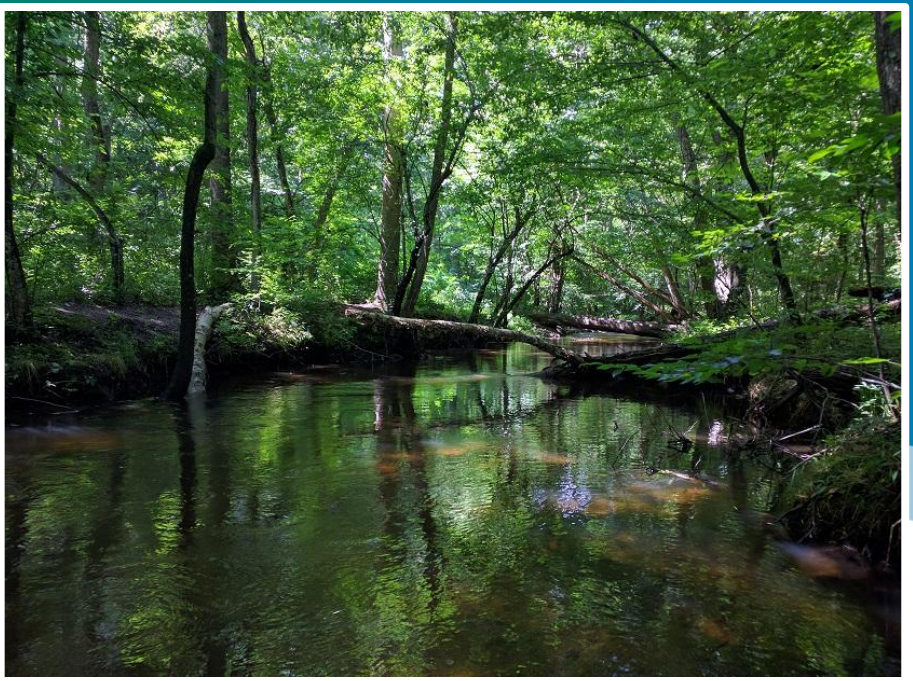

Determination of Filtration Capacity and Pollutant Removal of Freshwater Mussels in Delaware Streams

Final report for
DNREC Delaware Clean Water Advisory Council

Grant: CWQ 14-04

Partnership for the DELAWARE ESTUARY

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The Partnership for the Delaware Estuary brings together people, businesses, and governments to restore and protect the Delaware River and Bay. We are the only organization that focuses on the entire environment affecting the river and bay — beginning at Trenton, including the greater Philadelphia metropolitan area, and ending in Cape May, New Jersey and Lewes, Delaware. We focus on science, encourage collaboration, and implement programs that help restore the natural vitality of the river and bay, benefiting the plants, wildlife, people, and businesses that rely on a healthy estuary.

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Executive Summary

The status and restoration prospects of freshwater mussels in southern Delaware appears to be better than in piedmont streams of northern Delaware, which we have previously studied. Extant mussel populations were found in most surveyed streams and ponds of Sussex and southern Kent County, and at least two species were found in many locations. In contrast, earlier surveys in New Castle and northern Kent County revealed extant mussel populations in only a few locations, and typically just one species. As a generalization, piedmont streams are more impacted by stormwater, land use, and dams, compared to coastal plain systems. Hence, restoration prospects for freshwater mussels appear to be strong in southern Delaware.

Mussels collected from two source locations in southern Delaware were relocated to several candidate locations where mussels likely once existed but no longer do, based on our survey data. Relocations consisted of either PIT-tagged and free-released mussels or caged mussels. Their survival, movements (tagged) and fitness (caged) were then tracked for one year. Survival was consistently high for all treatment groups. Retention of tagged and released mussels in relocation sites was also high compared with other studies. Assessment of chronic fitness measures in caged mussels, such as condition index and the proximate biochemical composition within mussel tissues, revealed that the best fitness was actually achieved in one of the new locations, supporting slightly better mussel fitness than the best reference (source) site.

