with predatory chemical cues and determined physiological response based upon several different initial and final measures. Pointed Campeloma snails (*Campeloma decusum*) were exposed to chemical cues from a predator, the Rusty crayfish (*Faxonius rusticus*), to test the response of snail growth and shell strength in flow-through systems. Snail growth included measures of change in total shell length, operculum length, and weight throughout the experiment, and shell strength was obtained by measuring the amount of force needed to crush snail shells. Snail operculum growth was significantly affected by crayfish presence ($p = 0.0219$). Specifically, operculum growth was less in crayfish treatments. The total force needed to crush snail shells was also significantly different between treatment groups ($p = 0.0150$), with more force needed to crush snails exposed to predators. Snail total length ($p = 0.891$) and weight change ($p = 0.486$) were not significantly different across treatments. These results point towards snails allocating more energy into growing thicker, stronger shells as a predator avoidance tactic, implicating the role that predator presence has in shaping prey physiology.

Mechanics, ecology, and evolution of cranial kinesis in birds

Amber Wagstaffe, Philip Anderson, Roger Benson, Gavin Thomas, Peter Watson, Jen Bright

In neognathous birds, cranial kinesis allows the upper beak to rotate relative to the skull. Despite centuries of research into form-function relationships in birds, quantitative study of cranial kinesis has been largely overlooked, but may provide new insights.

Using a simple 2D four-bar linkage model, we modelled cranial kinesis using lateral images of over 300 extant bird species. Kinematic Transmission (KT) and gape measurements were compared with feeding categories. We also compared these metrics with link lengths, modelled their rates of evolution, and considered these results in the context of four-bar biological systems as a whole.

Although KT did not demonstrate strong relationships with feeding ecology, the addition of the beak to the 4-bar system permits significant separation of some dietary groups by accommodating gape differences within the models.

Additionally, we find that body size appears to constrain the feeding method employed by birds. Analysis of link lengths revealed that the output link (beak depth) is more strongly correlated with KT than the input (quadrate). This is despite the fact that within birds, the output link is not the shortest link, which was the trait previously believed to drive mechanical sensitivity in other biological four-bar systems. Apodidae (swifts and hummingbirds), Columbidae (pigeons), and shorebirds stand out in terms of rates of evolution.

The slimy & scaly surfaces of teleost fishes: hydrodynamic function

Dylan Wainwright, George Lauder, Bradford Gemmell

The skin of teleost fishes is a complex tissue that is made of connective tissue, hard bony scales, and a delicate epidermis that secretes mucus. Mucus is thought to have important functions in the immune system as it contains compounds that repel pathogens and continually sloughs off the body, making it difficult for pathogens to colonize the skin. Mucus is also hypothesized to have hydrodynamic effects – potentially reducing drag by modifying the boundary layer around fishes. We explored this idea by quantifying boundary layer dynamics of pieces of fish skin with and without mucus in an environment under steady controlled flow. We used the skin of the bluegill sunfish (*Lepomis macrochirus*) as our model, and we found that mucus changes the boundary layer in ways that are consistent with reducing drag. Mucus has two main effects in our experiment; first, mucus moves the initial formation of the boundary layer further away from the skin's surface, which could decrease drag on the skin. Second, mucus causes the velocity to increase more slowly as you move away from the skin's surface, which means that mucus creates lower shear stress between the water and the skin, which in turn suggests reduced drag. These findings mechanistically demonstrate how drag is being reduced by mucus in the boundary layer.

Amphibious locomotion and meniscus climbing in *Microvelia*

Holden Walker, Johnathan O'Neil, Pankaj Rohilla, Saad Bhamla

Microvelia, an insect amongst the family of Veliidae, possess the ability to traverse land and water at similar body speeds unlike other water-walking insects. These insects use an alternating tripod gait to locomote on both water and land. In this talk, we discuss the locomotive performance of *Microvelia* on different substrates (sand, sandpaper, water, and glass) of varying roughness and on water seeded with floating substrates (such as duckweed). Previous studies have mainly focused on water and smooth substrates, yet in nature, *Microvelia* often move on varying terrains such as water, duckweed, rocks, and sand. To understand how *Microvelia* adjusts to these different surfaces and the impact of different surface properties such as roughness, we use high-speed imaging and pose estimation software to describe the kinematics (body speed, stroke amplitude, and stroke frequency) of these tiny insects. Our results show that, on a solid substrate, traction is necessary to achieve a higher maximum speed but increasing roughness of sandpaper hinders the locomotion of *Microvelia*. Overall, our study provides a better understanding of amphibious capabilities in water-walking insects, the knowledge of which can be leveraged to design amphibious microrobots.

The Role of Habitat in Shaping Differences in Branchiostegal Morphology in Frogfishes

Kaaria Walker, Jaida Elcock, Stacy Farina

Jet propulsion is a type of movement performed by shooting a jet of water to generate thrust. This type of locomotion is common among many marine invertebrates. However, a small number of fishes, such as frogfishes, are capable of this behavior as well. Frogfishes are anglerfishes of the Antennarioidei suborder. They are benthic fishes found mostly in tropical and subtropical marine habitats. Frogfishes use their gill ventilation systems for jet propulsion, including their branchiostegals, which are large structures of the gill chamber that expand and compress with each breath. The goal of this research is to investigate if there are correlations between branchiostegal morphology and ecological parameters, to determine how jet propulsion may vary with habitat and ecology. We isolated and segmented the branchiostegals of 33 frogfish and 6 outgroup species using the CT-analysis software 3DSlicer.

We categorized each of the branchiostegals by anterior to posterior order (B1 to B6). Next, we performed a Generalized Procrustes Analysis with all six branchiostegals from each species superimposed onto each other. We then performed a principal component analysis and used phylogenetic ANOVA to examine how branchiostegal shape (PC axis 1 and 2) could be predicted by species-level ecological factors.

Links between coral thermal resilience and symbiosis across generations

Nia Walker, Hayley Luke, Spencer Miller, Darianne Kealoha, Erika Johnston, Carlo Caruso, Elena Mujica, Joshua Hancock, Crawford Drury

Coral heat stress resilience includes mechanisms to resist bleaching, and to survive without algal endosymbionts lost during bleaching and then eventually recover symbiont populations. Certain algal endosymbionts may also confer additional heat stress resilience to the coral holobiont. We investigated links between thermal resistance and recovery, pre-heat stress baseline growth rates, and symbiont type in *Montipora capitata*. We measured growth in *M. capitata* fragments for 3 months, and then we performed a two-week heat stress experiment, in which fragments were removed after observing a 30% decline in photosystem capacity (Fv/Fm). Following removal from high heat, fragments were placed into an ambient tank for 80 days of heat stress recovery. Higher resistant corals were dominated by *Durussinus* spp. symbionts, rather than *Cladocopium* spp. We also found that corals with higher baseline growth rates exhibited moderate heat stress resistance, suggesting a putative trade-off between high heat stress resistance and growth. High resistant corals were also more likely to survive during recovery. A subset of these adult corals was used for F1 spawning, to evaluate growth and heat stress resistance in juveniles. Juvenile heat stress resistance was significantly linked to egg donor but not sperm donor heat stress resistance. However, there was high variation in growth and heat stress resistance in juveniles within and among parent crosses, showcasing that heritability of such traits is highly complex.

Mate choice preferences in female Striped Killifish, *Fundulus majalis*

Nicolas Walker, Rachel Cuomo, Isaac Ligocki

Male and female striped killifish, *Fundulus majalis*, have distinct sex-specific patterning as well as differences in dorsal fin position. Additionally, there is wide within-sex variation in size in sexually mature male and female *F. majalis*. These complex and distinct differences may serve as cues that influence the outcome of competition for mates as well as mate preferences. In the present study, we investigated whether female *F. majalis* displayed a preference to associate with large or small male fish using both live stimulus fish as well as video recordings of fish that varied in relative size. Female *F. majalis* showed no preference to associate with large or small males in live or video trials. Interestingly, large female fish spent significantly more time with live conspecifics in general, regardless of size. We did not observe this difference in female fish exposed to video recordings of fish, or in small female fish. Our findings suggest that size alone likely does not influence decisions whether to associate with potential mates, and that video recordings may not adequately replicate a live stimulus fish in behavioral studies focused on this species.

Resistance vs Tolerance Response to *Serratia marcescens* Infection in the Common Eastern Firefly

Ryan Walker, Aidan Sullivan, Sarah Townsend, Sarah Lower, Moria Chambers

Resistance and tolerance are two strategies organisms use to deal with infections. Resistance is the capability of a host to eliminate or constrain a pathogen whereas tolerance is the ability of the host to minimize the negative effects of the pathogen. Recent work suggests that adult male common eastern fireflies (*Photinus pyralis*) are particularly good at surviving systemic bacterial infections even after a high infectious dose. Males caught early in the season and with good body condition (high mass:length ratio) were particularly good at surviving infection. To determine whether this improved survival was due to altered resistance or tolerance, we infected fireflies with *Serratia*

marcescens, which was one of the few bacterial species to increase mortality after systemic infection in previous work. We subsequently monitored survival and determined bacterial load post-infection at three time-points in a subset of the fireflies. Similar to the previous year, our results show that better body condition and early season-collected fireflies survived longer, indicating that these effects are robust across sampling years. Additionally, fireflies caught later in the season and with poor body condition had higher bacterial loads, suggesting that their resistance to infection has weakened. These results are important for firefly conservation as it suggests that environmental changes that result in poorer adult body condition may make it harder for fireflies to eliminate bacterial pathogens.

Impact of a biomimetic, ducted nipple on infant feeding function through ontogeny in an animal model

Skyler Wallace, Eliska Kaczmarek, Max Sarmet, Kendall Steer, Ani Smith, Hannah Shideler, Maressa Kennedy, Alex-Ann Velasco, Thomas Stroud, Alexane Fauveau, Morgan Blilie, Christopher Mayerl

Suckling is a defining character of all mammals and provides the sole source of nutrition for infants. It is, therefore, not surprising that in human infant care, breastfeeding is considered the optimal way to feed infants, from both nutritional and physiologic perspectives. However, many human infants are bottle fed, and bottles have a different internal structure than breasts. While the breasts of most mammals are soft tissue structures with ducts, bottles are cisternic (hollow). These structural differences are correlated with differences in physiology. For example, infants must generate suction during breastfeeding to acquire milk, whereas bottle feeding is more like cup drinking than breastfeeding. Here, we engineered a biomimetic (ducted) nipple to replicate the breast in an animal model, pigs. We raised a litter of infant pigs, with one group feeding on a cisternic nipple and the other group feeding on a biomimetic nipple. Through ontogeny, we found that pigs raised on a biomimetic nipple latched at a younger age, generated greater intraoral suction similar to breastfeeding behavior, and took longer sucks during feeding than the cisternic pigs. In contrast, feeding efficiency and growth were not substantially different between the two groups. Overall, our results indicate that a ducted artificial nipple may be used to elicit similar physiologic feeding outcomes to breastfeeding and may improve feeding outcomes in infants.

Building a modern denticle morphological and taxonomic database

Nicholas Wallis-Mauro, Leah Rubin, Elizabeth Sibert

Like other vertebrates, sharks and rays are covered in skin that serves a multitude of functions, such as physical protection, enhanced thermoregulation, and defence against predators. Shark skin is covered in dermal denticles, also called placoid scales, which represent some of the oldest known vertebrate fossils and are analogous to vertebrate teeth. Denticles found in the fossil record show a great amount of diversity in terms of morphology, which may relate to phylogeny, ecology, or environmental factors. Further, some fossil denticles look vastly different from anything observed on modern sharks. However, how these morphologies have varied through geologic time and how these varying morphologies reflect phylogenetic and ecological gradients remains poorly constrained. We present results from an ongoing denticle morphology database effort, a study in which we assess denticle morphology as it varies with ecology, taxonomy, and across the body of sharks. Preliminary results suggest that different ecological niches can correlate to differences in denticle morphology, however the system is complicated by both convergent evolution and phylogenetic correlation. Further, we find that certain areas of the shark have similar denticles across nearly all sampled eco-groups, despite significant differences in denticle morphologies across the body. This database and morphological-based framework will allow for study of how shark communities have changed over time and how they may continue to change into the future

Males parents are more responsive to female chick behavior in a facultatively siblicidal seabird

Stephanie Walsh, Morgan Benowitz-Fredericks

Black-legged kittiwake (*Rissa tridactyla*) chicks often hatch asynchronously, associated with competition for food and aggression from first-hatched alpha (α) chicks towards the beta (β) chicks. These behaviors can lead to facultative siblicide, but it is unclear whether parents facilitate or suppress them. Previous research has focused on chick behavior; here we investigate how and when parents interact with chicks' aggressive and food-soliciting behaviors, and consequences of parental behaviors for chicks. Because (1) chick sex may influence parental fitness, and (2) male and female adults may invest in reproduction differently, we tested the hypotheses that parent:chick interactions are affected by parent and chick sex. Previous research found females may invest less during chick rearing, generating the prediction that they will be less responsive to chicks. We continuously recorded behaviors of all nest occupants in 28 two-chick nests containing five day-old chicks for one hour. Male parents interacted more with chicks and did more preening than females, who vocalized more. Although chick behavior rates did not differ by sex, male parents tended to be more responsive to female chick aggression and feed them more compared to male chicks, whereas female parents tended to be more responsive to male chick aggression. We found no evidence of parental influence on siblicide: β chicks survived at 5 nests but behaviors were not significantly different at these nests.

Linking mechanical constraints with foraging choices in *Atta cephalotes* leaf-cutter ants

Olivia Walthaus, Finn Wagner-Douglas, Lina Rhmari-Tiemcani, David Labonte

Leaf-cutter ants are the prime herbivores of the Neotropics accounting for 15% of the total defoliation. Key to herbivory on this scale is efficient foraging - thousands of individual decisions combine to define the colony's resource intake. During foraging, polymorphic workers cut fragments from leaves to grow a fungus as a crop. Leaf-cutting involves mechanical constraints which vary across leaves and worker size: each worker has a maximum bite force they can produce, which scales with size, and each material is characterised by a minimum critical cutting force, which scales only weakly. The differential scaling of these forces places different demands on differently sized workers which cut the same material. So how does the cutting strategy of leaf-cutter ants vary across worker sizes and plant materials?

To address this question, we measured fragment size and cutting speed across worker size and for plant leaves varying in toughness. The total time spent per fragment was approximately constant across all conditions, but the cutting speed varied non-trivially with worker size and leaf toughness. We rationalise these experimental findings with a mechanical model that links muscle force-velocity properties with worker size, plant material properties and cutting speed; the quantitative agreement between model and data suggests that simple mechanical constraints may play a key role in driving complex foraging decisions

Winners don't keep winning, but alter signal use in secondary fights in brown anoles (*Anolis sagrei*)

Jay Walton, Matt Lovern

Agonistic encounters are an important component of intraspecific competition. Generally, winners of a primary contest win future contests, while losers keep losing. Two hypotheses have been proposed to explain these trends: that either individuals' physiology or internal perception of their fighting capacity is altered after a contest. The brown anole (*Anolis sagrei*) is a territorial lizard whose reproductive success is tied to maintaining a territory during the reproductive season, making them an excellent model to study winner and loser effects. I conducted staged encounters in the lab where focal males were placed in asymmetrical contests to force a winning or losing experience before a second contest 24hr later with a naive size-matched opponent. I collected plasma testosterone (T), progesterone (P), and cortisol (CORT), and compared multiple aspects of contest dynamics between winners and losers. I found no difference in hormones or likelihood of secondary contest outcomes.

Winners used significantly more signals and relied more on push-up displays in secondary contests. Loser effects could not be detected due to complications from the home field advantage reducing sample sizes in secondary contests. This data gives further support to the hypothesis that winner effects are driven by an individual's internal sense of fighting capacity, rather than physiological changes.

Impact of mandibular distraction on hyoid position in humans with Pierre Robin Sequence

Annie Wang, Peishu Li, Shelby Nathan, Hannes Prescher, Callum Ross, Russell Reid

Lower facial skeleton remodeling is hypothesized to impact pharyngeal dimensions across human evolution. Pathophysiology models offer a unique opportunity to examine changes in oropharyngeal morphology with craniomandibular defects. Pierre Robin Sequence (PRS) is a congenital condition in humans marked by micrognathia and posterior displacement of the tongue, which obstructs the oropharyngeal space and compromises feeding and respiratory performance. In severe cases, mandibular distraction osteogenesis (MDO) is performed to lengthen the mandible and enlarge the oropharyngeal space. Previous hypotheses suggest MDO improves oropharyngeal volume via anterior shift in hyoid position with mandibular lengthening. Using CT scans, we test this hypothesis by measuring 3D hyoid position relative to various craniomandibular landmarks in 38 PRS patients from one surgeon's practice (pre- and post-MDO) and 14 control patients. Control patients have significantly greater size-corrected hyoid-mandibular symphysis distances than pre-MDO patients. Post-MDO hyoid-symphysis distances fall between control and pre-MDO distances. Size-corrected hyoid-basiscranium and hyoid-vertebral column distances do not vary significantly between groups. We argue MDO does not enlarge the oropharyngeal space by moving the hyoid anteriorly relative to posterior pharyngeal wall. Instead, we hypothesize MDO moves the genioglossus attachment site forward relative to tongue base, thereby changing the geometry of tongue body and anterior pharyngeal wall. Our results provide new insights into the mechanism of mandibular distraction for PRS treatment, and highlight the complexity of craniomandibular remodeling on pharyngeal geometry.

Towards rapid generation and ready access of monoclonal antibody reagents

Linjian Wang, Taylor-Roy Sanchez, Juris Grasis, Chris Amemiya

Monoclonal antibodies are invaluable reagents for our community and are used extensively in basic biological research and in the biomedical industry. Monoclonal antibodies comprise a multi-billion-dollar industry annually, but the generation of these reagents is a very expensive and labor-intensive proposition for most academic researchers. An expedient and inexpensive platform to produce robust and stable monoclonal antibodies that can be distributed using an open-source model is highly desirable. To this end, we submit that we can exploit the lamprey immune system, innovative molecular cloning methods, and synthetic biology to rapidly and inexpensively generate robust monoclonal antibodies (lampribodies) to protein and glycan targets. Lampreys have evolved a convergent adaptive immune system compared to that of the jawed vertebrates (e.g., mouse, human) that employs leucine-rich repeats rather than VDJ segments for building their antibody repertoire. It is estimated that a staggering 10^{14} unique lamprey antibodies are theoretically possible, similar to that of the mouse antibody repertoire. We are developing a novel system that expresses lampribodies on the surface of *E. coli* cells that should greatly facilitate screening and purification for specific antigen binders. This platform would be much more expedient than the current methods for generating monoclonal antibodies and would provide a useful and affordable tool for various areas of research and medicine.

The influence of terrain roughness on cockroach walking and limb collisions

Ruiqi Wang, Glenna Clifton, Nick Gravish

Small invertebrates are able to navigate rough substrates even when moving at relatively high speeds. The cockroach *Blaberus discoidalis* is a model insect that frequently encounters uneven and loose substrates. When navigating rough substrates limbs encounter two main challenges: 1) encountering an obstacle in swing, or 2) missing stance-phase touch down due to uneven ground. The extent to which cockroaches engage in active leg position adjustments during such stepping perturbations is likely a function of locomotion speed and perturbation type. In this study we conducted experiments in which 6 cockroaches run on trackways featuring checkerboard patterns of varying sizes and heights (0.5in, 1in and 1.5in width with 0.25in height checkerboards). We collected high-speed stereoscopic videos and used DeepLabCut to measure body speed, leg dynamics, and foot positioning. At speeds 2.54cm/s, the frequency of front leg collisions increases, but the repositioning behavior of the other legs becomes less prevalent. The investigation contributes to a deeper understanding of cockroaches' agility and resilience in dynamic environments. Moreover, these insights hold potential implications for bioinspired robotic designs and locomotion strategies, harnessing nature's ingenuity to enhance mobility in challenging conditions.

Physical intelligence aids limbless locomotion in cluttered aquatic environments

Tianyu Wang, Nishanth Mankame, Anushka Bhumkar, Velin Kojouharov, Christopher Pierce, Daniel Goldman

Limbless locomotion in open water has been well studied biologically and robotically; the capabilities of limbless systems in cluttered aquatic environments remain less explored. Motivated by the remarkable simplification in control that physical intelligence (purely passive, mechanically controlled body-environment interactions) offers limbless systems in highly-damped environments [Wang et al. 2023], we hypothesized physical intelligence (PI) could also play a significant role in cluttered aquatic locomotion. Differing from robophysical modeling approaches that use sensory feedback and central pattern generators to mimic muscle-actuation mechanism, we developed a limbless robot ($L = 45\text{cm}$) with programmable body compliance, employing decentralized bilateral cable actuation inspired by organismal muscle actuation morphology. We investigated its swimming capabilities in a water tank with obstacles (10cm diameter posts, hexagonal pattern, 25cm center spacing). Our results indicate that, PI emerges when the robot is programmed with an appropriate level of anisotropic compliance, enabling open-loop swimming through obstacles; the robot without PI frequently bounces off upon collisions or gets wedged between obstacles. PI also allows the robot to locomote effectively across a wide range of shape parameters (wave amplitude, spatial frequency), making locomotion performance less sensitive to parameter choices than non-PI robots. Further, we tested performance across undulation frequency (0.03-0.3Hz) and found that, unlike highly-damped terrestrial environments where frequency has small effects on performance, the robot achieves the most effective locomotion (0.51BL/cycle) at an intermediate frequency (0.13Hz).

Twisting tale of termite's elastic mandibles: Conceptual modeling for mechanics

Yi-Yu Wang, Kuan-Chih Kuan, Tzu-Chia Liu, Chun-I Chiu, Hou-Feng Li, Kai-Jung Chi

Elastic mandible snapping is the fastest biological movement known to date, and has been found in ants and termites. *Pericapritermes nitobei* termite soldiers have asymmetric elastic mandibles and can generate snaps up to 132 m/s. Before snapping, *P. nitobei* soldiers use the right mandible to compress the twisted left mandible, during which elastic energy is stored in its two deformable "joints," located at its middle and base, respectively. To date, the geometrical constraints of such a "snap-jaw" system have not been thoroughly examined. In this study, we proposed 2D models by varying the relative lengths of and distance between the left and right mandibles to estimate the compression range and elastic energy stored in left mandibles of different shapes. We further identified the geometric features that could maximize energy storage

to the morphology of 45 extant termite species with asymmetric elastic mandibles. Our results show that the mandible geometry of extant termites could help maximize energy storage at both joints on their left mandible. We also investigated the effects of and interaction between the mandibles' relative lengths, shape, and their distance on energy storage. Results suggest that a longer left mandible (higher asymmetry) and a greater mandible distance could accommodate greater mandible bending and energy storage; whereas the left mandible's shape could modulate bending efficiency.

Insect wing mechanosensory neurons encode rapid bending across a range of wingbeat phases

Ziheng Wang, Alison Weber, Abigail von-Hagen, Bingni Brunton, Tom Daniel

Animals rely on sensory feedback to accurately execute desired movements, such as grasping an object or walking over rough terrain. In flying insects, mechanosensory structures scattered across the wings provide sensory information about the complex spatiotemporal patterns of their bending during flight. This feedback must be incredibly rapid and temporally precise in order to match the relevant timescale of behavior, during which wings beat tens to hundreds of times per second. We examine this mechanosensory feedback in an anesthetized hawkmoth preparation, in which we use a motor to control wing movements while monitoring neural responses extracellularly. In response to sinusoidal stimuli at the wingbeat frequency, response properties are heterogeneous, with individual neurons showing preferred response times distributed across all phases of the wingbeat. Approximately 25% of cells show bidirectional selectivity to opposite phases of the wingbeat (e.g., responding at both the peak and trough of motion), while the remaining cells respond to only a single phase. In response to band-limited noise stimuli, all neurons preferentially respond to frequencies much higher than the wingbeat frequency (typically 40-60 Hz) with striking similarity in the features of movement they encode. Taken together, these results support the idea that wing mechanosensors encode information about deviations from normal wingbeat motion, with individual neurons preferentially signaling at distinct phases of the wingbeat.

