

**Briefing Paper for 2008 (Year 4) for the Bird Study**  
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**Objectives for 2008 (extracted from proposal):**

1. Completion of swallow lifetime reproductive success study. a) Is reproductive success depressed in contaminated birds? b) Is lifespan shortened in contaminated birds? c) Is lifetime reproductive success related to individual lifetime mercury exposure?
2. Continuation of sub-lethal effects study on free-living swallows. A collaboration with Bill Hopkins, Dana Hawley and Haruka Wada from Virginia Tech.
3. New mercury-dosing study on captive wrens and sparrows to determine sub-lethal effects of relevant dietary levels. This project was not funded for 2008. A proposal to carry out this objective is forthcoming.
4. Completion of study to identify proximate source of dietary mercury: river or floodplain? a) First, we will characterize the stable isotope signatures of nitrogen and for wolf spiders along a transect parallel to the river and encompassing each of the river's four sewage treatment plants. b) The second approach will be to isolate spider gut contents during mayfly peak emergence periods and identify mayfly prey using PCR and mayfly-specific DNA probes.
5. New modeling study to determine the role of foraging behavior and habitat structure on exposure risk. This study will use simulation models to estimate the mercury intake for a theoretical bird foraging in a simulated floodplain habitat contaminated with mercury.

**Progress towards objectives in 2008:**

Objective 1) A portion of the population was manipulated for the collaborative biomarker study. To ensure that the return rate study was not affected by the manipulations, all females were caught during incubation (earlier than in previous years by 2-4 weeks). This allowed the identification of returning birds and their exclusion from the manipulation study. However, because blood samples from females were collected at an earlier date, the mercury levels in 2008 would be expected to be lower than in previous years, and this was observed (mean ~ 2 ppm in female blood, the lowest level found so far). There appeared to be less difference than in the previous two years in number of fledglings produced, but further analyses are required to determine if these data can be considered representative. Return rate continued to differ between contaminated and reference sites, with reference swallows more than twice as likely to be alive after 3 years of exposure than contaminated birds. There appears to be no strong "dose-dependent" type relationship between lifetime mercury level and lifetime reproductive output, although further analysis is needed because the current analysis does not take into account the number of years of exposure

Objective 2) These samples are still being analyzed, but we can definitely report that the ambitious experimental procedure (stressing swallows by enlarging broods) was a success and the target sample sizes were achieved.

Objective 4) The question to be answered is whether spiders show the same change in isotope signature above and below a tracer spike as the aquatic organisms directly exposed to the spike. If spiders do, and terrestrial-feeding crickets and caterpillars do not, this will suggest that aquatic prey comprise a large proportion of the spider's diet. Our first study involved collecting invertebrates above and below the two sewage treatment plants to compare isotopic signatures of aquatic and terrestrial organisms. Triplicate samples of large wolf spiders, plants, and crickets or caterpillars were collected at 12 points (10 pitfalls at each) stretching 1000 m above and below the treatment plants at Grottoes and Waynesboro. Triplicate samples of periphyton, snails, and mayfly larvae were also collected at each point. These have been freeze-dried and shipped to Colorado Plateau Stable Isotope lab for analysis of S, N and C isotopes. Results and analysis are expected soon. Our second study was a pilot study of whether PCR and gene sequencing can be used to identify aquatic organisms in spider guts. Spiders and larval mayflies and caddisflies were collected. These were sequenced in an attempt to develop a primer that binds to DNA of several aquatic prey organisms, but not spider DNA. Sequences properly identified species (based on GenBank searches), and during 2008/2009 a primer will be designed that will include several aquatic emergent species and exclude spiders and terrestrial prey. As a proof-of-concept, two wolf spiders were fed adult mayflies, and then dissected several hours later. The gut contents were sequenced, as well as the spider and mayfly. We will soon know whether the spider gut contents contained DNA that sequenced as mayfly. If so, the way is paved to apply the aquatic prey primer to unknown gut contents of spiders collected in the field, to determine if they contain aquatic prey DNA.

Objective 5) A lot of progress was made with mathematician Meagan McNulty on developing a spatially-explicit simulation model of bird foraging along the South River. This model will allow three types of investigation: 1) Do different foraging strategies (e.g. different species of birds) lead to different mercury exposure? 2) Do different sites have land cover characteristics that lead to different avian exposure? 3) Does altering land cover (i.e. remediation strategies such as planting trees) alter exposure? We have digitized each of our sites to delineate forest, pasture and developed land. We have written a program to upload GIS images into MATLAB. We have developed models of house wren, bluebird and Carolina wren foraging in MATLAB. We will be working with McNulty (now on the VMI faculty) to run the model simulation to answer the three questions above. Data from the soil surveys will be used to create realistic mercury gradients from river into floodplain.

#### **Dissemination of findings:**

Seven manuscripts have been submitted to journals from the bird study. 4-5 others manuscripts are in preparation for 2009/2009. Six more talks were given in 2008 at ornithological conferences, two by Kelly Hallinger and Mikaela Howie at the *Wilson Ornithological Society* meetings in Mississippi, and four by Dan Cristol, Mikaela Howie, Rebecka Brasso and Kelly Hallinger at the *American Ornithologists' Union* conference in Oregon, at a toxicology session chaired by Dan Cristol. The team will miss the 2008 SETAC meeting, but will contribute paper(s) to the Mercury as a Global Pollutant meetings in China in 2009 and the 2009 SETAC meetings.