Interestingly, one of the two mussel source locations (Brown's Branch) supported lower mussel fitness, and sizes of extant mussels are smaller there. Although this might simply reflect diminished food resources in streams compared to ponds, it was notable that higher fitness was supported in a candidate restoration stream having no current mussels (St. Jones). Although more analysis is needed, these findings suggest that conditions in St. Jones might have been more degraded in the past but natural recolonization by mussels might be currently impeded by blockages of essential fish hosts for mussel reproduction and larval dispersal. In contrast, suboptimal conditions in Brown's Branch could reflect a decline in current conditions, relative to the past.

Based on these findings, efforts to restore freshwater mussels to the studied streams and ponds where they had become extirpated can proceed to the next phase (e.g. release of hatchery propagated juveniles). And it is likely that many other similar sites in southern Delaware would yield similar, favorable prospects for mussel restoration readiness. The

seasonal physiological data from the current study, for two mussel species from various source locations, provide critical empirical information from which we can now model water quality outcomes from future restoration investments. Mussels living within Blair's Pond, Brown's Branch, and Ingram's Pond cleared water at the rate of 0.53 – 1.5 liters per hour per gram dry tissue, depending on season and location. These clearance rates are consistent with data from a variety of other bivalves, including freshwater mussels studied elsewhere as well as marine oysters and mussels. When compared to the amount of suspended particles in the studied systems, seston filtration rates varied from 0.8 – 12 milligrams per hour per gram dry tissue. Every 1,000 mussels living in these 3 systems is estimated to clear 2.8 million gallons of water per year, removing 173 kilograms of (dry) suspended particles. Considering that current populations of mussels in those 3 streams are far greater than 1,000 mussels, these results support the need to conserve extant mussel beds as a means of sustaining current water quality. More importantly, if streams and ponds such as Waples and St. Jones can be restocked with mussels, then the restored beds should promote water quality improvements. Hence, our results confirm that efforts to rebuild natural mussel populations would yield substantial positive benefits for water quality.

This study filled important gaps in our current understanding of freshwater mussel distribution, range, species richness, and ecosystem services within the State of Delaware. Use of a dual reintroduction protocol pairing tag/release methods with caging/fitness assessments refined and strengthened our comparative methods for gauging the restoration readiness of candidate locations, including both pond and stream systems. With this knowledge, future phases of mussel restoration are recommended, possibly including release and restocking of mussels using hatchery-propagated juveniles into streams and ponds that have been shown to support mussel survival and fitness. Future studies of the mussel-mediated ecosystem services that would result from such investments can now proceed using new empirical data on pollutant filtration rates by local mussel species, which to our knowledge are the first such measures assessed within the State of Delaware.

Introduction

Importance of Freshwater Mussels

In North America, over 70% of the near 300 native species of freshwater mussel species are endangered, threatened or of special concern (Williams et al. 1993) making them the most imperiled taxa nationally (Nobles and Zhang 2011). Freshwater mussels are also considered to be the most imperiled animal locally in the Delaware River Basin as well with declines in species richness, range of distribution, and population abundance (PDE 2012a, 2012b). The majority of the 12 native species to the basin are of concern and few areas support robust populations of common species (Table 1). Despite their decline, there are emerging data suggesting that freshwater bivalves are important for water quality and have other significant ecological roles. The ecosystem services contributed by these mussels depend on their population abundance and body sizes, similar to other filter feeding bivalves (e.g. clams, mussels, oysters) (Strayer 1999, Dame 2012). Due to their imperiled status and potential importance in ecosystem functioning and water quality, there has been a rise in national interest in protecting and understanding these animals.

This expanded interest is reflected by the greater diversity of state and federal agencies that are now attentive to freshwater mussel status and trends. In the past, the main groups that focused on mussel conservation and restoration were state heritage programs and a few federal agencies (USFWS, USGS), which focused on biodiversity preservation and the protection of listed species. Now, many other agencies (e.g., EPA) and water supply companies (e.g., Philadelphia Water Department, United Water) are focused on the water and habitat benefits that are furnished by healthy mussel beds in streams, rivers and lakes.