The rapid time-course response to heatwaves by heat-shock protein genes in *T. scripta* embryos

Clinton Warren, Madison Wilken, Hannah Warfel, Rachel Bowden, Ryan Paitz

Developing oviparous ectotherms are particularly susceptible to shifts in ambient temperatures. For many turtles, bouts of sudden heat ("heatwaves") are common in natural nests and can impact developmental outcomes depending on their timing and duration. Yet, few studies have considered the time-course response of genes involved with protecting cells and tissues against adverse thermal conditions during development. Heat-shock proteins (HSPs) are molecular chaperones that protect against adverse stimuli and are likely important in an embryo's response to heatwaves. Indeed, past data suggests that gonadal expression of several HSP genes differ between constant warm and cool conditions in red-eared slider turtle (*Trachemys scripta*) embryos. Here, we studied how quickly four HSP genes (*hsp90AA*, *90AB*, *90B*, and *110*) respond to naturalistic heatwave conditions ($31\pm 3^\circ\text{C}$) across multiple distinct tissues in *T. scripta* embryos, as well as how quickly their expression returns to baseline when temperatures return to pre-heatwave conditions ($26\pm 3^\circ\text{C}$). In addition, we investigated the potential effects of developmental stage and prior heat exposures on responsiveness by monitoring embryo-wide expression of HSP genes through the early stages of development following repeated, discontinuous heatwave exposures. These studies may provide a greater understanding of how turtle embryos rapidly respond to thermal shifts and how these responses may change throughout development.

A LC-MS/MS Concoction: Method Development for Steroid Hormone Quantification in Biological Samples

Claire Wasniewski, Kavita Sharma, Samjhana Pradhan, Devaleena Pradhan

Quantification of steroid hormones is crucial to exploring the field of endocrinology in a variety of systems and biological pathways. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a powerful technique that expedites the analysis of multiple hormones and biomarkers via high-throughput processing of biological samples. Here, we developed and validated an LC-MS/MS method to analyze steroid hormones in plasma, serum, tissue homogenates, and waterborne samples. Ten steroid hormones, including estrone, 17 β -estradiol, dehydroepiandrosterone, 11-ketotestosterone, 11 β -hydroxytestosterone, progesterone, hydroxyandrostenedione, pregnenolone, cortisol, and corticosterone were chosen for their presence in almost all vertebrate species and their relevance to behavioral neuroendocrinology. To analyze these hormones, we developed a method with the optimal combination of tools, program settings, sample preparation, and solvents to best separate and quantify the hormones. This included testing LCMS and MS parameters, such as fragment ions, collision energy, declustering potential, column types, and mobile phase gradients. The method was then validated with deuterated cortisol as the internal standard. A ten-point calibration curve was used, ranging from 0.05ng/ml to 500ng/ml for the steroid mixture. The limit of quantification, limit of detection, percent recovery, and repeatability, were also determined. To increase the accessibility of this technique, we also discuss breaking down the process of troubleshooting these steps of method development. Ultimately, our work is foundational for future steroid biochemistry and endocrinology work across various species and sample types.

Cultivating Solutions: Unraveling Biofilm-Mediated Larval Anchoring in Invasive Tunicates

Kai Watkins, Emily Lancaster, Markus Frederich

Many sessile tunicates are highly successful invasive species around the world. Tunicates are also important model organisms for chordate embryological studies, microbiological research, and investigations into the effects of pollutants such as microplastics. The mobile tadpole larvae settle and metamorphose into sessile adult forms quickly, and while it is known that the larvae prefer to settle on a surface with a bacterial biofilm, little is known about what bacteria or biofilm components attract the larvae. This project aims at understanding the biofilm conditions that encourage settlement of *Botrylloides violaceus* and *Ciona intestinalis*. Adults were collected from the wild and then spawned in the lab using light cues. Embryos/larvae were collected and raised to adulthood. Swabs were taken in their natural environment, and different bacteria were isolated to be identified through 16s rRNA sequencing. Based on colony morphology the microbial communities vary from the harbor, intertidal, and estuary areas, however there are several bacterial colonies that are consistent across all samples, with more significant crossover between intertidal and harbor samples. Ongoing experiments test for the settlement success in the lab on biofilms of different species. Understanding the ascidian-biofilm interactions will allow to develop novel antifouling methods and might help in invasive species mitigation efforts.

The ecology and evolution of decoys, a paradoxical antipredator strategy

Charles Watson, Christian Cox

Antipredator strategies include such well-studied phenomena as mimicry, aposematism, and crypsis. For each of these, the animal either poses a threat or perceived threat to the potential predator or seeks to avoid detection altogether. In contrast, decoy coloration, in which an animal draws attention of predators away from essential body parts to avoid lethal damage, may actually attract predatory attacks. Decoy coloration is paradoxical because it increases the likelihood of detection, which should lead to a higher likelihood of predation at a cost to fitness. We reviewed the literature on decoys in animals, and found that it has been documented in arthropods, echinoderms, fishes, amphibians, and squamate reptiles. Most decoys attract attention to autotomizable body parts, such as the limbs or tails, and frequently these body

parts can be regrown after removal or damage from predators. Some species leverage reflections or non-organismal structures (e.g., feces) to draw attention away from their body. We also found evidence for the context-dependence of decoys, with predator type, environmental attributes, and body size of the animal impacting both the presence and nature of the decoy. Given the variation, broad taxonomic distribution, and the paradoxical nature of this defense strategy, future research should focus on understanding the temporal and spatial dynamics of the evolution of decoys.

Transcriptomic responses of gecarcinid land crabs to acute and prolonged desiccation stress

Victoria Watson-Zink, Rachael Bay, Richard Grosberg, Joelle Lai

Terrestrial crabs have repeatedly and independently colonized land and many clades have convergently evolved similar solutions to address the unique demands of their novel terrestrial lifestyles. Because of their aquatic ancestry, desiccation arguably presents the greatest physiological challenge to the land crabs. Overcoming this stressor has resulted in the evolution of many unique adaptations across nearly every one of their major physical and physiological systems. We sought to detect the genetic pathways associated with the land crabs' response to acute and prolonged desiccation stress, focusing specifically on the gene expression responses of two tissues that play critical osmoregulatory and excretory roles – the posterior gills and the antennal gland. We also hoped to identify gene expression patterns in these tissues across species with differing degrees of terrestrial adaptation and relatedness. Will we see greater similarities in gene expression between land crab species that are more closely related or between species that show greater similarities in their relative degree of terrestrial adaptation?

To address these questions, we dehydrated and tracked gene expression changes in two congeneric land crab sister species from different terrestrial grades, *Tuerkayana celeste* (Grade III), and *T. magna* (Grade IV), and a highly-terrestrial confamilial species, *Gecarcoidea natalis* (Grade V). We found that while most of the differentially expressed genes were more likely to be conserved across all three species, duplicated genes and species-specific genes also appear to play a critical role in how land crabs adapt to the selective challenges that accompany a terrestrial life.

Increased winter temperature variability has contrasting effects on cold tolerance and energetics

Sarah Waybright, Michael Dillon

Ongoing climate change is resulting in both increases in mean temperatures and temperature variation, with important but less studied effects on insect physiology. Winter temperatures are expected to become more variable due to reductions in snowpack and more variable air temperatures. Variable winter temperatures will increase energy expenditure for dormant ectotherms; on the other hand, periodic exposure to low temperatures can increase tolerance of subsequent cold exposures and therefore offer freeze-avoidant insects additional protection against freezing. Therefore, we hypothesized that the effect of increased temperature variation is context dependent, with negative effects if periodic highs cause ectotherms to expend excess energy, and positive effects if periodic lows increase cold tolerance, reducing freezing risk. To test this hypothesis, we overwintered queens for 6-weeks in 1 of 9 treatments differing in means and daily temperature variation. We tracked mortality, mass loss, and supercooling points. As predicted, queens experiencing similar levels of temperature variation at higher means had higher mortality, mass loss, and supercooling points relative to those overwintered at cooler means. Periodic exposure to warm temperatures likely reduced cold protection and increased energy expenditure whereas periodic exposure to cool (non-freezing) temperatures increased cold protection. The effects on dormant ectotherms of increased temperature variability in winter will likely be context dependent; more data on thermal sensitivity of physiological traits will be critical to predict responses to climate change.

Predictors of arboreality from appendicular skeletons illuminate locomotor trends in early mammals

Luke Weaver, Jonathan Nations, David Grossnickle

Arboreal locomotion likely evolved independently in numerous mammalian lineages from the Mesozoic through Cenozoic, but inferring trends in early mammalian arboreality requires robust links between morphological traits and locomotor behaviors. To this end, we use a large comparative dataset of linear measurements taken on extant mammalian postcranial specimens to evaluate the skeletal proxies that best differentiate arboreality from other locomotor modes. Consistent with previous research, we find that measures of digit length and elbow dimensions are especially powerful predictors of arboreality. Due to strong phylogenetic signal, locomotor predictions based on single measurements or ratios are vastly improved with knowledge of phylogenetic placement and adequate sampling of closely related species. Nonetheless, combining multiple functionally relevant traits into our models yields robust predictions of locomotor mode even without incorporating phylogenetic information—this is especially encouraging for fossil groups with uncertain phylogenies. We then test these locomotor proxies in three early-mammalian case studies—Multituberculata, Plesiadapiformes, and Mesozoic Eutheria. Our results indicate that multituberculates had a wide locomotor breadth whereas plesiadapiforms were nearly all tree-dwelling, consistent with previous studies. However, we find less evidence for arboreality among early eutherians compared to previous hypotheses; many taxa in our sample are predicted to be primarily ground-dwellers. The predictive tools presented here will improve locomotor inferences for small mammals, further illuminating the prevalence and importance of arboreality in early mammalian evolution.

A call for studying mitochondrial bioenergetics of animal-infecting fungi

Ryan Weaver

Capture and transformation of energy from the environment is a foundational trait among living organisms and cellular solutions for meeting bioenergetic demands are diverse across major life-history strategies. Pathogens face variable—often harsh—environments across their lifecycles that span wide ranges of pH, temperature, oxygen levels, nutrient availability, and host immune responses, which pose challenges to metabolic machinery. In aerobic environments, oxidative phosphorylation-based respiration brings high yields of biological energy in the form of ATP, but also introduces vulnerabilities to environmental stressors that inhibit canonical mitochondrial electron transport chain (ETC) pathways. Respiratory stress can be deadly, so diverse bioenergetic solutions have evolved to allow for metabolic flexibility. That is, in addition to canonical ETC pathways, where electron flux from reducing equivalents terminates in complex IV to reduce molecular oxygen to water, many and diverse eukaryotes have a branched ETC that provides a second exit for electrons via the alternative oxidase (AOX). Here I argue that mitochondrial bioenergetics of pathogenic eukaryotes is an understudied yet critically needed component of our approach to treating emerging fungal diseases in wildlife. I use the amphibian-killing fungus *Batrachochytrium dendrobatidis* as an exemplar of how metabolic flexibility provided by a branched respiratory chain may be key to skin pathogenicity and highlight avenues for future research.

Acclimation of Cutaneous Evaporative Water Loss to Humidity

Savannah Weaver, Evan Odberg, Tess McIntyre, Taylor van-Rossum, Eric Riddell, Emily Taylor

Acclimation, the capacity for physiological change within organisms in response to their environment, informs our understanding of biodiversity and of potential extinction versus resilience to climate change. Our climate is changing much more rapidly than the speed of evolution, but plastic changes like acclimation have the potential to keep pace. There is a wealth of research on thermal acclimation in physiological traits, but relatively few studies have explored acclimation to the hydric environment and interactions between the thermal and hydric environment. We exposed Western Fence Lizards (*Sceloporus occidentalis*) to different temperature and humidity conditions

and measured cutaneous evaporative water loss (CEWL) before and after the experiment to assess plastic changes in CEWL. Lizards acclimated to humid conditions by increasing CEWL, while lizards acclimated to dry conditions by decreasing CEWL. Each acclimation temperature and humidity combination resulted in a unique vapor pressure deficit, the drying power of the air, to which lizards were exposed. We found a negative relationship between CEWL and treatment vapor pressure deficit. Lizards exposed to high vapor pressure deficit (dry air) typically acclimated by decreasing CEWL. The acclimation capacity that we measured will be essential for forecasting the activity and survival of animals in an increasingly warmer and drier environment.

Picking the Nose: investigating dermal denticle orientation at the nares of sharks

Amani Webber-Schultz, Brooke Flammang, Lauren Simonitis, Kayla Hall

Dermal denticles cover the entire body of Selachimorphs and play a critical role in directing flow around the body. While most fluid-denticle interaction research has been either nonspecific or constrained to the body, dermal denticle imaging and flow research has not examined the nares. Based on previous research, the initial assumption was that dermal denticles will be oriented in such a way that smoothly directs flow into the incurrent naris. Originally discovered on *Squalus suckleyi* (pacific spiny dogfish), we investigate a novel denticle patterning on additional species to determine if it is a recurring phenomenon through micro-computed tomography and scanning electron microscopy. These species include *Squalus suckleyi*, *Sphyrna tiburo* (bonnethead), *Carcharhinus acronotus* (blacknose shark), and *Rhizoprionodon terraenovae* (Atlantic sharpnose). We show a recurring dermal denticle patterning at the anterior region of the incurrent naris across species, implying that denticles may be playing a more complex role in narial flow than previously thought. Additionally, we 3D printed the incurrent naris dermal denticles and ran flow trials to visualize how water movement may be impacted by denticle orientation.

Shark Tales: Comparative Caudal Fin Diversity of Dermal Denticles

Amani Webber-Schultz, Brooke Flammang, Kaelyn Gamel

Propulsion in sharks is generated by the movement of their caudal fin. The shape of the caudal fin varies depending on the ecological niche and life history of the species. Similar to how their tails differ morphologically, the dermal denticles covering the caudal fin also exhibit variation in shape and size. Serving as the final point of contact for fluid passing over a shark, the influence of caudal dermal denticles on fluid flow is crucial for thrust generation. Dermal denticle morphology has also been observed to differ based on ecological niche. Benthic sharks are expected to possess larger, more rounded denticles, whereas pelagic sharks will have smaller, ridged denticles. To explore differences in surface topography among sharks of different niches, we used surface profilometry to investigate variations in surface roughness, denticle size, and denticle shape at different locations across the caudal fin.

Standardizing Stabbing: Quantifying Parasitoid Wasp Hosts' Resistance to Puncture

Abby Weber, Philip Anderson

Parasitoid wasps reproduce by depositing eggs into or on a host insect's cuticle. These host insects often live under or within various substrates, including fruit or wood, which the parasitoid must puncture with its ovipositor. The diversity of host substrates has led to a wide diversity of ovipositors in terms of both size and shape: ovipositors span an order of magnitude in length and two orders of magnitude in sharpness. We do not currently have a good understanding of how this diversity affects the performance of oviposition across substrates. We assume that the force required to puncture a substrate should not exceed either the buckling strength of the ovipositor, or the maximum muscle force that the wasp can produce. There is little data on the puncture resistance of these substrates to test this hypothesis. We determined the puncture resistance of a range of insect and plant materials commonly punctured during oviposition using a standardized tool. Surprisingly, we found a range of

puncture forces across substrates, up to six orders of magnitude greater than previous estimates of force these wasps could apply during oviposition. These results indicate that parasitoids are not simply pushing their ovipositor through these materials but may be using alternative strategies such as secreting enzymes to break down substrates, creating the initial fracture with one valve, or bracing the ovipositor with the sheath.

Growing a tail: cellular and genetic determinants that diversify tail proportion in mammals

Ceri Weber, Erica Gacasan, Alexander Weitzel, Talia Moore, Robert Sah, Kimberly Cooper

The axial skeleton provides a framework for diverse mammalian tail forms and functions, including various means of locomotion and communication. Extreme differences between species, such as the number and proportion of vertebrae, suggest the mammalian tail is highly evolvable, perhaps in large part due to its modular development and organization. Aspects of tail diversification are evident in the laboratory mouse and the bipedal lesser Egyptian jerboa, which diverged from a common ancestor about 50 million years ago. Despite having fewer vertebrae, the jerboa tail is 1.5-times longer than the mouse tail when normalized to body length due to disproportionate elongation of individual vertebrae. Using μ CT imaging and tissue histology of mouse and jerboa skeletons, we found that the initially similar neonatal tails diverge during a juvenile phase of disproportionate growth that is more rapid in jerboas. We show that disproportionate elongation is correlated with differences in proliferation and growth zone heights in vertebral growth cartilage and that the cranial and caudal growth plates do not contribute equally to longitudinal growth. Ongoing work will identify the molecular mechanisms responsible for the development and evolution of axial skeletal proportion in mouse and jerboa, which will form a basis to more broadly understand diversification of mammalian tail skeletal proportion.

Squamate preoviposition embryogenesis diverges from chicken and exhibits high diversity

Antonia Weberling, Natasha Shylo, Hannah Wilson, Seth Malloy, Melainia McClain, Marta Marchini, Katherine Starr, Thomas Sanger, Florian Hollfelder, Paul Trainor

Reptiles comprise an ecologically and evolutionarily diverse group of organisms. Their radiation and adaptation to nearly all niches on the planet occurred in concert with the accumulation of a remarkable diversity of morphological and behavioural features. But how did these features develop and evolve during embryogenesis? The more than 25,000 species of reptiles are presumed to develop like chicken embryos, following Eyal-Giladi-Kochav and Hamburger-Hamilton developmental milestones. Yet, few studies have explored non-avian reptile pre-gastrulation embryogenesis, due to the fact embryos have already developed to early organogenesis stages at the time of oviposition. Here, we provide the first characterisation of pre-oviposition morphogenesis in two representative squamates, the veiled chameleon (*C. calyptratus*) and the brown anole (*A. sagrei*). We discovered profound diversity in their modes of embryogenesis, which are distinct from chicken, and include a prolonged single cell stage of development. Furthermore, we also observed striking differences in pre-gastrulation morphogenesis. In chicken, a multi-layered blastoderm gives rise to a flat, monolayered epithelial disk, the embryonic epiblast, whereas the veiled chameleon forms an epiblast lumen via an inner cell mass-like structure, which is as hallmark of mammalian embryogenesis. In contrast, the brown anole forms a highly convex epithelial epiblast. Our studies of veiled chameleon and brown anole development can therefore provide novel insights into the evolution of cellular and molecular mechanisms driving early reptile morphogenesis and ecological adaptation.

Seasonal species distribution modeling and migratory estimates in *Tadarida brasiliensis*

Cara Webster, Michael Smotherman

Geographic distribution of an organism can vary seasonally due to changes in the local environment, usually from fluctuations in resource availability

or temperature. Many organisms use migration as a behavioral strategy in response to seasonal changes. *Tadarida brasiliensis* is a widespread, migratory bat that shows variability in migratory tendency across their range, but their movement patterns are poorly understood. To assess the level of variability of migration across the species' range and to test for possible sex differences, we will utilize both species distribution models (SDM) and migratory modeling techniques. We collected 2,457 presence records from the Global Diversity Information Facility from 1960-2020 in the United States and Mexico, with 1,011 records for female bats and 1,446 for male bats. From this data, we will generate SDMs for each season and sex to mark seasonal distribution based on several relevant environmental variables. We will use six SDM approaches (GLM, Maxent, Random Forest, Bioclim, Boosted Regression Trees) and consolidate for a final ensemble model. Additionally, we will use two models (circuit theory and least cost path analysis) to get estimates of migratory distance traveled. Accurate information on the movement ecology of a species is necessary for the most effective conservation measures. Also, improved detection of environmental drivers of bat migration will help predict how migration may shift in response to rapid global change.

Dual autonomous and conditional neural specification in two species of annelids

Nicole Webster, Allan Carrillo-Baltodano, Johnny Davila-Sandoval, B. Duygu Özpolat, Neva Meyer

Centralized nervous systems are animal innovations but their evolutionary history is unclear. In vertebrates and arthropods, neuroectoderm is specified by factors secreted from neighboring mesodermal cells during dorsal-ventral axial patterning, resulting in upregulation of the MAPK cascade and inhibition of BMP signaling. Recent data including work from our lab on animals in the third major bilaterian clade, Spiralia, have found varied including no involvement of MAPK activation and inhibition of BMP signaling in neural specification. To understand neural specification in spiralian, we isolated blastomeres in the annelids *Capitella teleta* and *Platynereis dumerilii* and assayed neural fate. In both annelids, first-quartet micromeres (8-cell stage) generate ectoderm of the episphere plus brain, while the 2d micromere (16-cell stage) forms ectoderm of the trunk plus ventral nerve cord (VNC). We found that these micromeres generated neural tissue in isolation, suggesting that inherited factors promote brain and VNC fate in both annelids. Furthermore, partial larvae arising from isolated animal-pole blastomeres (first- and second-quartet micromeres) failed to form a VNC; suggesting that additional external signaling affects neural fate. Our results suggest that CNS evolution may have been more complicated than a single centralization event at the base of Bilateria or that significant changes in CNS development occurred within Spiralia. This research was supported by NSF grant 1656378 to NPM and an MBL Whitman Early Career Investigator Fellowship to NBW.

Characterizing the sensorimotor transformation in the fly olfactory system to naturalistic stimuli

Samuel Wechsler, Vikas Bhandawat

Locating odor sources is a complex problem that requires flexible behavior and decision-making based on limited data in the form of sparse, transient odor packets. Foragers, nonetheless, are successful in locating biological necessities such as mates and food. The transient, variable nature of natural odor packets makes characterizing the stimulus during behavior difficult. We overcome this difficulty with optogenetics and the *Drosophila* olfactory system. Using a circular arena with a fixed-intensity central light zone, we optogenetically activate transgenically modified olfactory receptor neuron (ORN) classes in freely moving flies, producing noticeable behavioral changes. Track data and accompanying light intensities are used to replicate the fly's sensory experience in an electrophysiological rig to measure responses of first-order (ORNs) and second-order (PNs) olfactory neurons to well-controlled but naturalistic stimuli. The responses to these stimulus patterns are measured from multiple different ORN classes. From this, we can predict behavior based on the instantaneous firing rate and Δ firing rate (f, df) at the ORN layer. The responses of second-order projection neurons receiving inputs from an optogenetically activated ORN class either directly (cognate uniglomerular/uPNs) or indirectly (nonglomerular mPNs or multiglomerular/mPNs) show noticeable

differences in (f, df) when presented with the same stimulus, indicating the ORN's (f, df) is encoded by different PN classes. This data provides new insight into the nature of sensorimotor transformations in the context of naturalistic odor stimulus.

Arginine vasotocin influences androgenic effects on multimodal communication in foot-flagging frogs

Callie Weidman, Neshima Vitale-Penniman, Doris Preininger, Lisa Mangiamele

Foot-flagging frogs (*Staurois parvus*) use a variety of signals in their multimodal communication repertoire. Males primarily use a leg gesture, known as "foot flagging," to signal to rivals and potential mates. Vocalizations and other visually conspicuous posture, such as open mouth display, are also used. We previously found that foot flagging is androgen hormone-dependent, and that androgen receptor activation is necessary for a great diversity of signal transitions in males' multimodal displays. Yet, some signals, such as vocalization, appear not to be androgen-dependent in this species. This raises the question of whether other hormone systems, such as arginine vasotocin (AVT), could also play a role in the expression of multimodal signals. To address this question, we injected male *S. parvus* with either AVT, testosterone, testosterone+AVT, or vehicle. We elicited signaling behavior by pairing two males receiving the same injection with a female. We then analyzed the number of behaviors displayed and the escalation of agonistic encounters between males. Our results suggest an interaction between AVT and testosterone in regulating the production of multimodal displays, but they also indicate that some vocal and visual display behaviors may be influenced by AVT alone. Overall, our study supports a role for AVT in regulating male social signaling in this species, although it may interact with steroid hormones in complex ways to influence behavior.

Pliable perches: The effects of perch compliance on jumping performance of green and brown anoles

Sierra Weil, Austin Garner

In the southeastern United States, the invasive brown anole (*Anolis sagrei*) has entered the habitats of the native green anole (*Anolis carolinensis*). In the presence of *A. sagrei*, *A. carolinensis* move from their preferred habitat of tree bases, shrubs, and wide-leaved plants into higher perches and subsequently undergo character displacement. Several features of the structural habitat likely change alongside shifts in perch height, such as perch diameter or flexibility (compliance). While the impacts of perch diameter on locomotion are well-documented in *Anolis* and other squamates, fewer studies have examined the effects of perch compliance. Furthermore, the potential biomechanical factors impacting the invasive success of *A. sagrei* and the biomechanical consequences on *A. carolinensis* post-displacement have received little attention. Here we examined how jumping performance of *A. sagrei* and *A. carolinensis* is impacted by perch compliance. We sampled anoles from sympatric populations of *A. sagrei* and *A. carolinensis* and a population of *A. carolinensis* that rarely encounters *A. sagrei*. Anoles were coerced to jump off a dowel connected to springs with variable stiffnesses to modify perch compliance and jumping performance quantified via high-speed videography. Our findings will not only enhance our understanding of the drivers of invasive species success but will also identify potential biomechanical consequences experienced by native species.

Impact on House Sparrow Nestlings of Anthropogenic Materials in Their Nests

Brooke Weiss, Michael Butler, Olivia Asher, Mae Maddox

Nesting behaviors of birds evolved millions of years ago, but the ubiquitous access to human-made trash as potential nesting material is relatively recent. Birds incorporate trash into their nests, which is sometimes beneficial (e.g., cigarette butts in nests can reduce parasitism), but can also result in costs. However, we currently do not know how many of the specific types of trash items in nests affect nestlings. We collected data on 98 nests and 184 house sparrow (*Passer domesticus*) nestlings during the summer of

2023. Throughout development, we measured mass, wing chord, bill dimensions, tarsus length, and pin feather length. At the end of the nestling period, we measured circulating nutrient levels and stress levels (i.e., heterophil to lymphocyte ratio) using nestling blood. Our initial analysis suggests that an increasing amount of anthropogenic material in the nest is associated with a decrease in body mass. During the fall of 2023, we will perform additional analyses to test whether nestling morphometric or blood-based data is related to the specific types of trash found in the nest, rather than just the overall amount. Understanding how nest composition affects nestlings will allow us to describe the consequences of anthropogenic material on free-living birds and inform future conservation efforts for other species with a more threatened status.

Differential effects of salinization influence predator-prey interactions of tadpoles and insects

Allison Welch, Aubrey Anthony, Amanda Montgomery

Although much research on ecological stressors focuses on effects on individuals, less attention has been paid to how stressors impact community-level processes. Species interactions provide a crucial link between the individual level and community structure and function. Elevated salinity can be an important stressor in freshwater systems, owing to anthropogenic impacts including sea level rise, road salts, and agricultural practices. We investigated the relative tolerance of two species of anuran larvae and two species of predatory aquatic insects, as well as the impacts of sublethal salinity exposure on direct interactions between tadpoles and dragonfly nymphs. Dragonfly nymphs were more tolerant of salinity than was either species of tadpole, both of which were more tolerant than dytiscid larvae. At sublethal levels, salinity affected predator-prey interactions, resulting in greater rates of predation by dragonfly nymphs on tadpoles, as expected based on their relative tolerance. Our results indicate that differential effects of salinity can influence the relative abundance of predators and prey as well as the outcome of direct predator-prey interactions. Based on these results, we predict that increased salinity has the potential to not only decrease biodiversity in freshwater systems but may also alter community structure and ecosystem function. If the balance is shifted to fewer amphibian larvae, we predict less conversion of primary production to animal biomass. This prediction will be tested in future mesocosm experiments.

Actin Polymerization Increases in the DA During the Developmental Transition to Lung Ventilation

Hakeem Werra, Edward Dzialowski, Jessica Rippamonti

The Ductus Arteriosus (DA) is an embryonic blood vessel that connects the descending aorta and the pulmonary artery during fetal development. It functions as a right-to-left shunt diverting blood away from the nonventilated lungs and to the descending aorta. The DA serves a comparable physiological role in all terrestrial vertebrates during their embryonic development. Following birth or hatching, the DA constricts in response to increase in arterial oxygen tension brought on by initiation of lung ventilation, and it closes to stop the embryonic shunt. We examined the extent of actin polymerization in the DA during the transition to lung ventilation at hatching in the chicken. We isolated and fixed DA from developing chickens on embryonic days 15 and 19 (ED15 and ED19), during internal pipping (IP) and external pipping (EP), and in hatchlings. Vessel sections were fluorescently stained with Alexa Fluor 488 (DNase 1) for G-actin, Alexa Fluor 568 (Phalloidin) for F-actin, and TOPRO-3 for nuclei staining. Fluorescence microscopy was used to image the sections and quantify F-actin to G-actin levels within the DA for each age. From the images, F-actin to G-actin ratios were determined. Embryonic stages exhibited strong G-actin staining with minimal F-actin staining. During hatching, F-actin staining became more pronounced, exhibiting clearer fiber patterns in the tunica media. F-actin to G-actin ratios increased significantly in the DA from ED15 through hatching, suggesting actin polymerization in the DA increased as the chickens progressed from day 19 to external pipping (EP). These results suggest that increases in actin polymerization play a role in ductus arteriosus constriction at hatching in chickens.

The Perks of Eupercaria: Rapid Skull Shape Evolution in a Massive Radiation of Bony Fishes

JoJo West, Rose Faucher, Elizabeth Miller, Guillermo Orti, Lily Hughes, Sandra Alvarez-Carretero, Giorgio Carnevale, Aintzane Santaquiteria, Samantha Gartner, Mark Westneat, Carole Baldwin, Ricardo Betancur-R, Dahiana Arcila, Kory Evans

With over 6,000 species of teleost fishes, Eupercaria reigns supreme as one of the most spectacularly diverse clades of vertebrates, extant or otherwise. They have colonized all seven continents, their range extends to almost every aquatic habitat from freshwater to the deep sea, and they exhibit numerous distinct ecologies and morphologies. Accordingly, many questions remain about the mode and tempo of the Eupercarian radiation. Here, we use 3D geometric morphometrics, phylogenetic comparative methods and a novel phylogenetic hypothesis based on exon capture genomic data to quantify the tempo and mode of skull shape evolution across 600 species of Eupercarian fishes. We find a rapid burst in the rate of skull shape evolution and an increase in morphological disparity that roughly coincides with the KPG extinction event 66 mya, suggesting that this mass extinction event opened niches which ancestral eupercarians swiftly filled. We also find high rates of morphological evolution and disparity in Acanthuriformes and Tetraodontiformes while other clades like Perciformes and Labriformes exhibit intermediate levels of disparity and rates of skull shape evolution.

The neurogenomics of diversity in butterfly mate preference learning

Erica Westerman, Sushant Potdar, David Ernst, Gabrielle Agcoaili

Mate preference learning, where individuals learn to prefer or avoid phenotypes based on social experience, is pervasive across animal taxa. However, not all animals learn preferences for the same thing, whether in terms of trait learned or trait valence. To uncover the neurogenomics underlying variation in learning, we utilized two butterfly species: *Heliconius melpomene* and *Bicyclus anynana*. Lineages of *H. melpomene* exhibit differing degrees of aversive courtship learning, while female and male *B. anynana* both learn positive visual preferences, but learn preferences for different traits. To identify genes associated with this diversity in learning, we examined gene expression profiles of the brains, eyes, and antennae of male *H. melpomene malleti* and *H. m. rosina*, and the brains and eyes of male and female *B. anynana*, immediately post training and compared them to the same tissues of naïve individuals. In *H. melpomene*, we found more differentially expressed genes in the brains, suggesting that the strength of learned response may be driven by higher processing, while in *B. anynana*, we found more differentially expressed genes in the eyes, suggesting a role of the peripheral nervous system in imprinting-like learning. Genes that influence wing pattern (*B. anynana*), and genes adjacent to wing patterning (*H. melpomene*) were also differentially expressed. Our results suggest that different suites of genes are associated with diversity in what versus whether animals socially learn.

Biomechanics and Morphometrics: Computational 3D Modeling of Linkages in Labrid Fish Skulls

Mark Westneat, Samantha Gartner, Kory Evans

Computational modeling of musculoskeletal function can yield important insights into force transmission, kinematics, and evolution in functional systems. Many lever and linkage systems have limited mobility and may be modeled in 2D, yet 3D modeling is required to capture the full functional repertoire of most systems. The explosive increase in μ CT data sets for the skulls of fishes, through the #ScanAllFishes and #oVert projects, is a promising avenue for development of new computational biomechanical models. Here we developed a new three-dimensional computational model of lever and linkage biomechanics in the feeding mechanics of labrid fishes to yield novel mechanical traits, address questions of functional variability, and explore evolutionary patterns across recent phylogenetic trees. We used scans of 206 species of the Labridae (wrasses and parrotfishes), to collect over 200 3D coordinates quantifying the geometric morphometrics of the skull, including the jaw lever systems and the linkages of the anterior jaws, the opercular system, and the hyoid mechanism. Computational modeling shows that the

planarity of the anterior jaws system is high, with standard mechanical advantage and kinematic transmission similar to 2D analysis, yet the dynamics of linkage motion, expansion advantage, and path analysis reveal the importance of 3D modeling. Phylogenetic comparative analysis of novel linkage traits in labrid fishes reveals hidden pockets of functional diversity in the transmission of force and motion during feeding.

Hormonal Variation and Parental Care Dynamics in the Dyeing Poison Frog

Sarah Westrick, Eva Fischer

Individual variation in phenotypes is the fundamental substrate on which natural selection, and by extension adaptive evolution, depends. Individual variation in parental care is of particular interest because it can have life-long consequences for both parents and offspring. Historically, most research on parental care has focused on maternal and biparental care leaving a gap in our understanding of male-only paternal care. The dyeing poison frog (*Dendrobates tinctorius*) is characterized as a male uniparental species, with fathers caring for offspring by hydrating terrestrial clutches and transporting hatched tadpoles to ponds. Through detailed monitoring in the lab, we observed consistent individual differences among dads and surprising variation in the extent of female care, including regular take over the role of tadpole transport. To investigate the endocrine mechanisms underlying this variation and plasticity in both sexes, we measured hormones (testosterone, progesterone, cortisol, and corticosterone) and recorded behavior across the development of three clutches for eight breeding pairs. In addition to changes in individual parents, we characterized hormone synchrony between fathers and mothers. Identifying the mechanisms underlying variation and flexibility in parental care is an important step in understanding the downstream consequences of parental care and how parental care is differentially regulated between sexes.

ECOSTRESS 70 meter temperatures from space: a game changer for coastal/intertidal marine ecology

David Wetthey, Weidberg Nicolas, Sarah Woodin

The ECOSTRESS thermal infrared radiometer on the Space Station has a 70 m pixel size and a revisit time of hours to 5 days, depending on latitude. It is the precursor for the future TRISHNA (France-CNES / India-ISRO), SBG (NASA), and LSTM (European Space Agency) 70 m scale thermal missions planned for later in this decade. ECOSTRESS measures water and land surface temperature at spatial scales approximating patch sizes of organisms, and the scale of intertidal aquaculture sites. It resolves fine scale oceanographic features that are invisible in MODIS or VIIRS imagery. We measured fine scale gradients in low tide temperatures within intertidal shellfish beds in Spain and France. Shellfish beds in NW Spain had temperature gradients of up to 8°C between the low and high water lines at the time of low tide, enough to limit clam growth and reproduction in the upper shore. In Arcachon Bay, SW France, oyster culture racks in some areas reached temperatures limiting for oyster growth and survival. Larger scale geographic patterns of intertidal temperatures are consistent with physiological limitation of biogeographic distribution of the ecosystem engineering polychaete *Arenicola marina* on the French and Iberian coasts.

Impacts of Bacterially Mediated Chemical Interactions on Viral Success in Marine Ecosystems

Kristen Whalen, Oscar Garrett, Maya Cheam, Elizabeth Harvey, Marta Wilbrink

Bacterially mediated chemical interactions have far-reaching impacts on phytoplankton physiology and ecosystem dynamics. Likewise, viruses, the most numerous biological entities in the marine environment, are responsible for mortality of 20% of the oceanic photosynthetic biomass daily, dictating the flow and fate of carbon and nutrients. Here, we show two mechanisms by which different bacterial compounds alter viral success and the fate of phytoplankton hosts. Using the globally abundant, marine coccolithophore *Emiliania huxleyi* as our phytoplankton model, we demonstrate how a bacterial

communication signal isolated from the marine bacterium *Pseudoalteromonas* sp., is a potent inhibitor ($K_i = 2.3$ nM) of dihydroorotate dehydrogenase (DHODH), a fundamental enzyme in pyrimidine biosynthesis. Moreover, in vivo inhibition of *E. huxleyi* DHODH significantly decreased *E. huxleyi* virus (EhV) replication and nearly eliminated infectious virus titer. During normal EhV infection, newly replicated viruses acquire a lipid membrane upon budding from the host phytoplankton cell. Disruption of viral membranes by detergent-like compounds can compromise viral stability, resulting in nucleic acid leakage. Here, we identified a new antibiotic from the marine bacterium *Olleya* sp. which targets the destruction of the lipid bilayer in Gram-positive bacteria. When EhV was treated with the antibiotic, the titer of infectious virions significantly decreased relative to control. Together, these results demonstrate how bacterial compounds govern complex tripartite interactions between a phytoplankton host, a virus, and bacteria.

Phylogenetic Investigation of Phytolith Hardness and its Ecological Significance to Dental Wear

Niall Whalen

Phytoliths are minute silica deposits found within the tissues of all major vascular plant clades. Phytoliths, amongst many other roles, purportedly contribute to vertebrate herbivore deterrence by acting as tooth-wearing abrasives. This proposed ecological role inspired a classic coevolutionary hypothesis; the Cenozoic evolutionary arms race between ungulates and grasses—the former evolving high crowned, hypsodont cheek teeth coincident with the acquisition of increasing phytolith densities in the latter. Previous studies into poacean phytolith hardness suggest that such a scenario is perhaps plausible. These studies suggest phytoliths are unable to directly wear enamel but may indirectly contribute to its wear by abrading adjacent dentine. However, phytolith hardness—known from only very few, near-exclusively poacean taxa—remains poorly understood. It remains unknown if dentine-abrasion represents a derived material property unique to poacean phytoliths, or simply represents the basal condition common to all phytoliths. Here we examine the understudied yet highly ecologically diverse gymnosperms to gain a new understanding of the phylogenetic diversity of phytolith hardness and further explore the significance of dentine-abrading phytoliths in Poaceae. Our results suggest phytoliths of dentine-abrading hardness predate the poacean (and larger angiosperm) radiation, as gymnosperm and poacean phytoliths exhibit identical Mohs hardness values. This implies phytolith abrasiveness is not evolutionarily modified through changes in hardness, leaving only changes in phytolith abundance or morphology as potential biological avenues for altering plant abrasive capacity.

Microplastics Bioindicators! Using Wildlife Carcasses to Explore Plastics Pollution

Katie Wheeler, E. Olsen-Hodges, Karen Powers, Brian Walker, Sara O'Brien

Microplastics have become a widespread pollutant across the globe. Microplastic pollution originates from a variety of sources including the breakdown of larger plastic pollutants from consumer waste, small plastic particles from cosmetics, and industrial plastics of various sizes. Unfortunately, the widespread phenomena of plastic use and subsequent pollution means that many species across the taxonomic scale are exposed to microplastic pollution. While most research focuses on the pollution of microplastic in marine and aquatic environments, our study offers an opportunity to research the presence, abundance, type, and tissue distribution of microplastics in a variety of wildlife species. In partnership with the Southwest Virginia Wildlife Center of Roanoke, we receive carcasses of regional wildlife across a variety of taxa. Carcasses are sampled for GI Tract, liver, heart, and muscle. Carcass documentation contains location found, any subsequent feedings or medical attention, and body condition. Once tissue samples are collected, they are digested, suspended, and filtered to remove biological debris and highlight microplastics to be identified by number, shape, color, and type. Through this non-profit and academic partnership we hope to demonstrate the degree of microplastic pollution in Virginia wildlife.

Developmental impacts of environmental hypoxia on embryonic anemonefish

Nicole Whelpley, Kathryn Kavanagh

Climate change challenges coral reefs. As sea temperature increases, dissolved oxygen decreases. During the embryonic development, a large increase in oxygen consumption occurs primarily due to muscle tissue expansion. Exposure to chronic hypoxia during this time may induce phenotypic change that could affect larval performance and the fish's final form. To determine effects of environmental hypoxia on survival rate, morphological growth, and patterning during embryonic development, Amphiprion (Prenmas) biaculeatus embryos were exposed to dissolved oxygen treatments of 8 mg/L 'Control', 4 mg/L 'Low DO' or 2.5 mg/L 'Hypoxia' from day 1 to 6 of development. Notochord length and eye diameter were found to be significantly smaller in 'low DO' and 'hypoxia' treatment groups, while yolk area was larger, indicating reduced metabolism. Greater proportions of 'control' embryos survived to day 6. Future perspectives will address larval muscle development, and potential impacts this may have on swimming performance. Changes to morphology, survival, and performance seen during development may lead to new evolutionary patterns of diversity that are important to understand with the changing environment.

Daphnia devel-EYE-ment: A comparative analysis of compound eye size growth among Daphnia species

Alia Whiles, Christopher Brandon

Eye size is an important determinant of visual function. The freshwater microcrustacean, Daphnia, possesses a single compound eye that may aid in resource location, navigation, and orientation within the water column. In addition, the compound eye may be a target of visual predators as the dark pigmentation of the eye stands out against the transparent carapace of Daphnia. Thus, the size of the compound eye in Daphnia may be important for both visual and non-visual reasons. Daphnia grow indeterminately throughout their lifespan, where their eye size growth is correlated to increases in body size. To better understand the nature of eye size variation in Daphnia, it is worthwhile to investigate how the eye size may vary throughout its lifespan. In this study, we examine the growth patterns of the compound eye across a few species of Daphnia. Our results demonstrate some variation in growth patterns across Daphnia species. In addition, our results show that, overall, Daphnia invest more in eye growth relative to body size before the first instar, whereas the relative size of the eye remains constant after the first instar. Our findings draw a more complete picture of eye size variation in Daphnia that may give a broader understanding to former and future studies on eye size variation in Daphnia, and potentially other indeterminately growing species.

Effect of inertia on muscle force-velocity properties: Submaximal recruitment as a window to scale

Tyler Whitacre, David Labonte, Natalie Holt

Animal size carries important functional consequences. Recent theoretical work suggests muscle contraction speed (V_{max}) decreases with size due to greater inertia, as increases in mass (volume) outpace force capacity (cross-sectional area). Here we empirically test this, asking whether greater inertial loads, induced both by decreasing muscle activation level and increasing muscle mass, depress V_{max} . We predict that reduced muscle activation will decrease V_{max} , as the active force and thus acceleration will decrease while muscle inertia remains constant. Additionally, this activation-dependent velocity depression should be greater in larger species due to greater muscle inertia. We examined the effect of activation (100-60-40-10% recruitment) on the V_{max} of the plantaris muscle in-vitro in two species with a seven-fold difference in muscle mass; *Rhinella marina* (0.90 g) and *Hyla cinerea* (0.13 g). *Rhinella marina* results ($N=5$) indicate V_{max} at 60, 40, and 10% recruitment was 1.5, 1.7, and 3.0 times slower than at maximal (7.59 l-s). Shortening velocity was significantly slower between 40-100%, 10-100%, and 10-40% recruitment. Preliminary data will be presented for *Hyla cinerea*. This work demonstrates the role of inertia in muscle performance and highlights how emergent muscle properties can shape contractile dynamics and contribute to the effects of size on locomotor performance.

The Role of Symbiosis in Energetic Preparation for Asexual Development in Aiptasia

Erick White, Virginia Weis

Corals engage in an endosymbiotic relationship with dinoflagellates housed within their gastrodermis. Symbionts supply the host with photosynthates while the host provides a habitat and inorganic nutrients. Part of corals' success comes from their ability to rapidly repopulate via asexual reproduction. While adult energetics surrounding symbiosis is partially understood, it remains unclear how it works in reproduction in corals. The purpose of this study was to examine how adults provision asexual offspring for development based on symbiotic state and reproductive intent. We asked whether asexual offspring were better provisioned nutritionally if they were naturally or artificially made and whether the presence of symbionts affected nutrient level in the symbiotic anemone model system Aiptasia. Aiptasia form asexual offspring by pinching off a piece of the pedal disk to form a clone of the parent, which can also be formed artificially by cutting. We harvested naturally- and artificially-made offspring of the same size from (apo)symbiotic adult Aiptasia. Samples were pooled to produce homogenates that were used for total host levels of lipids and carbohydrates, respectively; protein levels via a Bradford assay and total algal counts were used to normalize data. We found no significant differences between any group in nutrient levels based on symbiotic state or method of production. Future studies will examine how the feeding regimen of the parents affects lacerate nutrient provisioning.

Soliciting SICB Community Feedback on a New Comparative Vertebrate Anatomy Text

Lisa Whitenack, William Ryerson, Amy Cheu, Vanessa Young

Current comparative vertebrate anatomy (CVA) textbooks are out of date, expensive, and often lacking in the human element of comparative anatomy - both in terms of anatomy and representation of the humans that practice in the broader field. Existing texts provide a brief historical overview; however, these overviews fail to acknowledge the diversity of scholars in the field, both historically and currently. They also lack an honest accounting of the history of the individuals who are included, as well as recognition of the impacts of their work on others. This narrow view carries over to the representation of the organisms featured in anatomy books as well, which tend to focus on male anatomy and binary sex. We are developing an open-access CVA textbook that will address these issues by including recent work on the systems studied in comparative anatomy, emphasis on the people who work in the field, a wider history of the field, and the impact of the work on people. Further, this book will contain more comprehensive female anatomy content than previous textbooks and will follow published recommendations for making anatomy more inclusive for marginalized groups. This interactive poster will serve as a place to collect feedback from the SICB community regarding the proposed scope, content, and pedagogical elements of this textbook.