As a National Estuary Program, the Partnership for the Delaware Estuary (PDE) is expected to establish measurable goals for sustaining and improving water and habitat conditions and to implement a Comprehensive Conservation Management Plan (CCMP) to protect and restore natural resources. PDE has elevated healthy freshwater mussel populations as one of a limited subset of "driver" goals that facilitate ecosystem-based restoration in the Delaware River Basin. This goal is based on the observation that mussels are long-lived (species dependent, upwards of 100 years) and are sensitive to disturbances to environmental and ecological conditions such as water quality, water quantity, riparian cover, and fish passage. Hence, to achieve multiple goals for water and habitat conditions in any given water body, a simplified focus on achieving a healthy assemblage of native freshwater mussel species living in abundance will drive positive decision-making in support of broader CCMP actions

and needs.

The water quality benefits of healthy natural mussel beds are only now being studied, but look to be sizeable. Subject to environmental conditions, each adult mussel filters gallons of water every day. Many streams that once supported abundant mussels no longer do. The loss of beds of filter-feeing mussels is thought to contribute to degraded water quality, representing a negative feedback for ecosystem health. Hence, mussel restoration should promote positive feedbacks to ecosystem health in the form of cleaner water, reduced erosion, and increased habitat complexity. For more information on freshwater mussel ecology, life history, and Delaware River Basin species, please refer to *Freshwater Mussels of the Delaware Estuary: Identification Guide & Volunteer Survey Handbook* (PDE 2014) and other information at the following website: http://www.delawareestuary.org/freshwater-mussels.

Table 1. Conservation status of freshwater mussel species in the Delaware River basin.

Freshwater Mussel Identification		State Conservation Status		
Scientific Name	Common Name	DE	NJ	PA
Alasmidonta heterodon	Dwarf Wedgemussel	Possibly Extirpated	Endangered	Critically Imperiled
Alasmidonta undulata	Triangle Floater	Possibly Extirpated	Threatened	Vulnerable
Alasmidonta varicosa	Brook Floater	Extirpated	Endangered	Critically Imperiled
Anodonta implicata	Alewife Floater	Critically Imperiled	no data	Vulnerable
Elliptio complanata	Eastern Elliptio	Secure	Common	Apparently Secure
Lampsilis cariosa	Yellow Lampmussel	Possibly Extirpated	Threatened	Apparently Secure
Lampsilis radiata	Eastern Lampmussel	Critically Imperiled	Threatened	Critically Imperiled
Lasmigona subviridis	Green Floater	no data	Endangered	Imperiled
Leptodea ochracea	Tidewater Mucket	Critically Imperiled	Threatened	Critically Imperiled
Ligumia nasuta	Eastern Pondmussel	Critically Imperiled	Threatened	Imperiled
Margaritifera margaritifera	Eastern Pearlshell	no data	no data	Critically Imperiled
Pyganodon cataracta	Eastern Floater	Apparently Secure	no data	Apparently Secure
Strophitus undulatus	Creeper	Critically Imperiled	Species of Concern	Secure

Although many current mussel populations appear to be extremely depressed and geospatially constricted relative to historic levels, numerous scientists and managers believe that this represents an opportunity to rebuild mussel populations. Countless streams and rivers that were once too polluted to support mussels have since been remediated to the point where mussel populations may again be sustained. However, blockages to fish passage, slow growth, and other impediments stand in the way of mussels being able to naturally re-disperse and colonize these habitats. Hence, assisted recolonization can directly augment and expedite recovery since the natural dispersal of native populations can be slow and unpredictable. It is also vital that any remaining mussel beds be afforded the greatest possible protection.

Freshwater Mussel Recovery Program (FMRP)

The FMRP was launched in 2007 by PDE with the goal of conserving and restoring native freshwater mussels within the Delaware Estuary. This program complements PDE's comprehensive watershed-based shellfish restoration strategy which also includes saltwater oysters and saltwater ribbed mussels. Together, these shellfish range from the headwaters to the Bay.