Developing a stickier skin: A novel role of leptin in mucociliary epithelial homeostasis

Kournie Whitfield, Robyn Reeve, Grace Curtis, Erica Crespi

Dysfunction in the respiratory tract's mucus barrier is a major contributor to the severity of respiratory diseases. Hyperplasia of mucus-secreting cells, mucus over-production, and mucus hypersecretion of the respiratory mucociliary epithelium (RME) are associated with respiratory distress and disease, but what causes these conditions is poorly understood. Our research explores the role of leptin as a modulator of ME differentiation and mucus secretion. Previous studies have shown elevated circulating leptin levels are associated with respiratory disease and adverse response to respiratory infection, and leptin signaling promotes mucus secretion in gut epithelium in mammals, but little is known about leptin's actions on mucus secretion or differentiation of mucosal cell types in the RME. Using the embryonic epidermis of the frog *Xenopus tropicalis*, an in-vivo model for mammalian RME, to test how leptin signaling affects mucus production, secretion, and embryonic cell-differentiation, we found leptin protein is highly expressed in small secretory cells (SSCs), a mucus-secreting cell type, suggesting autocrine and/or paracrine actions of leptin. Both immunoneutralization of leptin and CRISPR/Cas9 knockdown

of leptin reduces mucin production in the embryonic epidermis, which we confirmed with confocal and scanning electron microscopy to be the result of fewer SSCs. These findings are the first to support a role for leptin in RME differentiation of mucus secreting cells, and future experiments will investigate whether leptin signaling stimulates mucus production/secretion regulation.

Developmental plasticity of stress tolerance and the microbiome due to glyphosate-based herbicide

Jacob Whitlock, Zach Stahlschmidt, Paul Orwin

Glyphosate (GLY) is the most used pesticide worldwide, as well as in California where rising temperatures and extended drought are increasingly prevalent. Animals may be susceptible to GLY through their microbiomes because GLY targets the shikimate pathway, which is only found in plants and some microbes. The gut microbiome is integral to animals' physiological defenses systems (e.g., immune responses)—thus, it is important to understand how animals stress responses are affected by GLY exposure. Here, we exposed the variable field cricket (*Gryllus lineaticeps*), which is mainly found in California, to glyphosate-based herbicide (GBH) through development to examine GBH's effects on developmental plasticity for a range of life history traits (e.g., survival, development, body size, and investment into reproduction and flight capacity), stress (heat and desiccation) tolerance, and the gut microbiome. Our study is the first to examine the developmental plasticity of an animal's stress tolerance in response to GBH exposure, and it is among the first to examine developmental plasticity of the microbiome due to GBH. Therefore, we will provide new insight into interconnections among increasingly common environmental stressors (GBH, heat, and desiccation) and the gut microbiome.

Smallmouth bass individuals show differential performance in feeding and escaping across temperature

Katrina Whitlow, Grace Johnston, Molly Schneider, Emily Volpe

Successfully capturing food and escaping predation are essential for an organism's survival and fitness. The mechanics of prey capture and escape behaviors are well-documented in fishes, though our understanding of the determinants of this performance is limited, particularly in light of ongoing and predicted environmental changes. Furthermore, population-level trends may differ from individual fitness impacts. This study compares the effects of ambient water temperature (15, 20, 25, and 30°C) on predatory feeding performance and escape response performance in a heat-sensitive fish, smallmouth bass (*Micropterus dolomieu*). These studies found similar detrimental effects of extreme temperatures on strike success rates and responsiveness to predatory stimuli at the population level, but showed surprising individual variation. Specifically, many fish with high escape response rates also showed a low strike success rate — and vice versa. Extreme temperatures are overall detrimental to fitness at population levels, but which aspects of fitness are more dramatically affected appears to vary based on individual fish. Future work should also explore how differential thermal performance curves of predator and prey species may impact predator-prey interactions under future climate scenarios. This work reinforces the need for detailed analyses of climate impacts at all levels of biological organization to best predict future outcomes.

Bendy Hyoids: In-Vivo and Ex-Vivo Loading and Stiffness of the Hyoid Arch In Elasmobranchs

Cheryl Wilga, Lara Ferry, Elizabeth Dumont

Tessellated cartilage forms much of the skeleton of sharks and rays, many of which also regularly generate exceptionally high forces in the execution of day-to-day activities. Tesseræ are hypothesized to play a role in stiffening the cartilaginous skeleton for food capture and other activities that require the generation of high forces. In this study, the hyomandibula and ceratohyal cartilages, which support the jaw and throat regions of sharks and rays, were tested under compressive load in a material testing system to determine the contribution of tesseræ to stiffness. Second moment of area (I), a proxy for stiffness, was calculated from cross-sections. Young's Modulus (E) was calculated and used to evaluate cartilage stiffness in a range of elasmobranch species with and without tesseræ. In-vivo results show strong

evidence that the hyomandibula bends during mouth opening and closing. MTS results revealed that there was an abrupt shift in Young's Modulus for tessellated elements loaded in compression. We postulate that this shift, characterized by an inflection point in the stress-strain curve, is the result of the tesseræ approaching one another and compressing the intervening fibrous tissue, supporting the hypothesis that tesseræ function to stiffen these cartilages under compressive loading regimes. A suite of variables describing the cross-sectional shape, size and strength do discriminate among jaw suspension types in multivariate space (but less so between prey capture modes).

The Mammal Jaw as a Web Flange Structure: Towards a New Paradigm of Mandible Function

Alec Wilken, Austin Lawrence, Felipe Prado, Amanda Smith, Ana Rossi, Alexandre Freire, Zhe-Xi Luo, Callum Ross

The primary function of the tetrapod mandible is transmitting input muscle forces to a bite point. While the architecture and distribution of bony material has historically been linked to trajectories of biomechanical loads, methodological and imaging limitations have hindered a comprehensive correlation between the distribution of bony material and the exact sites of load transfer. Here we employ Finite Element Analysis (FEA) and a novel load path analysis to quantify sites of high force transfer in a wide array of mammal jaws, including *Didelphis*, *Canis*, *Panthera*, *Rattus*, *Tupaia*, and *Pan*. We used the R package Morphomap to quantify cortical bone thickness and compare mandible cross-sectional geometry against the location of the load path. We found that load paths are consistently located on the ridges in the mandibular ramus in our sample. These data provide a new framework for functional interpretations of the mandibular ramus. Ridges act as load paths to transmit forces from bony flanges acting as muscle attachment sites to the bite point and jaw joints. This study provides a framework for future research seeking to understand the relationship between skeletal morphology and biomechanical trajectories.

Elevated Dazl expression is associated with warmer temperatures and estrogens in *T. scripta* embryos

Madison Wilken, Bert Foquet, Clinton Warren, Ryan Paitz, Rachel Bowden

Primordial germ cells (PGCs) arise early in embryonic development, migrate to the gonads once they begin to develop, and ultimately give rise to oocytes or spermatogonia in many animals. For species with genetic sex determination, PGCs share sex-specific genotypic information with gonadal cells, while in species with temperature-dependent sex determination (TSD), PGCs arrive at a bipotential gonad that may still develop into a testis or an ovary. It was thought that as gonadal differentiation occurs, PGCs respond to gonad specific cues to differentiate towards oocyte or spermatogonia fates, respectively. However, recent research in the red-eared slider *Trachemys scripta* suggests that PGCs may actually respond to temperature before sex is determined, playing a more active role in sex determination. Specifically, these studies found female-producing temperatures (FPTs) tended to promote higher germ cell counts that coincided with subsequent ovary development. Here we characterize how PGCs respond to estrogens in *T. scripta* by quantifying the expression of a well conserved marker of meiosis (deleted in azoospermia-like, *Dazl*). We found higher *Dazl* expression under FPTs (31°C) than male-producing temperatures (MPTs, 26°C). We also found evidence that *Dazl* expression was induced by biologically relevant doses of estradiol prior to gonadal differentiation. These preliminary results provide insight into how PGCs may be influenced by the developmental environment before gonadal differentiation under TSD.

The Anolis gut microbiome: environmental influences and impacts on host physiology

Claire Williams, Carrie Alfonso, Karla Alujevic, Leah Bakewell, Samantha Fontaine, Jaden Keller, Yanileth Lopez, Nathaly Ponce, Alejandro Vivas, Kelly Wuthrich, W. Owen McMillan, Candace Williams, Christian Cox, Michael Logan

As rising temperatures threaten biodiversity, tropical ectotherms may be particularly vulnerable due to their narrow thermal tolerance ranges. Although

most studies of ectotherm responses to rapid environmental change focus on tolerance traits of the host, resident gut microbes may also affect thermal physiology and can change rapidly in response to the environmental conditions experienced by the host. Thus, shifts in gut microbiome communities might play a major role in adaptation of hosts when their thermal environments change quickly. Regardless, real-time field studies on the responses of gut microbial communities to changing environmental conditions are exceptionally rare. We transplanted slender anoles (*Anolis apletophthalmus*) from a mainland population to ten warmer islands in the Panama Canal. We collected fecal samples from all individuals prior to transplantation, and on four islands we repeatedly sampled the same individuals to observe shifts in their gut microbiomes in the weeks after transplantation. We then sampled F1 offspring from two of these same islands to characterize cross-generational shifts in microbiomes. We paired this field study with a controlled laboratory experiment where we altered lizard gut microbiomes with antibiotics and evaluated effects on physiological traits. We discuss how our discoveries about microbiome dynamics in warmer environments generate insight into host adaptation to our rapidly changing world.

The behavioral effects of 17 β -estradiol exposure on wood frog tadpoles.

Mitch Williams, Gina Ledermann, Miranda Miller, Isaac Ligocki

Understanding the effects of chemical pollutants on wildlife is critical to more adequately assess and potentially mitigate human induced environmental change. Many chemical pollutants are considered endocrine disrupting contaminants (EDCs); numerous studies have described estrogenic effects of particular EDCs, although what constitutes such a change vary widely within and between species. To better understand potential impacts of EDCs and generate predictions regarding the impacts of compounds thought to have estrogenic effects, we exposed wood frog (*Lithobates sylvaticus*) tadpoles to 17 β -estradiol to determine whether tadpoles differed behaviorally depending on treatment. We exposed tadpoles to one of two concentrations of 17 β -estradiol (10ug/L and 100 ug/L), a vehicle control, or fresh water for 48 hours, after which tadpoles underwent repeated behavioral tests over the course of several months until metamorphosis. We measured activity levels of the tadpoles by placing a single individual into a 20cm by 30cm arena with a 5 cm grid printed on it and counted the number of gridlines crossed by the tadpole as it swam over the course of a 10-minute observation period. We found no significant differences in activity levels between treatment groups or across the sampling timeline. While these concentrations of 17 β -estradiol did not elicit differences in activity level, these findings contribute to our general understanding of the behavioral and physiological impacts of exposure to steroid hormones which will ultimately contribute to a more comprehensive understanding of the potential impacts of EDCs on wildlife.

Genetic Sex Determination in Octopuses

Sierra Joy Williams, Kirt Onthank

Cephalopods are an ecologically and commercially important group of marine organisms. However, there is, surprisingly, no published information regarding the mechanisms of sex determination in any species of cephalopod, and minimal information published regarding molluscan sex determination in general. To address this issue, we used ddRAD-seq (double digest restriction-site associated DNA) using EcoRI and MspI to search for possible sex-specific genomic loci. We obtained gDNA samples from twenty-eight ruby octopuses (*Octopus rubescens*), eighteen phenotypic males and ten phenotypic females, in order to illuminate the genetic sex differences of octopuses for the first time. These data on the genetic bias for sex determination in octopuses could be used not only to reveal the mechanisms on sex determination in this important group of animals, but could also form the basic assays to determine sex in life stages that do not have phenotypic sex markers, such as hatchlings.

Nematostella vectensis as a new meiosis research organism

Stefanie Williams, Matt Gibson, Scott Hawley

Sexually reproductive species use meiosis, a specialized cell division that produces haploid gametes which fuse to produce diploid progeny. While

the principles of meiosis are the same between species, there are notable differences in the mechanisms that control the segregation of homologous chromosomes. Additionally, even though meiosis has been studied for a century, there are lots of questions that have been left unanswered, some of which are: How do homologous chromosomes pair? How is crossover distribution molecularly regulated? What is the role of sexual dimorphisms? And how diverged are meiotic processes from an evolutionary perspective? It is hypothesized that many species-specific differences during meiosis are driven by the synaptonemal complex (SC). In 2012, Fraune et al. (PNAS, 2012) showed that the major SC components SYCP1 and SYCP3 are conserved in basal metazoans such as *Hydra magnipillata* and *Nematostella vectensis*. *Nematostella* has features that make it a useful organism to study meiotic processes: It possesses stem cells that provide a continuous supply for gametogenesis upon environmental changes in temperature and light and per spawning event each animal can release several hundreds of gametes. Furthermore, its translucent nature and the large size of the gonadal region relative to its whole body size provides potential for live-imaging studies that enables us to study the dynamics of its processes.

Extreme elevational migration spurs cryptic speciation in giant hummingbirds

Jessie Williamson, Ethan Gyllenhaal, Selina Bauernfeind, Emil Bautista, Matthew Baumann, Chauncey Gadek, Peter Marra, Natalia Ricote, Thomas Valqui, Francisco Bozinovic, Nadia Singh, Christopher Witt

The Giant Hummingbird (*Patagona gigas*) bends the rules of life. It is two-fold larger than any other hummingbird species, and it represents the longest terminal branch of the hummingbird tree of life (~14 Myrs). It is also unique among hummingbirds for its broad range, which spans 35 degrees of latitude and >4,200 m of elevation in South America. At tropical latitudes, giant hummingbirds reside year-round in the high Andes; however, southern temperate populations breed at sea level and vanish from breeding areas during the austral winter. Darwin speculated that they migrate to “the harsh deserts to the north”, but their migratory routes and wintering range have remained unknown. It is also unknown whether differences in elevational range and migratory behavior within *Patagona* are associated with genetic differentiation or cryptic diversification. Here we combined satellite and geolocator tracking with whole genome sequencing to uncover the migratory routes and wintering grounds of southern Giant Hummingbirds. Tracking revealed an epic circular migration, encompassing extreme elevational shifts and protracted stopovers. High-resolution satellite tracking illustrated a dramatic upward ascent of the Andes punctuated by periodic upward bursts, resembling acclimatization strategies of mountain climbers, and accompanied by similar blood adjustments. Genomes showed that migratory and non-migratory forms are strikingly differentiated. The two forms are separate species that occur side-by-side for half the year and differ almost imperceptibly in size, plumage, and respiratory traits.

Do Insects Sniff with Their Wings?

Mark Willis, Shivansh Dave, Vivian Wheeler, Peter Coggan, Kim Thompson

When more information is necessary to detect or identify an odor, individuals in many species, including humans, sniff to increase the amount of odor molecules encountering their olfactory sensors. Insects may exhibit a similar behavior by fanning their wings to increase the flow of air and odorants over their odor-detecting antennae. During courtship behavior, males of the oriental fruit moth (OFM), *Grapholitha molesta*, fan their wings continuously. This behavior increases the flow of pheromone-bearing air over the males' antennae. To test the idea that wing fanning is an important odor sampling behavior for these insects, we surgically removed the wings of a group of OFM males and compared their ability to track a female moth's sex pheromone up a wind tunnel in varying wind speeds to OFM males with intact wings. All moths in our experiment located the pheromone source in the highest wind speed. As the wind speed decreased to 0 m/s, the importance of wing beat induced flows became apparent. In zero wind only 56% of the males with their wings removed located the pheromone source while 93% of intact males located the source. These results suggest that the wing fanning behavior of

walking OFM males aides in detecting and tracking odors by increasing the air flow and delivery of pheromones to the antennae. In other words, OFM males sniff with their wings.

Scaling analysis of taenidia in beetle (*Zophobas morio*) tracheae

Sara Wilmsen, Sasha Zaslavsky, Devyn Hopkins, Brock Palm, Saadbin Khan, Viktor Nikitin, Pasha Shevchenko, Anne Staples, Jake Socha

Insects rely on a network of tubes called tracheae that directly deliver oxygen to the tissue. Along the length of most tracheae, ring-like structures called taenidia line the inner wall. Taenidia have been commonly understood as structural features that strengthen the tracheal wall. However, it has been shown that in many insect species, tracheae collapse periodically with pressure changes in the body cavity, producing bulk flow of air within the tracheae. This dynamic deformation challenges our understanding of the functional role of taenidia in the tracheal system. Based on their location and geometry, it is possible that the taenidia may influence airflow patterns. However, to study airflow, we must first understand the three-dimensional geometry of taenidia and how they scale with tracheal size. Here, we analyzed taenidia from darkling beetles (*Zophobas morio*) across a range of tracheal sizes (diameter, 1-300 μm). Tracheae were imaged using synchrotron x-ray imaging at Argonne National Laboratory. Tomographic images were segmented and rendered, then measured using imaging software. The scaling analysis included taenidial width, height, and spacing, with cross-sectional area as a measure of tracheal size. Preliminary results suggest that taenidial geometry scales hypometrically with tracheal size, but spacing and geometry scale isometrically. These findings are being used for the creation of idealized tracheal models for computational fluid dynamics studies, whose functional implications may impact design of insect-inspired microfluidic devices.

Ear Me Out: The Role of the Mandible in Mysticete Auditory Reception

Madison Wilson, EW Misty Paig-Tran, Petr Krysl, Ted Cranford

Baleen whales, or mysticetes, rely mainly on bone conduction to funnel sound into their inner ears. These whales have lost the ability to hear some of the higher ultrasonic frequencies, and thus specialize mainly in hearing within lower frequency ranges than the toothed, or odontocete whales. Odontocete auditory reception works by using a combination of bone conduction and a system that utilizes the lipid fat bodies that reside in their mandible. Mysticetes do not retain these fat bodies-and the nature of the mechanism by which the mysticetes hear is still unknown. This study used finite element analysis software and CT scans to investigate whether the mandible plays an integral role in mysticete auditory reception. A CT scan of a juvenile fin whale was used in conjunction with BoneShaker software to computationally model the effects of sound waves on the tympanic bulla. Our models simulated a full field force excitation, wherein a range of sound frequencies encountered the entire skull and the subsequent movements of the skull were quantified in Paraview software. Virtual sensors were placed at the footplate of the stapes to quantify the changes to the velocity relative to the outer skull under stimulation from 10-700 Hz and with varied jaw connectivity (fused, jointed, and disarticulated). We found that varying the morphology of the jaw connectivity did not have a measurable effect on sound reception (stapes movement).

Gestating at the top of the world: Adaptive evolution of reproductive traits at high elevations

Kathryn Wilsterman

A major goal of biology is to understand how, at ultimate and proximate levels, organismal diversity has emerged across the landscape. Adaptive evolution is a major force in such diversification and has generated remarkable diversity in physiology and environment-organism interactions. As the ultimate arbiters of fitness, reproduction and reproductive traits (contrasted here with performance and survival-related traits) are a critical to these patterns; the ability to reproduce in novel environments is a requisite step to adapting and persisting therein. Nonetheless, few examples of adaptive evolution in reproductive traits

in females have attempted to link genetic variation to reproductive physiology and function. My lab uses high elevation-adapted deer mice (*Peromyscus maniculatus*) as a model to investigate how reproductive traits evolve and adapt in response to environmental challenges. Hypoxia at high elevations leads to lower offspring birth weights and increased mortality across mammals, including in humans and deer mice. However, high elevation-adapted populations appear to be relatively protected from these adverse outcomes. In this talk, I will discuss our recent work investigating the physiological and genetic basis of gestational adaptations in deer mice, and I will preview current and up-coming work that expands beyond placental adaptations to consider fetal-maternal interactions more broadly.

Using Bayesian multi-level modeling to predict habitat importance in Ruminantia

Alexa Wimberly, Jonathan Nations, Graham Slater

Identifying the form-function relationships that exist in extant species is crucial for inferring the ecology and evolution of extinct taxa. However, studies seeking to predict habitat use from morphological traits typically use discrete categories, which may be effective for statistical analyses but fail to account for the multivariate nature of the environmental contexts in which mammals exist. Here, we employ Bayesian multi-level modeling and rank the importance of 7 different habitat types for 208 living ruminant artiodactyls based on IUCN Redlist data. We use two functional indices from the hindlimb to develop predictive models of habitat importance: the calcaneal gear ratio indicative of in-lever mechanics of the hindlimb and metatarsal/femur (MT/F) ratio as an index of cursoriality. Results from the predictive models using body mass and phylogeny as covariates show that differences in gear ratio did not strongly predict habitat importance outside of the grassland habitat, where mean and large bodied species with a low gear ratio (indicating a relatively short calcaneal tuber) have a higher probability of inhabiting grasslands. However, MT/F ratio strongly predicted preference for forest, savanna, and grassland habitats with body mass showing a strong, but varied, effect on the relationship between MT/F ratio and the relative use of each habitat. These results suggest that more nuanced prediction of habitat use in extinct ruminants is possible.

Life Cycle Transitions in the Freshwater Jellyfish, *Craspedacusta sowerbii*

Kent Winata, Jonathan Zhu, Jessyn Langguth, Chloe Davis, Micah Perry, Nadine Folino-rorem, Paulyn Cartwright

The invasive jellyfish *Craspedacusta sowerbii* can be found in freshwater bodies on all continents excluding Antarctica. *C. sowerbii* has a complex life cycle including a planula larva that transforms into a polyp. The polyp can asexually bud other polyps to form a small colony as well as bud frustules that disperse and develop into new polyps and colonies. Polyps bud the medusae stage (jellyfish) which upon reproductive maturity will spawn gametes and upon fertilization repeat the life cycle. Polyps can also produce podocysts, which are a dormant stage that can eventually transform into new polyps. The aim of this study was to determine the optimal laboratory conditions for growth of and transition to different *C. sowerbii*'s life cycle stages. Frustules were established at different temperatures and observed for 3 weeks to determine the optimal temperature for transitioning from the frustule stage to polyp. Podocysts were subjected to different environmental stresses to determine the environmental cue(s) for dormancy and transitioning to a polyp. Finally, after exploring both nutrient-abundant and nutrient-deprived conditions in mature colonies, it was determined that nutrient deprivation induces medusa budding. This research will not only enable *C. sowerbii* to be more easily reared in the laboratory, but provides insights into the conditions in which this invasive jellyfish can spread and thrive in a wide range of environmental conditions.

Unseen Opportunity: Do Hermaphrodite Cues Influence Male-Male Aggression in Mangrove Rivulus Fish

Molly Wingard, Lindsey Wells, Andrew Fuller, Giovanna Lopez, Mark Garcia

Aggressive contests in fish often occur in the presence of bystanders, which can affect contest dynamics and outcomes depending on the sex of the audience members. For example, a female audience can elicit increased use of low-intensity displays and decreased use of high-intensity attacks between the male combatants. Mangrove rivulus (*Kryptolebias marmoratus*) fish occur naturally as either self-fertilizing, simultaneous hermaphrodites or functional males. Male rivulus' reproductive success thus depends on their ability to obtain outcrossing opportunities with hermaphrodites. Our study's aim is to examine whether hermaphroditic chemical cues in the absence of visual cues influence the intensity of male-male rivulus contests and if unequal access to these cues provides a competitive advantage. We dosed focal individuals with either 10ml of DI water (control) or 10ml of water laced with hermaphroditic cues (treated). Following, we created size-matched pairings that resulted in three combinations of contestants; control-control, control-treated, and treated-treated. Contest intensity and outcomes were then quantified and compared across combinations. We predict that control-control pairings will display higher levels of high-intensity aggressive behaviors compared to treated-treated pairings. Further, we predict that treated males will decrease their use of high-intensity aggressive behavior and thus be at a disadvantage when faced with control males. Results of our study may provide insights into whether the unique reproductive strategy of the mangrove rivulus influences male-male aggressive contests.

The Effects of Climate Change and Urbanization on Hummingbirds' Ranges

Sierra Winter, Chris Clark

Finding the effect of climate change on vulnerable, widespread taxonomic families-like the hummingbird family, Trochilidae-may help us to better understand how much the climate can change in a region before influencing species' abilities to live there. To predict responses to climate change, we modeled the future ranges of the 14 hummingbird species that breed in the United States. Using the online Global Biodiversity Information Facility database, we downloaded historical (1970-2000) occurrence data for each of aforementioned species. Using WorldClim, we compiled climatic data for the hummingbird occurrences' GPS locations. We then used species distributional models in RStudio to create a map of their ranges from 1970-2000. Based on the climatic influences on their historical ranges, we mapped climate-driven future ranges for each hummingbird species based on WorldClim's climatic predictions for 2030-2050. Certain hummingbird species are affiliated with urban areas, most likely due to the resource subsidies (hummingbird feeders and gardens) that humans provide, while other species avoid urbanized areas and prefer more rural or undeveloped habitat. To test the effects of urbanization on the predicted future ranges, we then layered the historical and projected range maps and plotted modern (2000-2020) occurrences on top of them, which should be in-between the different ranges. For areas where a hummingbird species was incongruently absent or present, we analyzed whether that area was urbanized or rural.

Hierarchy, tempo and mode in primate body size evolution

Anna Wisniewski, Graham Slater

A species' body size relates to its ecology and physiology in myriad ways. Correspondingly, the evolution of body size has received much attention both as a proxy for various aspects of species ecology and as a trait of interest in and of itself. However, patterns of body size evolution seem to be clade-specific and it is difficult to derive general macroevolutionary predictions for how body size evolution should proceed over a clade's evolutionary history, despite there being several descriptive models, e.g., Cope's rule and early burst patterns. Primates are an ideal model system to investigate patterns of body size evolution because of their outstanding historical and modern ecological diversity and great variance in body size. Here, I evaluate competing hypotheses for the tempo and mode of primate body size

evolution using a comprehensive phylogeny and data set of body sizes of 600+ species of living and extinct primate. Preliminary results suggest that primate body size evolved under a fractal-like early burst pattern, with individual subclades experiencing rates of body size evolution that are rapid early in the group's evolutionary history and decline exponentially over time.

A Robotic Model Organism to understand the Multi-medium Locomotion of Flying Fish

Aimy Wissa

Locomotion strategies vary significantly across species, even within the same medium. Understanding such strategies is especially challenging when the organism moves in multiple mediums and at the interface. In this talk, we present our recent efforts in developing physical and analytical models to investigate the aerial-aquatic locomotion and transition of flying fish. Flying fish have evolved this unique ability to taxi along the water's surface as they transition from swimming to gliding. During this transition, the fish is partially submerged in the water while its enlarged pectoral fins are fully deployed above the water's surface. Rich physics governs this transition, including hydrodynamic and aerodynamic forces and fluid-structure interactions between the flexible fins and the fluid mediums, to mention a few. The presentation introduces a robotic model organism (RMO) that is biologically relevant to the flying fish regarding shape, material properties, and non-dimensional fluid parameters. The RMO was instrumental in uncovering the role of the pectoral fins during the gliding locomotion mode through wind tunnel experiments and the effect of the caudal fin flexibility during swimming and taxiing through water channel experiments. An RMO is a powerful approach to answer key questions and uncover critical questions about locomotion. It allows us to design controlled experiments to test form-function hypotheses and explore a large parameter space beyond what is observed in nature to test evolutionary hypotheses.

Hawk and Roll: Aerodynamics of Harris's Hawk-Inspired Wingtips In and Out of Ground Effect

Hannah Wiswell, Aimy Wissa, Girguis Sedky

Among birds, some are specialists. For example, seagulls rely mainly on gliding flight and have high aspect ratio wings. In contrast, Harris's hawks and other birds of prey are flight generalists capable of agile and efficient flight. They perform frequent take-offs and landings, carry significant payloads, fly near solid and free surfaces, and can hover for extended periods of time. Some wing features of birds like the Harris's hawk are moderate aspect ratio wings, which are associated with maneuverability, and wingtip slots that are thought to enhance efficiency by mitigating tip vorticity effects. The wingtip slots are formed by the emargination of the primary feathers. This emargination also shifts the center of shear of a given feather relative to its center of mass, coupling the feather's out-of-plane bending with torsion. In this study, we experimentally evaluate several biologically relevant wingtip designs, investigating the aerodynamic effects of the wingtip slots and bending-torsion coupling. Additionally, we investigate the effect of the wing tip devices when the wings are operating in ground effect by placing various solid surfaces under the wings at varying distances. Experiments are conducted in the 4'x4' wind tunnel facility at Princeton University at $Re = 2 \times 10^5$. Time-resolved forces and flow field measurements will be used to understand how the wingtip devices alter the vortex structures, especially in the presence of strong ground effects.

Sex differences in the genetic basis of fitness and their implications for sexual conflict in anoles

Tyler Wittman, Rachana Bhawe, Heidi Seears, Aaron Reedy, Robert Cox

Studies of wild populations often infer intralocus sexual conflict from sex differences in selection on phenotypes, but few measure it directly by estimating the between-sex genetic correlation for fitness. We used 6 generations of mark-recapture and genetic parentage data from an island population of brown anoles (*Anolis sagrei*) to measure selection on body size, a sexually dimorphic trait that has been implicated as a source of sexual conflict. To directly test for intralocus sexual conflict, we used genetic pedigrees and Bayesian

animal models to measure additive genetic (co)variances for body size and fitness in each sex. Across five annual breeding seasons, males consistently experienced strong directional selection for large size. Although selection was weaker in females, it also favored large size, suggesting that body size is not a major source of ongoing sexual conflict. Body size was strongly genetically correlated between sexes, but the between-sex genetic correlation for fitness was effectively zero, despite significant additive genetic variance for fitness in each sex. Body size was genetically correlated with fitness within each sex, indicating that selection acted on genetic variance in size. However, body size and fitness were not genetically correlated between sexes. We conclude that sexual conflict is relatively weak and unrelated to body size in this population, and that the loci currently segregating genetic variance for fitness are different in each sex.

The diversity of resonance in insects

Ethan Wold, James Lynch, Jeff Gau, Brett Aiello, Nick Gravish, Simon Sponberg

Most insects indirectly actuate their wings by deforming an elastic exoskeleton with their flight muscles. The spring-like function of the insect thorax has led to the conception of insects as mechanical resonators, flapping at resonance to reduce the large power requirements of flapping flight, at the potential cost of frequency-modulation capacity. While it is known that insect wingbeat frequencies vary with wing inertia in a way consistent with resonant mechanics, it remains unclear whether resonance tuning constrains flight performance and control across taxa. We explore the roles of muscle and exoskeleton in determining insect resonant mechanics and integrate them with 'spring-wing' models. By comparing species of slow-flapping Bombycoid moths, we demonstrate that an insect's resonant frequency is not always strongly impacted by thoracic stiffness, and that many insects need not flap at resonance. Unlike moths, the wingbeat frequencies of fast-flapping insects like bees are not set by the nervous system, and are emergent by virtue of specialized flight muscles. To understand resonance in fast-flapping insects, we measure the elasticity of bee thoraxes, and show how interplay between muscle physiology and exoskeleton can give rise to emergent, supra-resonant wingbeats. Finally, we explore how evolutionary transitions may have occurred between slow-flapping and fast-flapping flight. Our results suggest insects are constrained by, but not bound to, their resonant frequency, enabling agile locomotion across many frequency orders of magnitude.

Divergent evolution of learning and memory brain structures in Pancrustacea

Gabriella Wolff

Mushroom bodies, lobed brain structures mediating learning and memory have been extensively studied in insects, but are they a novel evolutionary trait particular to this taxon? The discovery of insect-like mushroom bodies in mantis shrimp (Stomatopoda) suggests that these brain structures may have been inherited from a common ancestor with crustaceans. However, little is known about the brains of crustaceans apart from a few lineages including lobsters and crayfish which possess dome-shaped structures called "hemielipsoid bodies", analogous to mushroom bodies. A more comprehensive survey of malacostracan lineages allowed us to trace the evolution of mushroom body morphology in this clade. Mushroom bodies are described in the earliest evolved malacostracan lineages and persist in the later-evolved reptantians. In some lineages, lobed mushroom bodies are subsumed into lobeless structures or "hemielipsoid bodies." In other lineages, mushroom body characters have been secondarily lost or significantly reduced. Further supporting the hypothesis that mushroom bodies were present in the last common ancestor of Pancrustacea, we recently discovered evidence of these structures in ostracods which belong to the early branching superfamily Oligostraca. Conservation and loss of mushroom body circuitry will be discussed in the context of sensory ecology and learning and memory behaviors.

DNA-Barcoding Marine Invertebrate Diversity of Oman: phylum Nemertea

Audrey Wong, Ethan Kahn, Christina Ellison, Irina Cherneva, Svetlana Maslakova

Oman is surrounded by the Gulf of Oman and Arabian Sea — regions of high diversity and endemism. The boundary between these two bodies of water is among the sharpest biotic transitions. However, marine biodiversity of Oman remains largely undescribed. Nemerteans are soft-bodied marine invertebrates characterized by an eversible proboscis used for defense and predation. Despite their significance as predators, biomedical potential as toxin producers, and ubiquitous presence in marine ecosystems, they are among the lesser-known phyla, with ~1300 described species, and some 90% that remain unknown. No nemertean species has ever been reported from Oman. Based on recent sampling along the coast of Oman, Cytochrome Oxidase I gene sequencing, and species delineation analysis, we report 98 operational taxonomic units (OTUs, putative species) of Nemertea. Of those, only three can be confidently assigned to a described species (97% are undescribed), 93% are sequenced for the first time, 80% are not found outside Oman, and at least 43% are morphologically cryptic. Four distinct morphospecies have not been sequenced, bringing the total number of putative species to 102. Diversity estimates suggest that 42-44% of OTUs are yet to be sampled. Our work illustrates the vast magnitude of undescribed diversity on Earth, and highlights the importance of biodiversity surveys and methodological advances that expedite species discovery. Furthermore, the documentation provides a baseline for conservation and sustainable use of biodiversity.

The effect of wing morphing on the vibration properties of feathers in relation to air flow sensing

Jasmin Wong, Shane Windsor

Birds have a remarkable ability to adapt during flight to changing behavioural requirements and environmental conditions by morphing their wings. Flight control by wing morphing is thought to require reliable sensory information about the surrounding flow. As air flows over the wings, feathers deform dynamically in response to local aerodynamic forces. However, the mechanical receptors that may encode this vibration-based flow information are only found in the follicles at the base of the feathers. The goal of this study is to answer the following questions: 1) How can the feathers collectively modulate aeroelastic flow information passed to the receptors, and 2) How does wing morphing behaviour modify this structural signal processing? To address these questions, we dried zebra finch (*Taeniopygia guttata*) wings in various combinations of elbow and wrist flexion. First, we characterised how specific frequencies were processed by feather arrangement by measuring the frequency response along the length of select feathers in the wing as a result of sinusoidal acoustic stimulation ranging from 20-2000Hz. Following this, we related the aeroelastic behaviour of the flight feathers to changing flow conditions in a wind tunnel. These data can help us understand how structural modification through wing morphing can tune the range for flow sensing prior to further neural processing and inspire the development of morphologically "smart" distributed flow sensing technologies.

Phylogenetic inference from whole-genome sequences in bee hummingbirds (Mellisugini)

Jenny Wong, Chris Clark, Alan Brelsford, Sam Mansour

Hummingbirds in the Mellisugini tribe ("bee hummingbirds") are a model clade for the evolution of sexually selected morphology and behavior. Available phylogenies are based on a small number of primarily mitochondrial loci, which may not reflect true relationships due to incomplete lineage sorting and gene flow between species. To improve the accuracy of inferred phylogenetic relationships, we obtained whole-genome sequence data from 97 individuals of 29 species of bee hummingbirds, along with two other hummingbird species as outgroups. We explore the stability of inferred relationships to variation in the choice of reference genome for read alignment (ingroup vs. outgroup reference genome), tree inference method (maximum likelihood vs neighbor-joining), genome partition (whole genome, sex chromosome, or

ultra-conserved elements), and genotype filtering parameters. Finally, we scan the genome for loci that have experienced introgression between species using the Population Branch Statistic within trios of closely related species.

Characterizing dark gene functions in corals through microscopy and scRNA-seq approaches

Kevin Wong, Natalia Andrade-Rodriguez, David Ehrens, Gabriela Hage, Ellyn Darke, Nikki Traylor-Knowles

The advancement of molecular biology has provided tools to inform marine biota conservation, however interpretations are limited due to the lack of genomic resources for non-model organisms. This particularly poses issues when utilizing genomic approaches to understand the mechanisms underlying coral stress-responses, such as bleaching. For example, immune related genes are highly correlated with bleaching in corals, however these gene networks are poorly understood due to the lack of annotation of important regulatory genes or highly interconnected nodes. The genes that lack functional annotation or validation in non-model systems are commonly referred to as “dark genes”. This study aims to identify dark genes in the coral, *Galaxea fascicularis*, related to different mechanisms of bleaching to further characterize innate immune pathways involved with this phenomenon. We conducted two parallel experiments to simulate different mechanisms involved during bleaching: 1) thermal stress to induce apoptosis and necrosis, and 2) menthol exposure to induce symbiophagy. For both experiments, samples were taken for transmission electron microscopy to visually confirm the induced mechanisms, bulk RNA-seq to obtain a global profile of dark genes associated with each visual mechanism, and single-cell RNAseq to describe specific cell-type associations. With increased knowledge of functional annotations, we can further describe the role of the innate immune system during coral bleaching, and also provide greater genomic resources for characterizing novel cell types in corals.

Is the motor program of faster insects more precise?

Leo Wood, Simon Sponberg

Can faster insects generate motor programs with greater spike timing precision, or do other factors contribute to allowing some animals to locomote at high frequencies? Appendages move in cycles, so muscles have to be orchestrated relative to where the limb is in a cycle. But as an animal's frequency of locomotion gets faster, orchestrating muscles relative to the phase of a limb requires the nervous system to become increasingly more precise. We sought to uncover how nervous systems scale their precision with frequency by studying Bombycoid moths, a clade of insects sharing similar anatomy despite remarkably different flight styles, ecological niches, and, crucially, wingbeat frequencies. We recorded the comprehensive flight motor program in tethered individuals from 4 different Bombycoid species: 2 wild silkmoths, *Actias luna* and *Automeris io* (wingbeat frequencies of 13Hz and 22Hz, respectively), and 2 hawkmoths, *Manduca sexta* and *Hyles lineata* (24Hz and 41Hz). We measured how neural precision changes with wingbeat frequency, within and across species, by quantifying changes in spike timing variance. Within and across species, we found spike timing became more precise with increasing wingbeat frequency for most muscles, but that this precision scaled slower than wingbeat frequency alone would predict. So as insects increase wingbeat frequency, spike timing does become more precise, but there are constraints on this precision and other physiological changes are necessary to produce fast locomotion.

Performance Variation in the Cold Across a Subtropical-Tropical Latitudinal Gradient

Riley Wood, Ryan Earley

To survive increasingly common cold fronts, organisms must escape or contend with thermal challenges. Mangrove rivulus fish, native to Florida mangrove forests, tolerate highly variable environments and jettison from water to thermoregulate. By gradually decreasing water temperatures and maintaining different ambient temperatures, we compared behavioral thermoregulation to cold water temperatures between subtropical (Tampa) and tropical (Florida Keys) populations. Here, we use fish lineages from these

strongly genetically differentiated populations to explore whether Tampa populations might have adapted to better withstand the cold relative to Keys populations. We hypothesized that Tampa animals would remain in cold water longer and be more active in cold air temperatures than Keys animals. We also hypothesized that behavioral responses to temperature would be heritable and thus, have the potential to evolve in response to future selection. Each treatment - cold and control - employed the same genetic lineages from each population and measured the same behavioral responses. Water temperature was decreased from 27.5°C to 12°C over 12 days in cold trials but was maintained at 27.5°C in control trials. Water temperature treatments had three air temperature sub-treatments - cold (4°C), warm (27°C) or hot (36°C). We present preliminary data that provides key insights into whether, and to what extent, temperature-induced behavioral plasticity has diverged between two genetically differentiated populations that experience novel cold weather events of different magnitudes.

Crocodile talk: structural analysis of American and Morelet's crocodile vocalizations in Belize

Helena Wood-Barron, Athena Rycyk, Jonathan Triminio, Marisa Tellez

Crocodiles are known to use vocalizations for social interactions, such as courting and territorial defense, as well as distress calls. There are two species of crocodiles living in Belize, the American crocodile (*Crocodylus acutus*) and the Morelet's crocodile (*Crocodylus moreletii*), along with American-Morelet's hybrids. The overall aim of this project was to analyze the structure of calls made by both American crocodiles and Morelet's crocodiles and to analyze any differences between the calls made by both species. Crocodiles were temporarily captured and restrained during ongoing capture surveys, and any vocalizations made during capture were recorded. Morphometric data was also collected during this time. Afterward, each recording underwent acoustic analysis in RavenPro to determine the sound structure of each call. This was done by collecting data with the following parameters: total duration (DT), duration of the first quartile (D1/4), max frequency (Fmax), beginning frequency (Beginning), the frequency at the first quartile (F1/4), end frequency (Fend), the slope of the first quartile (Slope 1), and slope of the remaining three quartiles (Slope 2). The call structures were then compared between species, age classes, and individuals. No clear species or age class difference was observed. However, there was great variation found amongst the seven individual crocodiles analyzed, suggesting that these vocalizations are more complex than previously thought and possibly individually distinct.

Male water availability affects mating outcomes in wolf spiders

Jacob Woods, Noah Leith, Kasey Fowler-Finn

Variation in climate often crucially impacts reproduction, yet we know little about how water availability affects reproduction compared to other climatic factors like temperature. Here, we investigated how access to water resources in male wolf spiders affects multiple features of male and female reproductive success. We assigned adult *Schizocosa stridulans* males to one of two water availability treatments. Then, we randomly paired them with adult females that received a constant water availability treatment. We then tested for differences between male watering treatments in the likelihood for males to copulate with a given female, which likely shapes a large proportion of variation in male reproductive success in this species. To explore factors that shape reproductive success in both sexes, we measured various traits related to fecundity and offspring viability. We found that males deprived of water before mating were significantly less likely to mate with any given female. However, when water-deprived males did mate, their female partners had higher fecundity than females that mated with non-deprived males. This finding implies that water-stressed males may invest more resources into successful copulations, because they obtain successful mating opportunities much less often. Water availability may therefore generate variation in reproductive strategies in variable or altered environments.

Tail-Assisted Climbing in Rats

Brian Woronowicz, Murtaza Hathiyari, Shahin Lashkari, Noah Cowan

Understanding the mechanisms behind tail-assisted climbing can shed light on the versatile locomotion strategies employed by animals, providing potential new insights for robotics. Here, we investigate how rats utilize their tails to aid in climbing up onto a ledge. We constructed a horizontal bottom platform 10 inches below a ledge. A quarter-inch diameter “pullup bar”, that could be freely rotating or fixed, was placed at the ledge. A force plate under the ledge measured tail forces the animal used to help propel itself over the ledge. Over 134 trials for one rat, we observed that when the pullup bar was locked, a lower ditch exit impulse led to larger tail force during exit ($t(71) = -7.61, p < 0.05$). However, when the bar was freely-spinning, the rat categorically altered its exit strategy, exhibiting a reduced vertical impulse to exit from the bottom platform and increasing the likelihood of tail use for climbing assistance by 62.5% (Fisher’s exact test: $p < 0.05$). The tail exerted substantial moments of up to 145% of the opposing gravitational moment caused by the rat’s center of mass, demonstrating the clear biomechanical significance of the tail’s contribution to climbing dynamics. This project provides novel insights on the utility of tails during climbing, reinforcing the literature that demonstrates that tails are general-purpose appendages, helping animals accomplish myriad locomotor tasks.

Mock tags cause a short-term decrease in takeoff velocity in House Sparrows

Natalie Wright, Katherine Crawford, Abigail Garcia, Olivia Rataezyk

To assess both short- and long-term impacts of tags/transmitters on takeoff flights, we affixed House Sparrows (*Passer domesticus*) with mock tags ~5% of body mass. We elicited escape-style takeoffs immediately before tagging, immediately after tagging, after 5 and 10 days with tags, and immediately after tag removal on day 10. Half of the birds had tags attached via harnesses, the others via glue. The first takeoff with a tag had a significantly slower average velocity than the takeoff before tag attachment, but on days 5 and 10 with tags, average takeoff velocity was similar to that of the initial, tag-less takeoff. After tag removal on day 10, takeoff velocity was greater than in the initial, tag-less takeoff. Wingbeat frequencies were greater in all tagged flights and the final flight after tag removal on day 10 than in the initial untagged flight. Birds harnessed tags resumed pre-tagging velocities quicker than birds with glued tags. Our results indicate that while lightweight tags do significantly affect the flight of songbirds, birds appear to adjust to their presence, compensating via elevated wingbeat frequencies, and resume typical takeoff velocities fairly quickly.

The Morphology and Function of Oral and Pharyngeal Teeth in the California Moray Eel

Savanna Wright, Rita Mehta

California morays, *Gymnothorax mordax*, are resident benthic predators in the southern California kelp forest ecosystem. Few prey are invulnerable to their sharp teeth and large vertical gapes. Moreover, moray pharyngeal jaws also lined with sharp teeth are highly mobile and facilitate swallowing prey. Therefore, prey that are apprehended receive two bites – one from the oral and another from the pharyngeal jaws. Little research has explored the morphological differences in teeth and thereby their function between the two jaws. We hypothesized that the degree of tooth curvature varied across the oral jaws, while curvature remains more consistent within the pharyngeal jaws. We examined tooth curvature in two regions of the upper jaw and the dentary and the upper and lower pharyngeal tooth plates. Our results indicate that teeth along the peripheral inner maxillary region of the oral jaws are the most curved. However, pharyngeal teeth exhibit the greatest curvature compared to any teeth in the oral jaws and the pharyngeal teeth vary little in their curvature. This data supports the idea that the pharyngeal teeth of the moray are used to ensnare prey, while teeth in the oral jaws vary in function depending on their position in the oral cavity. These results provide a baseline to compare morays from different regions, and refine aspects of tooth shape and curvature that may vary intraspecifically and across species.

Exploratory Experiments of Cap Pushing in the Behavior of Messor Ants

Liam Wrixon, Charles Abramson

Ants fascinate biologists with incredible feats of farming, hunting, and engineering achieved through complex colony behaviors. While in an NSF-REU program in Lesvos Greece, we investigated problem-solving behavior of *Messor* ants. Specifically, we examined colonies’ reactions to their nests being repeatedly covered and compared the consistency of response behaviors both among and within colonies. We conducted these experiments utilizing the Cap Pushing Response (CPR) method in which a small bottle cap was used to cover the colony entrance. The CPR method has been used in honey bees to examine a variety of behaviors associated with different stimuli. Preliminary experiments were run in order to optimize CPR in *Messor* ants. A variety of cap types were tested because most caps were too large for the ants to manipulate directly. The experiment was replicated across five separate colonies using the standardized methods developed in the preliminary experiments to block colony entrances. Each colony differed in their respective responses to the caps blocking their entrances, with minimal behavioral overlap across colonies. However, most individual colonies were relatively consistent in their responses to repeated experimental iterations. Here we present the study design, behavioral response results, and discuss broader implications to our understanding of coordinated behavior in ant colonies.

Promoting Indigenous Students in NSF REUs

Liam Wrixon, Charles Abramson

The National Science Foundation (NSF) sponsors hundreds of Research Experiences for Undergraduate (REU) programs. The goal of REUs is to bring hands-on research participation and experiences to undergraduates across the country. NSF is especially interested in increasing diversity in research and giving opportunities to those from institutions with limited research programs. Unfortunately, communication of these opportunities to potential native candidates can be lacking and indigenous students are often underrepresented in scientific fields. We aim to promote NSF REUs to native communities and students. Here we will offer guidance to potential indigenous candidates and their mentors/PIs on how to pursue REU opportunities, as well as the firsthand benefits one can experience from an REU program.

Segmentation and regeneration of the cauda of the sabellariid annelid *Phragmatopoma californica*

Cheng Hsing Wu, Bruno Pernet

Phragmatopoma californica is a common intertidal reef-forming annelid in southern and central California. Like most annelids, *P. californica* is segmented, with segments delimited internally by septa and indicated externally by the presence of parapodia and chaetae. However, only the anterior part of the body shows obvious segmentation; the posterior ~1/3 of the body (the “cauda”) is a smooth-walled cylinder with no parapodia or chaetae, leading many to call this region “apparently unsegmented”. If the cauda is unsegmented, sabellariids must add anterior body segments during growth in a way different from other annelids. We sought to determine how cauda length is related to body length, whether the cauda can regenerate after amputation, and if internal anatomy might help determine if it is segmented. Measurements of body and cauda length in field-collected worms showed that the cauda increases in length with body size. After partial or full amputation of the cauda, worms rapidly regenerate a new cauda. Scanning electron microscopy of sagittally-sectioned caudae demonstrated that septa were present; further, the spacing between septa was similar to the spacing between adjacent lateral blood vessels in the cauda. These observations strongly suggest that the sabellariid cauda is segmented. It seems very likely that despite their unusual morphology, sabellariids grow by teloblastic growth – adding body segments to the posterior end – as do other annelids.

A thermoconforming lizard alters heat-shock protein network expression in response to thermal stress

Kelly Wuthrich, Albert Chung, Adam Rosso, Michael Logan, W. Owen McMillan, Christian Cox

Increasing temperatures caused by climate change are predicted to be particularly harmful for tropical thermoconforming ectotherms, which have evolved in a historically constant climate and do not use behavior to regulate body temperature. These organisms can initiate cellular- protective mechanisms by upregulating expression of genes such as those in heat-shock protein networks to cope with increasing temperature. However, whether expression of heat-shock protein networks can be altered in response to acute thermal stress has not been documented in most organisms. We studied how genes across heat-shock protein networks were differentially expressed in response to increasing temperature in the slender anole (*Anolis apletophallus*). Specifically, we tested how genes associated with the hsp40, hsp70, and hsp110 networks changed in expression in brain, muscle, and liver tissues in response to both a mild (32°C) and more extreme (35°C) heat shock that persisted for 3 h. We found that both treatments caused upregulation of some but not all genes in each network, and that there was substantial overlap in the genes that were upregulated among tissues and between treatments. Our results highlight the importance of heat-shock protein network expression in the response of tropical, thermoconforming ectotherms to increasing temperature, and have implications for understanding how these species might persist in a rapidly changing environment.

Fine kinematic analysis of anemonefish larvae reveals mechanisms enabling pelagic dispersal

Claire Wyart, Noah Locke, Vincent Laudet, Olivier Mirat

Although kinematic analysis of fish swimming has been investigated in adults, the swimming abilities of larval forms are less understood. One exception is larval zebrafish, extensively studied in the field of neuroscience. Five days post fertilization, larval zebrafish explore their environment via discrete bouts covering 1 mm with the tail beating at 25 Hz for four oscillations, and interspersed with second-long intervals when the larvae remain motionless. We characterize here the kinematics of larval forms of *Amphiprion ocellaris* (A.o.) and *Amphiprion clarkii* (A.c.), two of the six species of anemonefish found in Okinawa. We selected larvae 7 to 8 days after hatching, when their body length is comparable to 5 day-old zebrafish. Using a high-speed camera, we analyzed the spontaneous swimming patterns of these larvae either horizontally in a circular arena, or vertically in a rectangular water column. We found that the locomotor frequency and tail beat amplitude range deployed by larval A.o. and A.c. were remarkably similar to larval zebrafish's of the same size. In contrast, larval anemonefish can sustain hundreds to thousands of tail beats, with bouts lasting minutes long. By doing so, anemonefish larvae a week after hatching can swim at velocities of 5 body length per second and sustain swimming for minutes in order to achieve large distances in the ocean consistent with the distance of ca. 35 km that fish can travel in the ocean during their larval life. Further fine kinematic analysis of larvae through development and when facing currents will provide insight on how these reef fish can migrate tens of kilometers during the pelagic phase.

HCR in situ hybridization reveals new complexity and detail in the nervous system of Lymnaea

Russell Wyeth, Yulia Reunov, Victoria Tweedie-Pitre

Studies of gastropods have contributed substantially to understanding how nervous systems function to control animal behaviors. Neuroanatomy based on immunohistochemistry has underpinned much of this research. However, a long-standing challenge has been availability and specificity of antibodies able to target neurons in gastropods. To circumvent this issue, we have chosen to explore labelling neural-specific gene expression, exploiting sequence data to (theoretically) label a much wider array of potential targets. Previously, we used quantitative PCR to verify expression of genes involved in the production of neurotransmitters. Here, we used hybridization chain reaction (HCR) in situ hybridization labelling in wholemounts of both the brain and cephalic sensory organs of the pond snail, *Lymnaea stagnalis*. Following optimization, probes

targeting tyrosine hydroxylase (involved in the production of catecholamines; e.g., dopamine) labelled patterns exactly as expected from previous immunohistochemistry and traditional in situ hybridization. Expression of tyramine beta hydroxylase (involved in the production of octopamine) was found in a small but consistent contingent of central neurons and in sparsely distributed peripheral cells in the lips and tentacles. Observations revealed anatomical detail far exceeding traditional in situ hybridization, although, unfortunately, neurites were not labelled as they are in immunohistochemistry. Overall, we suggest this approach has great potential for advancing studies of nervous system structure in gastropods, as well as providing a basis for future genetic manipulation of neural function.

Foraging Methods and Head Shape in Two Sympatric Sea Turtles

Jeanette Wyneken, Michael Salmon, Tommy Cutt, Alexander Gaos, Don McLeish

Vertebrate head shape varies with diet, ontogeny, and phylogeny. Robust heads with wide, v-shaped jaws are common among the Cheloniidae. However, two cheloniid species are distinct in jaw shape. Green turtles (*Chelonia mydas*) are short-faced with u-shaped mandibles. In contrast, hawksbill (*Eretmochelys imbricata*) faces are elongated, with narrow, V-shaped jaws. The two species occur sympatrically on shallow reefs where both forage on algae. We hypothesized that these extremes in head morphology reflect foraging differences. We measured skull shape and collected similar measures of head shape in live immature and adult turtles, focusing on facial vs. neurocranial proportions and jaw shape. The proportional length differences in facial vs. neurocranial portions were most pronounced in juvenile hawksbills. Adult facial proportions were less extreme than in juveniles but are longer than in green turtles. In green turtles, facial length vs. neurocranial length proportions were less extreme in juveniles and adults. To provide functional contexts for head shape, we observed feeding by both species on the same or similar algal species at the same Hawaiian reefs. Foraging behavior differed. Hawksbills fed on algal colonies located in crevasses (often removing obstructions to access the algae). Green turtles scraped or nipped algae from open, flat hard surfaces. Turtle facial proportions and shapes reflected differences foraging behavior and specific types of forage locations on the reefs.

A candidate Giant Fiber Neuron underlies mosquitoes' responses to visual threats

Nicole Wynne, Karthikeyan Chandrasegaran, Richard Rust, Clement Vinauger

Despite the associated risks, female mosquitoes have no choice but to feed on mobile and defensive hosts to acquire the proteins and nutrients required to produce progeny. During the time they search and drink our blood, these female mosquitoes are exposed to the risk of being detected and either hurt or killed by our swats. Their ability to detect and avoid these threats is thus directly related to their fitness and reproductive success. While visually guided escape responses have been extensively analyzed in classical model species such as *Drosophila melanogaster*, how mosquitoes detect and respond to visual threats has only started to be unraveled. In this context, we combined behavioral and immunohistochemical methods to determine how *Aedes aegypti* females respond to visual threats in both free and tethered flight. Using an LED arena and LCD displays to introduce looming stimuli designed to mimic a swatting hand, we observed that visual threats do not prevent in-flight mosquitoes from landing on a host mimic, but trigger an oriented escape response in landed mosquitoes engaged in blood feeding. Investigations into the neural basis of these responses revealed the presence of large descending neurons comparable to the Giant Fiber Neuron system identified in fruit flies. These results will be discussed in the context of mosquitoes' adaptations to feeding on live and defensive hosts.

Effects of urbanization on locomotion in Western Fence Lizards (*Sceloporus occidentalis*)

Daisy Xiong, Victoria Vang, Ramiro Barajas, Sofia Hernandez-Corona, Emily Spain, Bree Putman, Angela Horner

With increasing urbanization due to rising human populations, organisms may be forced to adapt rapidly. This study investigates the impact of urbanization on Western Fence Lizards (*Sceloporus occidentalis*) and their adaptations in such settings. These lizards are known to thrive in both urban and natural environments and adapt quickly. Previous studies showed that although anoles had longer limbs in urbanized habitats Western Fence Lizards have shorter limbs in urbanized habitats, highlighting the importance of studying multiple species in urbanization studies. We captured lizards from three locations in inland Southern California with both urbanized and natural habitats in order to compare the localized effects of urbanization. We hypothesized that limb and toe lengths in Western Fence Lizards would be correlated with locomotion ability, and predicted that urban lizards would have shorter limb and toe lengths, and thus run slower when compared to lizards living in natural habitats. We measured the speed and stride characteristics of each lizard by filming them (480 fps) running across a 90 centimeter trackway a. After the lizards ran at least four trials on the track, each lizard was weighed and measured. We digitized speed, stride length, and contact time using DeepLabCut and then compared to the lizard morphology data. Preliminary results suggest that both locality and habitat influence lizard morphology and locomotion, suggesting Western Fence Lizards are highly adaptive.

Hydrodynamic and acoustic noise measurements of shark skin-inspired surfaces

Nicole Xu, Kaushik Sampath, Jonathan Stocking, Jason Geder, Ravi Ramamurti

Improvements in hydrodynamic performance and reductions in flow-induced acoustic noise are important to design energy efficient, minimally invasive unpiloted underwater vehicles (UUVs) for ocean applications. One approach is to use inspiration from shark skin, which has tooth-like microstructures called denticles, that reduce skin friction drag in turbulent flow. Previous studies demonstrated that both natural and bioinspired denticles can reduce drag and increase lift-to-drag ratios in hydrofoils at low angles of attack in laminar flow. However, the performance of bioinspired denticles has not yet been tested at higher Reynolds numbers (Re), which are more relevant for larger UUVs or high-speed mini-UUVs. Using laboratory experiments and computational fluid dynamics, we address how bioinspired denticles can improve the performance envelope of UUVs. Bioinspired denticles manufactured using state-of-the-art stereolithography techniques. Force measurements of NACA0012 hydrofoils with denticle panels were collected in a recirculating water tunnel (Re ranging from 85,000 to 350,000). Acoustic measurements over flat and denticle-covered panels were collected in the U.S. Naval Research Laboratory's HydroAcoustic Flow Channel facility (Re ranging from 32,000 to 64,000). Results of a 3D unsteady Navier-Stokes solver are validated against these experiments and used to visualize the flow over denticles. Future applications include improving the efficiency and anti-biofouling properties of UUVs for enhanced persistence or use in more sensitive marine environments, in which higher noise signatures could potentially disrupt more delicate organisms.

Comparison of mitochondrial performance of mice in laboratory, semi-natural, and wild populations

KayLene Yamada, Kang Nian Yap, Natalie Harris, Shelby Zikeli, Vimala Kaza, Hippokratis Kiaris, Andreas Kavazis, Wendy Hood

Environment plays a formative role in many physiological processes. Historically, physiology studies have been conducted in laboratory or field settings. Laboratory studies provide highly controlled environments, but animals responses to these environments do not always mimic responses in natural contexts. Energetic demands of an individual under laboratory conditions are often low due to limited space and steady food sources. Field studies capture physiology in the context animals evolved but are complicated by the difficulty of following individuals over time. The energetic demands of

wild individuals are often greater because of necessary activities such as foraging. Recently, semi-natural enclosures have been used as an alternative, allowing researchers to control many aspects of the environment while maintaining ecological relevance and creating wild-like energetic demands. We determined if the mitochondrial physiology of white-footed mice *Peromyscus leucopus* in semi-natural enclosures more closely mimics wild or laboratory-maintained animals. We isolated mitochondria from skeletal muscle and liver and measured respiratory control ratio and compared markers of oxidative damage to evaluate differences in cellular bioenergetics. We predicted that the physiology of mice in the semi-natural enclosures will more closely mimic the wild than lab. Preliminary results show semi-natural mice have higher muscle respiratory control ratio than wild or laboratory groups. Our findings will help researchers identify the best context for future experiments by providing information on how housing environment impacts physiology.

The tale of two tails: material properties of kangaroo rat and lab rat tail tendon fascicles

Emily Yamauchi, Viktor Gevirtzman, Miles Valencia, Craig McGowan, Manny Azizi

Tendons are dense, collagenous tissues that connect and transmit energy between muscles and bones. While the mechanical properties can vary between species, few studies have linked this variation to function. Here, we use a unique model system to investigate how variation in tail function may correlate with variation in the mechanical properties of tendons. Here we compare the properties of tendon fascicles from lab rats (*Rattus sp.* BNx344) to those from kangaroo rats (*Dipodomys deserti*). Kangaroo rats are known to whip around their tails to coordinate their bodies midair during escape responses. For this study, we investigated whether increased control in tail muscle-tendon units is associated with tendon mechanical properties. The properties of tendon fascicles were measured using cyclic-loading, stress-relaxation, and ramp-to-failure tests. Preliminary results suggest that kangaroo rat tail tendon fascicles have lower stiffness and relaxation percent compared to lab rats. In addition, lab rat fascicles reached failure at lower strains compared to kangaroo rats. Despite these differences, both groups have similar hysteresis and failure forces. The results of this work combined with future studies aimed at measuring tendon dynamics *in vivo* will allow us to relate tendon variation to the mechanics and control of tail movements.

Physiological and behavioral mechanisms underlying the mate familiarity effect in Cassin's auklet

Amy Yanagitsuru, Thomas Hahn, John Wingfield, Haley Land-Miller, Amanda Spears, Mike Johns, Frédéric Angelier, Christopher Tyson, Rebecca Forney, Elisha Hull

We explored the mechanisms that underly reproductive benefits of perennial monogamy in Cassin's auklet. We hypothesized that observed fitness benefits of retaining a mate is due to physiologically-mediated differences in parental behavior across experience levels and traced candidate mechanisms from physiology to behavior and reproductive outcomes. We uncovered significant differences between males and females, with incubating males responding to their mates' nest attendance in a pair-experience dependent way, and a relationship between prolactin and attendance that depended on female behavior. Female incubation attendance was correlated with the attendance of her mate, but there was no evidence that her responsiveness varied with pair bond duration and was instead correlated with her body condition and the interaction of prolactin and corticosterone. Male behavioral responsiveness to bond duration and the behavior of his mate has important fitness implications, as male attendance was an important predictor of both hatching success and chick weight. Female attendance had no effect on hatching success and only correlated with chick weight when the female entered the breeding season in good body condition. These results imply that the benefits of mate familiarity are conferred primarily by males in Cassin's auklet and that the ability of the female to provide care is constrained by energetic limitation and the demands of the egg.

Physiological and morphological correlates of sexually dimorphic growth in sablefish (*Anoplopoma fimbria*)

Yuzo Yanagitsuru, Edward Hayman, William Fairgrieve, J Luckenbach

Sexually dimorphic growth (SDG), where one sex grows faster, is well-documented in fishes and has been hypothesized to be caused by earlier onset of puberty in one sex and a concomitant investment of energy towards reproductive development over growth. To test this hypothesis, we characterized when SDG occurs and investigated the potential underlying biological mechanisms in sablefish (*Anoplopoma fimbria*), an emerging aquaculture species exhibiting faster female growth. Males and females were tagged and individual growth rates tracked over 24 months, and blood, pituitary, liver and gonads sampled bimonthly. Both sexes had similar growth rates until 340-500 mm length, after which both sexes exhibited reduced growth rates with more pronounced reductions in males, resulting in divergent growth rates (i.e. SDG). Concurrent to the lengths when SDG started, there were increases in gonadosomatic and hepatosomatic indices, and transcripts for several growth-related genes in liver (*igf1*, *ghr1*, *ghr2*) for both sexes. There were also sudden increases in plasma estradiol and pituitary growth hormone transcripts in females, but not males. Gonadal histology revealed that all females had not advanced beyond the primary oocyte stage and no males initiated spermatogenesis, indicating that no fish entered puberty during the experiment, despite observing SDG. We suggest that SDG may be related to increases in female estradiol and growth hormone production, but the mechanisms underlying faster female growth remain unknown.

Body size and light environment modulate flight speed and saccadic behavior in free flying *Drosophila melanogaster*

Haoming Yang, Vahid Tarokh, Jamie Theobald, Simon Sponberg

The size of holometabolous insects varies depending on dietary conditions during larval development. Insect compound eyes, which grow proportionate to body size, are limited by their anatomy and function is dependent upon features like facet count, facet diameter and eye area. Therefore, smaller individuals may be impaired to effectively use ambient light for visual behaviors. In this study, we were interested in comparing the fly trajectories of *Drosophila melanogaster* flies of disparate sizes under the same light conditions. We manipulated fly size by restricting their food availability as larvae. Using a high-speed camera free flight assay, we tracked flight trajectories and analyzed saccade patterns throughout the day for several days at a time. We further analyzed flight patterns at four distinct light niches: dawn, day, dusk, and night. We found that larger flies saccade significantly faster than smaller flies. To test whether this was due to smaller flies having limited visual function, we ran trials with subjects reared to become large adults where the light environment during the day always remained dim – 25% of the original light intensity used previously. When comparing the fly trajectories in dimmer light to those under bright light, we found that the individuals in brighter conditions where significantly faster. Our results indicate that small flies behave most like regular flies under dim conditions, suggesting that light processing is diminished when the eye morphology is reduced.

Metabolic Consequences of Freezing in the Intertidal Mussel, *Mytilus trossulus*

Josh Yang, Katie Marshall

The bay mussel, *Mytilus trossulus*, is an intertidal bivalve found along the west coast of North America which risks freezing during low tides in the winter. Despite being freeze tolerant, the energetic costs required to survive freezing are unknown and may affect its role as an ecosystem engineer. Using a closed respirometry system, we measured their oxygen consumption as a proxy for metabolic rate before and after single and repeated freezing events. We compared these responses to hypoxia exposures as tissues are not perfused when frozen which largely inhibits gas exchange. In general, we observed that mussels' oxygen consumption rates decreased after freezing but increased after exposure to hypoxia. This decrease after a freezing event may be linked to the extent of damage accumulated and represent damage to the oxygen cascade which can limit the uptake of oxygen. Alternatively, this may represent a deliberate metabolic suppression to limit oxidative damage. Interestingly,

repeated freezes in the winter did not cause the same decrease in oxygen consumption, suggesting that periods of repair may be crucial to survive freezing. Further work will elucidate the mechanisms underlying the main driver of this response and the broader ecological implications to the intertidal zone following cold spells.

Female foraging strategy co-evolves with sexual harassment intensity in the Trinidadian guppy

Yusan Yang, Swanne Gordon, Andrés López-Sepulcre, Eleanor Grant

Sexual harassment is a widespread evolutionary outcome of sexual conflict over mating rates. Male harassment can impose costs on females, and females often change their behaviors to avoid unwanted attention. In Trinidadian guppies (*Poecilia reticulata*), males use either sneak mating behavior or courtship displays as reproductive tactics. Both behaviors can be sources of sexual harassment, but sneak behavior is likely more harmful. Males adapted to low-predation habitats use more courtship and fewer sneak tactics than their high-predation ancestors. Here, we tested whether female foraging strategy co-evolves with less severe male harassment as guppies colonize low-predation environments. We set up outdoor stream mesocosms with common-garden-reared males and females from either a high- or a low-predation population in a 2 × 2 design, and tested whether populations diverge in female response to male harassment. We found that both sneak behavior and courtship display reduced female foraging, but the effect of sneak behavior was more extensive. Furthermore, the negative effect of sneak behavior was more pronounced on high-predation females. Our results suggest that female foraging strategy coevolved with divergence in male mating strategy: females under more severe sexual harassment evolved a foraging strategy that is more sensitive to varying harassment levels.

Expression of Five Mineralization Genes in the Shell-less *Wirenia argentea* (Mollusca, Solenogastres)

Meghan Yap-Chiongco, Katharina Stracke, Aina Børve, Andreas Hejnol, Kevin Kocot

Aside from the characteristic shell, members of the Mollusca produce an array of mineralized structures for diverse functions such as defense and reproduction. The worm-like Solenogastres lack a shell and are instead covered in aragonitic spines and scales called sclerites. Sclerites, also found along the girdle of chitons (Polyplacophora), are an assumed synapomorphy for Aculifera (Aplacophora + Polyplacophora) however their homology to the shells of other molluscs is unknown. In fall 2022 and 2023, ~200 individuals of the solenogaster *Wirenia argentea* were collected via epibenthic sled from Haudglandsosen Fjord near Bergen, Norway. Expression of five genes known to be involved in shell mineralization in conchiferan molluscs (*soxC*, *gata2/3*, *engrailed*, *notch*, and *bmp2/4*) was investigated via in situ hybridization at various stages of larval development of *W. argentea*. Expression *bmp2/4* begins 4-5 days post hatching (dph) and appears to be associated with *gata2/3* and *soxC* expression in mineralizing tissue of the trunk as well as in pedal nerve development. *Engrailed* expression is concentrated in the trunk beginning 7-10 dph and is consistent with developmental timing of sclerite formation. *Notch* is expressed across all time points within the trunk and prototroch and may have a role in nervous and sclerite development. Here we present the first findings from this study aimed at elucidating the evolutionary origins and the underlying "mineralization toolkit" within Mollusca.

Nudibranch behavioral responses to dangerous prey are location-dependent, not state-dependent

Kassidy Ye, Kate Otter, Laurenzia Cairo, Paul Katz

Toxins are generally to be avoided; however some animals, such as nudibranch molluscs specialize on dangerous and toxic prey. For example, the nudibranch, *Berghia stephanieae*, feeds exclusively on the anemone, *Exaiptasia diaphana*, which eject barbed, toxin laced harpoons called nematocysts used for defense and prey-capture. Here we observed *Berghia* interacting with *Exaiptasia* to determine its behavioral responses to touches on different parts of its body. We had hypothesized that the valence of the stimulus changes

depending upon hunger state and predicted that hungry animals would turn towards an Exaipstasia, whereas sated animals would flee. Yet, contrary to our hypothesis, contact location on the Berghia was found to be the main predictor of behavior; contact to the oral tentacles and proboscis resulted in reliable appetitive responses, whereas contact with other bodily locations resulted in mostly aversive responses. Overall, it seems that perceived valence of contact with the dangerous prey is contact location dependent, rather than hunger-state dependent. One confound is the decrease in time, and thus contact, that the Berghia experienced when sated. Ongoing work seeks to use body-fixed Berghia and controlled contacts for further investigation.

The Geometry of Prey Capture Across Diverse Praying Mantis Species

Jaime Yockey, Danielle Taylor, Daisy Dan, Yiheng He, Gavin Svenson, Joshua Martin

Praying mantises (Mantodea) are a relatively small but diverse order of insects. They all share a crucial characteristic: they are all predators that use their forelegs to capture prey. This common task is accomplished by a variety of ecomorphs specialized for a substrate (grasses, soil, bark, etc.), hunting strategy (ambush vs pursuit), or mode of movement (climbing vs running). Here, we investigate how the geometry of the grasping foreleg impacts the optimal prey size for a mantis species. We have adapted a machine-learning algorithm (DeepLabCut) to identify 24 landmarks on the femur and tibia of over 500 species of mantises. Using additional image processing techniques, we define the shape of the leg between these points, and generate a two-dimensional model of the prey capture area between the tibia and femur. We begin with a simple, geometrical model developed and behaviorally verified for a single species by Holling (1964), that uses only the angle of the tibial spur and the length of the tibia. We solve this for all species, and map the estimated optimal prey size across the morphospace for both extant and non-extant shapes. We find that the majority of species occupy an isocline of optimal prey size in the morphospace, with a minority set of species climbing a gradient towards larger optimal prey size, and another minority set falling down the gradient towards a smaller optimal prey size. We next extend this analysis using a more complete model of the foreleg, which includes the femoral spines. We find that individual species throughout the phylogeny increase the optimal prey size by adaptations of the femoral spines.

Improvements towards North Atlantic right whale science communication, outreach, and education.

Zaphillia Yost, Ashley Bowers-Macrander, Jason Macrander

Preventing the extinction of endangered species requires public awareness of the issue and implementation of proper conservation management strategies. The North Atlantic right whale (*Eubalaena glacialis*) is a critically endangered species found along the eastern seaboard of the United States. The major drivers of their decline are vessel strikes and entanglement in fishing gear. Currently, the right whale population sits at just under 350 individuals, a number that has been declining after the population peaked at 480 individuals in 2011. This decline suggests a failure in one or both aspects of conservation. My project explores different methods of scientific communication to find effective ways to increase public awareness of the status of the North Atlantic right whale.

Replaying the tape of life: Comparative transcriptomics of phenotypic convergence

Becca Young

Evolution often repeats itself as organisms converge on similar phenotypic solutions to similar ecological challenges. But, is repeated evolution enabled by shared genomic mechanisms? Numerous studies have attempted to link DNA sequence variation to convergent or parallel evolutionary changes in complex traits. However, such complex traits, by definition, result from the function of many genes as well as their interaction with each other and with environmental factors. Thus, key insights connecting genotype to the phenotype can be gained by characterizing the readout of the genome, i.e., how

gene expression evolves in association with phenotypic variation. When such 'omics-level inquiries are carried out across species, the discovery of generalizable rules underlying repeated phenotypic evolution becomes possible. We show how combining phylogenetic comparative methods with transcriptomics provides a powerful tool to identify the molecular pathways that may have evolved repeatedly in association with convergent evolution complex behavior. We identify neuromolecular correlates of a fascinating example of interspecific cooperation, mutualistic cleaning behavior in coral reef wrasses. We characterize neural gene expression diversification across six wrasse species that vary in cleaning behavior and identify robust correlates of cleaning behavior. In addition, our results reveal a gene expression network that is shared among species that independently evolved mutualistic cleaning behavior. In sum, our research provides insights into the molecular basis of repeated evolution of behavior and complex phenotypes more generally.

Investigating Circadian-regulated Structural Plasticity of the Suprachiasmatic Nucleus

Edward Young, Alex Neitz, Keun-Young Kim, Mark Ellisman, Horacio de-la-Iglesia

Circadian clocks control the timing of bodily functions and behaviors, allowing them to align with the 24-hour solar day. In mammals, circadian rhythms are coordinated by a central brain pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN relies on intrinsic neural network properties to act as a robust central pacemaker. The central circadian pacemaker neurons of *Drosophila* undergo circadian structural changes in axonal and dendritic processes, and our hypothesis is that this 24-hour plasticity is also a defining property of the SCN. We focus on a subpopulation of SCN neurons that express the neuropeptide vasoactive intestinal polypeptide (VIP), vital to synchronizing the SCN neuronal network. We have already shown that these cells undergo 24-hour changes in fiber density at the confocal microscopy level, and our goal is to determine whether these changes result in changes in synaptic connectivity. Using mice expressing tdTomato within VIPergic cells housed under a 12:12-hour light-dark cycle, brain samples were collected 3 hours after either lights-ON or lights-OFF. Subsequent tdTomato fluorescence imaging followed by correlational serial block electron microscopy analyses was conducted to trace VIPergic neurons. Mapping of synaptic contacts on the 3D-reconstructed VIP neurons will allow us to assess whether changes in fiber density result in 24-hour changes in pre- or postsynaptic connectivity within the SCN neuronal network.

Do n-3 polyunsaturated fatty acids reduce energy costs in western sandpipers (*Calidris mauri*)?

Kevin Young, Morag Dick, Catherine Ivy, Christopher Guglielmo

Fatty acids can influence metabolism directly as fuel and by influencing metabolic rate through signalling or physical action by incorporation into membranes. Long-chain n-3 polyunsaturated fatty acids (n-3 LCPUFA) are strong signalling ligands for lipid catabolism pathways, and increase membrane fluidity of ectotherms living in cold environments. Western sandpipers (*Calidris mauri*; WESA) are marine-associated shorebirds that feed on biofilm, including algal primary producers of n-3 LCPUFA, during migration. We previously demonstrated that n-3 LCPUFA reduce flight costs of WESA. In this study we used respirometry and implanted temperature sensitive PIT tags to investigate the impact of dietary n-3 LCPUFA and acute cold exposure on resting and summit metabolic rates, and body temperature. Metabolic rates were similar between sandpipers fed high and low n-3 LCPUFA diets. However, when exposed to cold n-3 LCPUFA fed sandpipers had lower body temperatures, and shorter assay durations. Resting metabolic rate and body temperatures were similar in sandpipers between 27 and 5°C. However, metabolic rate was positively related to body temperature in birds fed n-3 LCPUFA and independent of body temperature in low-n-3 LCPUFA-fed sandpipers. Although WESA express a similar range of active body temperatures independent of diet, lower body temperatures are associated with reduced energy costs on n-3 LCPUFA and this may be important for maintaining metabolic flexibility and energy savings during endurance flights.

Climbing is hard (for literally everyone).

Melody Young, Jon Gustafson, Edwin Dickinson, Michael Granatosky

The ability to climb vertical surfaces has developed separately among most limbed animals, yet few anatomical features define climbing animals. This disconnect presents an opportunity to comprehend why climbing mechanics prevent species from making significant structural changes in response to climbing. In this study, we combine a phylogenetic and manipulative experimental approach to understand the external forces imposed on the limbs during climbing, and assess the metabolic costs and center of mass dynamics associated with this behavior. We collected single limb forces from fourteen disparate taxa (including insects, amphibians, lizards, birds, and mammals). Further, we collected center of mass movements to calculate mechanical power from five taxa, and measured the metabolic cost of climbing in humans across three experimental conditions. Universally, climbing requires animals to generate entirely propulsive fore-aft forces, whereas the normal-plane forces are split between an exclusively tensile forelimb and predominantly compressive hindlimb. Climbing mechanical costs were primarily driven by potential energy, but all species experienced kinetic energy fluctuations that added to the overall cost. Lastly, we found the metabolic costs of climbing were found to be largely indistinguishable between simple and complex climbing conditions. These results demonstrate that irrespective of phylogeny, body size, or anatomical specialization, climbing is largely constrained by basic physical laws that constrain the ability to “optimize” the costs associated with this behavior.

Comparative grip strength across arboreal and non-arboreal tetrapods

Melody Young, Edwin Dickinson, Michael Granatosky

The animals that inhabit arboreal environments face distinct challenges, due to the intricate three-dimensional landscape of this environment, including surfaces of different sizes, levels of flexibility, and orientations. As such, many arboreal vertebrates have evolved a myriad of morphological adaptations on their distal autopodia (e.g., claws, suction cups, setae, etc.), and are thought to possess stronger grasps to maximize stability on arboreal substrates. In this study, we collected in vivo grip force data from humans and nine distantly-related arboreal species (e.g., parrots, sloths, tree frogs, and lemurs) (body size range: 0.028 – 4.6 kg) across three major tetrapod lineages (mammals, amphibians, birds) using custom built grip-strength testers mounted to a force platform. These data were compared to other readily available data on grasping forces collated from literature sources. Contrary to our predictions, the gripping forces generated by arboreal primates (~40% body weight) are significantly lower than tree sloths (~80% body weight) and arboreal tree frogs (~100% body weight). However, all the above lineages are significantly weaker than either climbing (~150% body weight) or raptorial (~170%-300% body weight) birds. These results shed light on the need to reclassify the long-standing primate-defining characteristic of a “strong grasp” based on evidence.

Molecular asymmetry in amphioxus embryos: implications for the early patterning events in chordates

Jr-Kai Yu

How animal embryos determine their early cell fates remains an important question in developmental biology. Studies in various model animals have shown that asymmetrically localized maternal transcripts play important roles in axial patterning and cell fate specification. In cephalochordates (amphioxus), an early branching chordate group, maternal transcripts of germline determinants form a compact granule that is inherited by a single blastomere during cleavage stages. Blastomere separation experiments also showed that other transcripts associated with the granule are likely responsible for organizing the posterior structure in amphioxus. To obtain a global transcriptomic blueprint for the early-stage amphioxus embryo, we applied high-throughput RNA sequencing using separated blastomeres to examine asymmetrically localized transcripts in two-cell and eight-cell stage embryos. This dataset represents a valuable resource for guiding characterization of molecular players in early amphioxus development, and for comparative studies on testing conservation

and divergence of developmental programs during chordate evolution. One example is that we observe differential enrichment of maternal transcripts associated with mRNA metabolism between the animal-tier and vegetal-tier blastomeres, which may result in the diverged post-transcriptional regulatory functions among blastomeres and lead to the nuclear accumulation of β -catenin in the vegetal side of the amphioxus embryo. This result is in line with other invertebrate and vertebrate model systems, and provides important information to our understanding on the evolution of early patterning mechanisms in chordates.

Direct pouncing behavior of jumping spiders Phidippus regius in web invasion

Xiao Yu, Zhenjie Jiang, Qian Kun Liu, Yaqing Wang, Chen Li

Jumping spiders exhibit various web-invasion behaviors when hunting orb-weavers. While *Portia* is known for detouring and mimicry, *Phidippus* mainly utilizes direct attack, yet the corresponding pouncing locomotion is rarely investigated beyond takeoff. Here, we study the pouncing attack of jumping spiders (*Phidippus regius*) on orb-weavers (*Larinioides cornutus* or *Neoscona arabesca*). We used a branch for the jumping spider to approach and leap toward a dead or alive orb-weaver on an artificial web (N = 4 individuals, n = 21 trials). We observed two jumping modes after similar preceding behavior of advancing and exploring in alternation. In “pounce” mode, the jumping spider visually locked on prey, charged, pounced with frontal legs retracted initially and expanded near contact, and eventually bit the prey. In “leap” mode, the jumping spider leaped with all legs spread. The jumping spider frequently chose “pounce” for an alive orb-weaver (81%) and “leap” for a dead one (80%, $p < 0.05$, GLMM), and an ethogram shows that this choice was unrelated to the preceding actions ($p < 0.005$, Chi-squared test). We speculated that “pounce” allows all legs to grasp the prey simultaneously to increase the capture rate. We are tracking 3-D kinematics and analyzing frontal leg movement while jumping to investigate its effect on midair body pitching, and reconstructing spider eye-view to examine the role of visual feedback in web-invasion behavior.

Genetic Variations and Olfactory Adaptations in Mollusks: Insights into Environmental Perception

Giovanna Yumi-Scorsim-Omura, Laurel Yohe

Gaining insight into the molecular basis of olfaction is essential for a comprehensive grasp of biological processes, adaptation, and evolution, thereby unveiling the intricate mechanisms that underlie environmental perception. The Mollusca phylum encompasses diverse species with specialized sensory organs like tentacles, osphradia, and photoreceptors. Examining the genetic variations underlying these adaptations offers insights into the functioning of unique sensory organs often arising from gene duplication events. Mollusk morphological evolution diverges in freshwater and marine ecosystems, yielding distinct olfactory cell forms resulting from these genetic variations. We hypothesize that marine mollusks harbor a higher abundance of olfactory receptors due to the greater chemical diversity in their habitat, potentially driven by gene duplication events. To probe the ecology and evolution of G-protein coupled receptors (GPCRs), including olfactory receptors, we performed an extensive search for candidates from literature and 16 mollusk genomes using TBLASTN. Sequences were aligned, trimmed, and subjected to IQTree analysis. UPhO aided in identifying orthologs and paralogs in gene family trees, enabling the detection of gene duplication events. Early results shed light on olfactory receptor presence and distribution in mollusks, contributing to a deeper grasp of GPCR diversity and adaptations. This research lays the groundwork for further exploration into the sensory biology of these remarkable organisms while spotlighting the significance of gene duplication in shaping olfactory systems.

Role of substrate in terrestrial locomotion in young American eels

Miriam Yushavaiev, Jasleen Kaur, Amanda Horung, Rita Mehta, Andrea Ward

Anguilla rostrata (American eels) are unique because they are catadromous, transitioning from marine to freshwater habitats during their early life stages. Like many elongate fishes, American eels are known to move terrestrially, which can happen during that initial upstream migration. Although glass eels have been known to climb the walls of dams during their upstream migration, dams are obstacles to their successful migrations. In this study, we compared the interactive effects of incline and substrate on terrestrial locomotion of eels in order to develop better eel passages that may help American eels overcome the obstacles of dams in their migration routes. We hypothesized that incline and substrate affected terrestrial locomotion in young eels. Migratory American eels were collected from local dams and kept individually in the laboratory. Eels were tested moving uphill and downhill on 4 inclines across 3 different substrates. Based on previous studies we predicted that the eels would move most efficiently on a flat non-compliant substrate (fixed rocks) We found that glass eels and elvers move most efficiently on non-compliant substrates, especially at low inclines. Moving uphill on any substrate required more lateral motion of the body. Our study also indicates how body size affects the efficiency of locomotion across different substrates. These results will help inform the creation and implementation of eel passages that are effective for multiple-size eel classes.

"Lions are extremely adept at doing nothing": a new ecological categorization scheme for Carnivora

Hartrich Zack, Alexa Wimberly, Jonathan Nations, Graham Slater

Quantifying ecological variation is a fundamental problem in functional morphology. In mammals, most locomotor categorization schemes use univariate categories such as fossorial, aquatic, ambush predator, or pursuit predator. Although these categories can be extremely useful, their simplicity and inconsistency erases much of the nuance of animal behavior that could contain important ecological signal. Loss of signal when using these categories could underlie the difficulties in recovering clear relationships between ecology and morphology. We propose a new multivariate behavioral categorization scheme based on ranked importance scores for different substrates, using Carnivora as an example. For each species, we determined the importance of four substrates (terrestrial, aquatic, subterranean, arboreal) by examining the literature for key words and phrases and ranking each substrate on a scale of 1 to 5 (1=unimportant, 5=vital). Using these rankings, we performed polychoric PCA and cluster analyses to assess our scheme and to compare it to previous categorizations. The primary variation along both PC1 and PC2 is between the most arboreal and most subterranean taxa whereas the highest terrestrial scores occupy the center of the PC space. Cluster analyses indicate that the optimal number of groups is nineteen, far more than typically used. With this framework, we will be able to analyze the relationship of ecology to gross morphology and bone microstructure with more granularity and replicability, further elucidating form-function relationships.

You are what you eat: urban soil lead predicts *Turdus migratorius* blood lead in Flint, Michigan

Dorothy Zahor, Jamie Cornelius, Kenneth Glynn

High levels of pollutants can occur in urban environments and pose a threat to human residents as well as local wildlife. Many urban centers suffer from lead-contaminated drinking water due to the corrosion of pipe infrastructure. Irrigation with this water may contribute to soil lead levels. The American robin (*Turdus migratorius*) is a widespread songbird in North America, well-known for hunting earthworms in urban lawns. This earthworm specialization results in the ingestion of large amounts of soil by robins. This study investigated the impact of the Flint, Michigan (MI) water crisis, during which the city water supply was contaminated with lead, on American robins during their breeding season in southeast MI by comparing soil lead levels (SLL) and blood lead levels (BLL) of birds captured at irrigated sites of Flint to those captured at non-irrigated sites in Flint and control sites. BLL were elevated in irrigated sites of

Flint relative to the irrigated urban control and non-irrigated rural sites. Further, median robin BLL were positively and strongly correlated with lawn SLL across our seven study sites suggesting that high BLL in American robins can predict elevated soil lead levels. Further research should address how lead might impact urban wildlife and whether robins can serve as a bioindicator of lead exposure for other neighborhood inhabitants.

Effect of circadian rhythm robustness on sleep and epilepsy in a mouse model of Dravet syndrome

Hannah Zaini, Asad Beck, Glorianna Gutierrez, Horacio de-la-Iglesia

Impaired circadian regulation and sleep disturbances are common in patients with epilepsy and are associated with both increased seizure frequency and decreased quality of life. We used a mouse model of Dravet syndrome (DS), a genetic form of severe childhood-onset epilepsy, shown to have impaired circadian regulation of sleep. We examined whether the addition of a 24-hour ambient environmental temperature cycle, alongside the pre-existing light-dark cycle, would strengthen the robustness of circadian rhythms and lead to better epilepsy symptomology and sleep quality in DS mice. In order to monitor seizure activity and sleep, we implanted DS and wild-type mice with two encephalography and one electromyography electrodes to record neural and muscular activity, as well as a wireless temperature sensor to record core-body temperature. Animals were initially housed in constant temperature (22°C) for a week and then cyclic temperatures (22°C daytime; 15°C nighttime) for two weeks. Interictal spikes (a neural signature of epilepsy associated with increased seizure risk) and sleep stages were autonomously scored using machine learning algorithms developed in-lab. Our results suggest that increased structure of environmental temperature correlated to improvements in sleep quality and rapid eye movement (REM) sleep possibly due to influences on circadian rhythm. We anticipate this will lead to reduced prevalence and frequency of interictal spikes and seizures.

What I learned identifying 100,000 lizards on iNaturalist: the potential of community-based science

Peter Zani

We are in a race we are almost certainly losing day-by-day to document biodiversity at genetic, behavioral, and spatial scales. Doing so elucidates priorities, needs, concerns, and will likely document what has been lost. Integrating community observations with scientific identifications capable of revision is at the heart of the iNaturalist social-media site. Biology professionals, para-professionals, and talented amateurs each play an important role in the community-science endeavor from submitting natural history observations to identifying individuals or types of behavior. In addition, the resulting dataset is increasingly being used to illustrate important patterns such as the pace of invasive species, novel occurrences, and climate-driven changes in geographic distributions. As part of various projects using the iNaturalist.org platform, in the past year I have identified 100,000 lizards ranging from the depths of the Grand Canyon to the heart of the Amazon rainforest. This talk is an attempt to communicate the lessons learned, illustrate some of the tools available that aid in identification, and outline the challenges and rewards of thousands of micro-interactions focused on identifying often rare or even undescribed species. Finally, because there are increasing attempts to bring iNaturalist into the classroom or lab, I will attempt to offer tips that enable easy entry into the often-esoteric world of socialized natural history.

Disease and temperature synergistically impact the thermal biology of a critically endangered frog

Jakub Zegar, Carolina Lambertini, Michael Logan, Jamie Voyles, Michel Ohmer

Global amphibian populations have experienced severe declines due to chytridiomycosis, a disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), and recent work suggests that climate change may worsen disease outbreaks. Bd has had devastating impacts on the Panamanian golden frog (*Atelopus zeteki*), which experienced population declines in the early 2000s, and subsequent captive breeding programs were established

with the goal of eventual reintroduction. To understand the role of temperature and disease on the future resilience of this species, we examined the influence of acclimation temperature (17°C and 25°C) and Bd infection on *A. zeteki* thermal physiology. We found that acclimation temperature and infection intensity independently and synergistically impacted thermal traits. Bd-infection reduced critical thermal maxima (CT_{max}), regardless of acclimation temperature. However, over the course of a six-week period, this reduction in CT_{max} was observed sooner in frogs acclimated to 17°C than those acclimated to 25°C. Furthermore, CT_{max} was negatively correlated with Bd load, indicating a dose-dependent effect. Finally, frogs acclimated to the higher temperature had reduced jumping performance with increased acclimation time, indicating that this species may be sensitive to a warming environment. This work improves our understanding of how disease can impact a species' ability to withstand climate change, and how previous thermal history may ameliorate or worsen these effects.

Behavior of humpback whale yearlings in Hawaii and comparisons with newborn calves

Julia Zeh, Marc Lammers, Adam Pack, Susan Parks

As adults, humpback whales (*Megaptera novaeangliae*) exhibit a variety of behaviors on their breeding grounds. In addition to breath holding dives, both sexes produce a diverse vocal repertoire, males physically compete and have a complex song display, and females give birth to single calves who they nurture through a migratory cycle until weaning, at approximately 12 months. Limited data are available on the behavior of these newly independent yearlings compared to newborn calves. To explore behavioral ontogeny, we characterized and compared diving and vocal behavior recorded on suction cup acoustic and movement tags deployed on 5 yearlings and 3 calves in Maui, Hawaii in January and February 2022-2023. Yearlings were tagged in a variety of social contexts and stages of weaning. We found that yearlings dive significantly deeper and longer than calves and spend less time at the surface between dives than calves, suggesting greater aerobic capabilities by the end of year one. More frequent vocalizations and more complex call types were recorded in groups with yearlings than those with calves, demonstrating the expansion of the vocal repertoire over the first year. These behavioral differences help illuminate the ontogeny of humpback whale behavior over the first year of life, which can be used in a comparative context to explore phenomena like vocal learning and mammalian diving physiology more broadly.

Population Genetics of *Nymphon australe* in the Southern Ocean

Jessica Zehnpfennig, Candace Grimes, Kenneth Halanych, Andrew Mahon

There has been growing support for the hypothesis that a major collapse of the Western Antarctic Ice Sheet 1.1MYA resulted in the opening of transantarctic seaways(s). This significantly shaped the history and evolution of Antarctic marine fauna and impacting the distribution and mixing of populations of benthic invertebrates in the area. Pycnogonids (sea spiders) are speciose in the Southern Ocean and have a remarkable amount of recently diverged lineages, resulting in high rates of endemic species in the region. *Nymphon australe*, the most abundant species of pycnogonid in the Southern Ocean, has a previously reported circumpolar distribution and is an optimal representative to study drivers of the speciation processes in Antarctica due to their brooding reproductive strategies and slow ambulatory capabilities of the adults. This study uses single nucleotide polymorphisms (SNP's) derived using double-digest restriction site-associated DNA sequencing (ddRAD-seq) from 217 *Nymphon australe* individuals sampled from twelve distinct geographic locations including the Western Antarctic Peninsula, Bellingshausen Sea, Amundsen Sea and Eastern Antarctica to examine genetic diversity, population connectivity, population differentiation, effective population size, and gene flow in *Nymphon australe* collected from the continental shelf regions surrounding Antarctica. The results from this study will help contribute to knowledge about the population structure of *Nymphon australe* as well as test the validity of the transantarctic seaway hypothesis.

Principles of muscular packaging

Yu Zeng, Stephen Deban

Skeletal muscles exhibit diverse morphology and functions, with the internal architecture coupled with the performance. Among various constraints underlying muscular design, the issue of packaging within a confined volume has not been well evaluated. Here, we address two key questions: (1) how does packaging affect muscular capabilities? and (2) how can muscles optimize performance within confined spaces? Modeling muscles as assemblies of hydrostatic, contractile units, we investigated the interplay of force dynamics and mechanical output as functions of (1) whole-muscle shape and (2) arrangement of pennate fibers. Our findings highlight that the specific mechanical potential of a muscle is contingent upon its pennate arrangement within a defined shape. Furthermore, we emphasize the pivotal role of bulging in muscle architecture, which influences the arrangement of muscle fibers and eventually impacts energy transmission efficiency. Consequently, optimal energy efficiency in pennate muscles is achieved through a range of pennate angles near 40°, which was in accordance with morphological evidence. Our results provide insights on the principles underlying diverse muscular designs and shed light on the emergence of novel muscular systems tailored for specific performances, such as the ballistic tongue projection in lungless salamanders.

Form-function relationship in biological puncture: the effect of speed, sharpness, and curvature

Bingyang Zhang, Philip Anderson

Puncture by the insertion or penetration of a sharp, pointy object into biological tissues is a widespread mechanism in nature. Organisms across phyla utilize puncture tools that are highly diverse in form and scale to achieve complex biological functions. A better understanding of the mechanics underlying the diversity and evolution of these biological puncture systems necessitates systematic characterization and quantification of their form-function relationships. To elucidate the complex interplay between tool geometry, material response, and impact dynamics, we establish a highly controlled framework for biological puncture combining both dynamic and static puncture experiments and finite element simulations. Our results show that the puncture performance is sensitive to the sharpness/slenderness of the puncture tool at lower puncture speeds. However, the strength of such dependence diminishes at higher rates within a bio-relevant dynamic range. Moreover, finite element visualization reveals that, within a bio-relevant range, the structural curvature of the puncture tool has a relatively small influence on puncture energetics compared to the sharpness in a homogeneous soft material. The effect of curvature mainly affects the initiation of penetration, as validated by quasi-static puncture experiments. Overall, these findings highlight the complexity of biological puncture and offer important biological implications: The rate-mediated form-function relationship may allow certain puncture systems to release from mechanical constraints and gain higher levels of diversity and adaptability.

Design to Learn: Creating visual representations of biological events may deepen understanding

Ke Er Zhang, Jodie Jenkinson, Gaël McGill, Susan Keen

Visual representations are essential for communicating an understanding of complex biological phenomena. However, representations are necessarily simplifications, including or excluding aspects of a structure or process that sometimes create misconceptions in viewers. Training biologists to analyze the use of layout, color, size, scale, and other graphical elements of visual materials, as well as the process in which these components are combined, may improve the learning value of visualizations.

We created a Visual Science Communication Toolkit that uses multimedia (2D animations and an interactive activity) to teach the principles of visual science communication design to undergraduate life science students. Toolkit users apply what they have learned through an interactive design task. Our goal was to produce more critical consumers and designers of visual materials.

We tested the Toolkit with both biology students and faculty. Completing the design task, especially when done collaboratively in pairs, provided users with

the opportunity to verbalize their understanding of scientific processes and to think critically about design choices as sources of misconception. In this talk, we will demonstrate key elements of the Visual Science Communication Toolkit and discuss what user responses taught us about how to improve visual literacy in biology.

Bugs and Robots: Investigate the External Reaction from Clicking Mechanics Using Dynamic Simulation

Liyan Zhang, Teagan Mathur, Aimy Wissa, Marianne Alleyne

As one of the few organisms in nature capable of jumping without using legs, the click beetle uses a unique morphological feature present between its mesothorax and prothorax. This feature enables it to rapidly convert stored elastic potential energy into kinetic energy.

To investigate the influence of this structure on the beetle's aerodynamic characteristics and its interactions with the surrounding environment, we created a dynamic simulation model. This model simplifies the beetle's structure into several robotic components, such as links, revolute joints, and torsional springs. By solving the ordinary differential equations (ODEs) related to the system's equations of motion, we can observe system responses over time under various initial conditions and parameters, including contact point curvature, energy storage, and initial angular velocity. The simulation enabled a more detailed investigation into the forces exerted during the click beetle's clicking motion and its impact on surrounding materials.

The simulation also demonstrated how beetle-related variables, such as elytra curvature and center of mass, affect the take-off angle, take-off velocity, and consequently, jumping capability. Our findings provide further insight needed for the design and fabrication of legless jumping robotic mechanisms.

Creating successful zoo-academic collaborations through education, enrichment, engagement

Margaret Zhang, Cassie Shriver, Jonathan Erickson, Joseph Mendelson, Andrew Schulz

Zoo collaborations can benefit research collaborations involving conservation or bioinspiration, especially when studying specialist species, non-native animals, or megafauna. Collaborations between zoos and academic institutions can benefit both parties, as zoos can assist in research while institutions can assist in technological improvements. However, zoo-academic collaborations are often challenging as it is difficult to identify common areas of interest between the two organizations. Over the past few years, Georgia Tech (GT) engineering students have fostered effective and beneficial collaborations with Zoo Atlanta in Atlanta, Georgia, through multiple pathways focusing on education, enrichment, and engagement. To facilitate education, we hosted Zoo Biomechanics Day, a collaborative scientific public outreach event that hosted 8,000 attendees where GT researchers presented their research related to biomechanics and animals. To enhance animal enrichment, undergraduate students created low-cost habitat advancements including an automatic feeding system for terrestrial forager species and an arboreal feeder for tree-dwelling species to improve animal access to natural feeding habits. To develop university-student engagement in interdisciplinary research and conservation, students created a student organization, Tech4Wildlife@GT. Through Tech4Wildlife, students collaborate with Zoo Atlanta to implement alternative design construction methods, such as 3D printing, to enable access to low-cost fabrication techniques. Altogether, beneficial engagement between zoos and institutions can be built through short-term educational courses and long-term programs to strengthen collaboration and create a foundation for future research partnerships.

Lack of circadian entrainment by time-restricted feeding in a day-active rodent

Victor Zhang, Cassidy Austin, Jim Kenagy, Horacio de-la-Iglesia

While extensive research has been conducted on food entrainment in nocturnal rodents, knowledge regarding the effects of time-restricted feeding (TRF) on circadian rhythms in diurnal rodents is limited. We examined the effects of TRF on general locomotor activity, wheel running, and core body

temperature (Tb) rhythms in a diurnal rodent species, the antelope ground squirrel. Animals were entrained to a 12:12 light-dark cycle and subjected to a 10-h feeding regimen (being the minimum duration required for maintaining normal, adequate food consumption) centered around either the middle of the light phase (light feeding "LF"; N=9) or middle of the dark phase (dark feeding "DF"; N=6) for up to 20 days. Compared to LF animals, DF animals experienced substantial reductions in both body weight and daily food consumption across the duration of the study. Although DF squirrels exhibited small amounts of nocturnal activity during TRF, neither LF nor DF squirrels showed food-anticipatory locomotor activity, indicating that overt activity rhythms were not entrained by TRF. Moreover, three of six DF animals exhibited mild hypothermia, with daily Tb minima declining 5-7°C below normal. These results demonstrate that food-anticipatory activity is not induced by nocturnal feeding in antelope ground squirrels.

Schooling fish save energy by filtering environmental turbulence

Yangfan Zhang, Hungtang Ko, Michael Calicchia, Rui Ni, George Lauder

Understanding the physical principles underpinning vertebrate locomotion is key to clarifying the ecological and evolutionary benefits of collective movement. We hypothesize that active directional swimming in fish schools results in altered kinematics of individual fish and reduces the energetic cost of group swimming compared to individuals swimming alone. This is the 'environmental turbulence filtering' hypothesis. We test this hypothesis by quantifying energetics and biomechanics for schools of giant danio and solitary individuals swimming in control and turbulent conditions over the speed range of 0.3–8 body length sec⁻¹ (BL s⁻¹). The length scale of turbulence eddies is on the same order of magnitude as fish body depth (~1cm). Fluctuation of lateral velocity increased by ~300% with mean velocity, suggesting a much higher turbulent disturbance effect on the travel trajectory of the animals. We discovered that, at higher speeds, by reducing 3-D school volume by 68% in turbulence, fish schools saved 47% of the aerobic cost and non-anaerobic energy by 8 folds, reducing the total cost of 190–378% over 3–7 BL s⁻¹ compared to solitary fish. Fish within the school spend ~25% less kinematic effort (tail beat amplitude*frequency) to swim in turbulence at higher speeds. We suggest that the reduced locomotor cost stems from the schooling dynamics that filters the externally imposed turbulent eddies into more coherent and smaller eddies within the fish schools.

Characterize Cellular Senescence in Avian Species

Yufeng Zhang, Joshua Shirazi, Reihane Eric, Amir Hosein Sanjari-Nia, Birgit Schilling, Judith Campisi, Chidambaram Ramanathan

A striking example of a prime driver of aging phenotypes and pathologies is cellular senescence – a complex stress response that causes an essentially irreversible arrest of cell proliferation while maintaining metabolic activity and development of a multicomponent senescence-associated secretory phenotype (SASP). The accumulation of senescent cells has been associated with numerous age-related conditions, such as cancers. Over the past two decades, extensive research has focused on cellular senescence primarily in laboratory rodents and human cell lines. However, our understanding of cellular senescence in other species, especially in avian species, is extremely limited. This study aims to characterize cellular senescence in two avian model species domestic Chicken *Gallus gallus* and Zebra Finch *Taeniopygia guttata*. We first isolated and cultured primary dermal fibroblasts from these two avian species and induced cellular senescence using X-irradiation and overexpression of oncological RAS, causing DNA damage and activation of oncogene in cells respectively. Then, we quantified cellular senescence by measuring senescence associated β -galactosidase levels. We also measured SASP profile using newly developed proteomic approach. We found that cells from these two species could be induced senescence by both approaches similar to their rodent and human cells counterparts. However, even though some similarities were observed in the secretory profiles across most species, but each species manifested a unique SASP profile.

Chemical Characterization of Cuticular Hydrocarbons in Diurnal and Nocturnal Fireflies

Yiyu Zheng, Katie O'Connor, Nathan Peot, Greg Fahrner, Zhangyi Wu, Sean Halloran, Jocelyn Millar, Gregory Pask, Sarah Lower, Douglas Collins

Fireflies are renowned for their distinct flashing patterns, primarily utilized for mating; however, not all fireflies emit light. Diurnal fireflies have lost this ability as adults and are thought to rely on pheromones for communication. Field observations indicate that male diurnal fireflies are attracted from a distance and land near females, using their antennae to sample the air and the females' elytra prior to mating, suggesting the use of both long-range and contact-based mating signals. In contrast, while adult nocturnal fireflies generally use bioluminescent signals to locate females, they antennate females upon approach, suggesting that they may also employ contact pheromones, such as cuticular hydrocarbons (CHCs), as a mating signal. In this study, we investigate species- and sex-dependent differences in the composition of CHCs among both diurnal and nocturnal fireflies. Whole-body hexane extractions were collected from 10 firefly species found in the eastern United States, which were then analyzed with gas chromatography-mass spectrometry (GC-MS). The relative abundance of CHCs was typically the most pronounced difference between sexes, while the identity and/or diversity of compounds varied more substantially between different species. These findings support fundamental studies of mating signal evolution in fireflies.

Physical intelligence in centipede limbs facilitate reliable locomotion on rugose terrain

Baxi Zhong, Juntao He, Kelimar Diaz, Tianyu Wang, Daniel Irvine, Daniel Soto, Yasemin Ozkan-Aydin, Grigoriy Blekherman, Daniel Goldman

In terrestrial legged locomotion, foot slip is often considered detrimental and, consequently, actively avoided through feedback controls (e.g., reflexes). However, recent research [Chong et al. PNAS 2023] illustrated on robots that foot slipping is not only inevitable but also beneficial for systems with many legs. Here, we investigated how centipedes (*Scolopendra polymorpha*) navigate rugose terrains with randomly distributed step heights (thus a high probability of missed footsteps and slips). We recorded high-speed videos of centipedes and used DeepLabCut to systematically assess locomotor performance, analyze body and limb kinematics, and measure tarsal tip slippage, with subsequent comparison to their performance on a flat surface. Surprisingly, we observed that centipedes exhibited similar body and limb kinematics across all terrains with almost identical locomotor performance (measured in body lengths per cycle). Further, slippage profile analysis suggests compliant "physically intelligent" mechanics: upon collision with a block, the limbs yielded and passively slid through the block. Our results suggest these animals negotiate limb-substrate interactions and navigate complex terrains leveraging the innate physical intelligence of their limbs to simplify control. Finally, we demonstrate in robophysical studies that directionally compliant limbs facilitate effective multi-legged locomotion over noisy landscapes even with simple open-loop control.

Individuality in captivity: Intercellular motility of a dinoflagellate symbiont in an acoe host

Grace Zhong, Manu Prakash

Biological individuality has long intrigued philosophers and scientists. Symbiosis - often marked by persistence and adaptation of the community, but reproduction of the individual constituents - offers a unique lens to this concept. One aspect of agency is motility - the ability to control one's motion - as opposed to mere mobility, where one's motion is controlled by one's host. In symbiosis, in the context of multicellular hosts, inhibition of symbiont motility is common in order for the host to control symbiont motion to achieve community goals such as prescribed distribution within the host. We introduce *Waminoa* sp., an acoe worm - without a gut, flat in morphology, and hosting symbiotic dinoflagellates - as an ideal platform to probe questions surrounding symbiont motility and individuality. Here, we report the discovery of impressive motility of dinoflagellate symbionts in *Waminoa* sp.. We quantitatively analyze motility patterns of symbionts within the host tissue, at cellular to organismic scales, and by comparing symbiont motion to passive elements

within the host, we delve into the interplay between mobility and motility. We further probe how tissue architecture and properties may enable the observed motility. Our findings contribute to our understanding of agency in symbiosis and raise intriguing questions about its implications at both the individual and community levels.

Modulating gaze control by manipulating multisensory input in *Drosophila melanogaster*

Nobel Zhou, Michael Rauscher, Jessica Fox

The integration of multiple sensory modalities is necessary for behavior. In humans, gaze control is achieved by integrating visual signals with mechanosensory feedback from multiple sources, mainly the vestibular system. In flies, the reduced hindwings known as halteres play many of the same roles in gaze stabilization as the vertebrate vestibular system. We hypothesized that gaze stabilization behaviors are linear combinations of fast mechanosensory responses and slow visual responses. We tested this hypothesis by providing a pseudorandom stimulus in both modalities and observing the head movements of tethered fruit flies (*Drosophila melanogaster*) in response. In the absence of visual stimuli, the yaw rotation of the fly was sufficient to produce a compensatory gaze stabilization reflex, indicating mechanosensory involvement. By treating the underlying gaze stabilization as a linear model, we were able to discern the magnitude of the response in regard to stepwise yaw rotation; we found that haltere removal made this response significantly weaker.

Computational inference of thermal tolerance across insect taxa

Sophia Zhou, Fernan Perez-Galvez, Nick Teets

It is imperative to understand how climate change effects arthropods, the most diverse and abundant animals on earth. Climate change is impacting the distribution of arthropods and facilitating the establishment of invasive species. Accurate quantification of thermal limits can improve predictions of species distributions and invasion risk, but current methods can be labor intensive and error-prone. We recently developed an open-source Python command line application, DIME (Detector of Insect Motion Endpoint) to automatically assess thermal limits in *Drosophila melanogaster* from videos of flies exposed to heat or cold, and here we assessed whether this method works across diverse insect species. To achieve this objective, we 1) selected a group of arthropods inhabiting various ecological niches, 2) carried out the thermal limit assay, 3) analyzed the data with both manual and computational approaches (i.e., DIME). We collected upper thermal limit (CT_{max}) data for 6 insect species and compared them to previously published thermal limits, and our data were consistent across 5 of the 6 taxa, supporting our hypothesis that the assay developed for the *Drosophila* is applicable to other species. For remaining species, however, there has yet to be an apt standard to which the results would be comparable. Nevertheless, our results demonstrate that DIME can produce high-throughput, accurate measurements of thermal limits, which can contribute to ongoing efforts to predict ectotherm responses to climate change.

Higher Phase Variation in Nematodes than in Cockroaches Implies CPG's Role as a State Estimator

Yishun Zhou, Eleni Gourgou, Shai Revzen

Rhythmic animal locomotion in vertebrates and invertebrates is generated by central pattern generators (CPGs), whose activity is modulated by both mechanical and sensory feedback. Previous studies have investigated the balance between feed-forward and feed-back in CPG operation, and also on designing controller models and numerical simulations to produce animal-like locomotion behavior. We examined the frequency stability of a kinematically derived phase estimate in rapid motions of *B. discoidalis* cockroaches and *C. elegans* nematodes to better understand the role of CPGs. At $n=3$ cycles into the future, nematodes exhibit -1.65 to 1.29 radians (iqr; 17 animals; 462 trials; 4920 total cycles) of phase variability, whereas cockroaches exhibit only -0.67 to 0.92 radians (iqr; 7 animals; 19 trials; 247 total cycles). The greater frequency stability of cockroach CPGs may be caused by their coupling

to the mechanical oscillations of the body described by the SLIP and LLS models, whereas nematodes crawl in a low Reynolds number regime where the system has no significant mechanical inertia preserving the frequency. If a CPG is a feed-forward pattern generator, its frequency stability should not depend on mechanical coupling to a “memory” of the frequency in the form of inertia; if a CPG is primarily a phase estimator, its frequency stability will be strongly influenced by such “memory”. Our evidence weighs toward the interpretation that CPGs are state estimators rather than pattern generators.

LoCo-Pipe: an automated pipeline for population genomics with low-coverage whole-genome sequencing

Zehua Zhou, Peter Sudmant, Runyang Nicolas Lou

Low-coverage whole genome sequencing (lcWGS) is a cost-effective tool to characterize the patterns of genetic variation across entire genomes. It has become increasingly popular for both addressing basic evolutionary questions and solving applied biodiversity conservation problems. However, the further adoption of this tool is hindered by its reliance on a suite of complex software programs. These programs can account for genotype uncertainty through a probabilistic framework, but they present a steep learning curve for beginners. To lower these barriers, we created LoCo-Pipe, an automated Snakemake pipeline that streamlines a set of essential population genomic analyses (e.g. principal component analysis, genetic diversity estimation, etc.) and can be launched with a single line of code. LoCo-Pipe incorporates several best practices and filtering steps, and it enables users to easily make their own adjustments by editing a configuration file. It could also take advantage of high performance computing clusters to maximize efficiency through massive parallelization. Other advantages include built-in software package management and a well-organized data structure. We provide users with comprehensive documentation, including extensive in-line annotations and a detailed tutorial. A small toy dataset is included in the tutorial, with which users can quickly test LoCo-Pipe and visualize its results before applying it to their own dataset. LoCo-Pipe will be available on GitHub and we welcome all users to contribute and bring more functionalities to it.

The Frogolotl: A frog-axolotl chimeric model system to study multi-scale morphogenesis

Gabriel Zimbler, Vasilios Nanos, Michael Levin

An organism can be viewed as a system of modular subunits organized in a nested multi-scale hierarchy, ranging from the molecular to the cellular, tissue, organ, and whole-organism levels. During morphogenesis, the body is constructed by the interactions of those subunits across all levels of organization. To elucidate the principles that govern these interactions, we ask whether combining genetically distinct subunits into one chimeric organism results in the generation of new chimeric morphology. Therefore, we developed a new chimeric model organism between frog (*X. laevis*) and axolotl (*A. mexicanum*) named ‘frogolotl’ by means of xenoplastic transplantation of the tail bud at early embryonic stages. This new chimeric model system, in combination with modern molecular biological tools, will allow us to better understand how the specification of target morphology is dependent on genetic and epigenetic factors. We use geometric morphometrics to elucidate the role of tail-body communication in tail morphogenesis and regeneration by comparing growth rates, allometric relationships, and shape changes of grafted tails to those same measures of tails in the wild-type condition. Furthermore, we combine those morphometric measures with RNAseq analysis to acquire a more comprehensive understanding of how organs adapt to their new environment on the molecular level. Overall, these experiments provide novel insight into the role of tissue-tissue interactions in development and regeneration.

Heading for a breakdown: Assessing evolution through the hybridization of two sexual systems

Diamanda Zizis, Melody Sain, Tanisha Williams, Chris Martine

The plant genus *Solanum* represents an ideal system to investigate how hybridization between two different sexual systems impacts the reproductive and phenotypic biology of the hybrid progeny. Hybrids between Australian

Solanum species *S. dioicum* (dioecious) and *S. ultraspinosum* (andromonoecious) were grown to track what happens when you cross two taxa with different sexual systems. Morphometrics were conducted on 18 morphological characters. Hybrid crossing failure was evaluated using fluorescent microscopy. The only successful hybrids from the original crosses were those derived from *S. dioicum* as the pollen donor and *S. ultraspinosum* as the pollen recipient. Due to strong maternal effects, all F1 hybrids resembled *S. ultraspinosum*, thus all F1 plants were andromonoecious. The F2 and F3 hybrids demonstrated variability in inflorescence architecture, specifically the persistence of cosexual flowers in the staminate position of an andromonoecious inflorescence and the abortion of staminate buds, which may be suggestive of a change in sexual system. Nearly all attempts at F3 and F4 hybrid generations failed—suggesting hybrid breakdown. The observation of pollen germinating but failing to reach the ovary suggests that pollen tube abortion in the style is contributing to hybrid breakdown. Hybridization between taxa with two distinct sexual forms may shed light on the evolution of non-normative reproductive strategies in this clade.

Thermography and behavioral analyses reveal novel mechanisms of seasonal acclimatization in birds

Juan Zuluaga, Raymond Danner

Studies on seasonal acclimatization in birds have primarily involved metabolic measurements, few of which have investigated behaviors, and none have investigated changes in peripheral heat exchange. Using thermography and behavioral analyses, we examined the seasonality of heat exchange and thermoregulatory behaviors in chipping sparrows (*Spizella passerina*). We captured cold-acclimatized individuals in winter and heat-acclimatized individuals in summer and collected thermal images of these birds at 15.0°, 27.5°, and 40.0°C. We found that heat dissipation through the bill and legs changed seasonally, but surprisingly, both were higher in winter than in summer. We found that heat dissipating behaviors were more common in winter, whereas heat conserving behaviors were more common in summer. Behaviors associated with resource costs or predation risk showed more distinct differences between season than low-cost and low-risk postural adjustments. The seasonal adjustments to behaviors suggest that non-acclimatized birds must use costly thermoregulatory behaviors more frequently than acclimatized birds. The use of thermography catalyzed the discovery of one completely novel behavior and the first instance of a known behavior in a new species. Both novel behaviors aided in evaporative heat loss and occurred more commonly in winter, supporting the presence of seasonal acclimatization as evidenced by behavioral adjustments. These results provide novel insights to the process of acclimatization and suggest a role of behavioral adjustments in seasonal acclimatization.

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