The FMRP consists of 8 areas of focus (Fig. 1):

- **Surveys** of freshwater mussels (qualitative and quantitative) to identify potential restoration sites and provide data on extant populations.
- **Conservation** of current mussel populations and their habitat.
- **Restoration** of freshwater mussel populations through tactics such as reintroductions to candidate waters.
- **Propagation** using hatchery methods to seed streams for water quality uplift and bolster mussel abundance.
- **Habitat** suitability for freshwater mussels to aid in restoration practices.
- **Research & Monitoring** to understand mussel life history, ecosystem services, and their interaction with future environmental conditions.
- **Remediation** of negative impacts on freshwater mussels and their habitat.
- **Outreach** to educate the public about conservation and restoration of freshwater mussels.

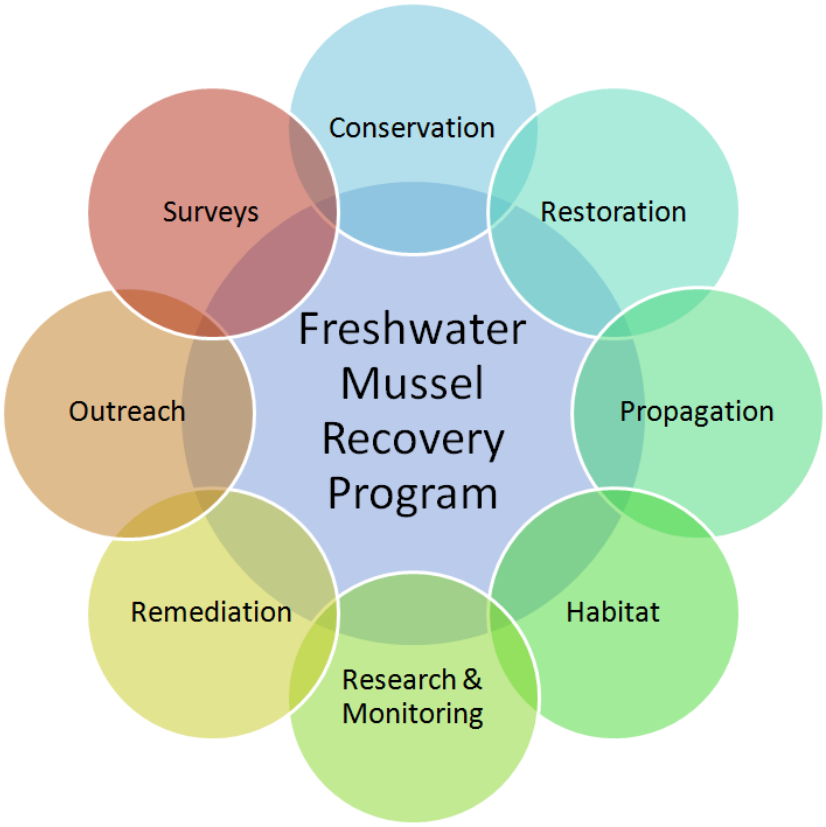

Figure 1. The FMRP bubble graphic highlights each area of focus.

Project Overview

This research project builds upon previous research by Partnership for the Delaware Estuary on the status and potential restoration of freshwater mussels in northern Delaware (Kreeger et al. 2014, Cheng & Kreeger 2015) and expands our understanding of freshwater mussels throughout the state of Delaware. A variety of research activities were performed to complement each other, helping to guide future protection, restoration and research with regard to freshwater mussels within the state.

Mussel surveys were conducted to fill key data gaps regarding the current status of mussel populations in southern Delaware, helping to discern protection and restoration needs. In streams and ponds where mussels no longer existed or were low in species richness, transplant and caging studies were conducted to compare their restoration readiness. Finally, mussel physiology experiments were conducted to provide quantitative data regarding the capacity of some dominant mussel species to filter suspended matter, including pollutants, so that we can better understand the benefits of mussel protection and restoration. More information for each research activity is summarized below and methodologies and results are reported in subsequent sections.

Qualitative Surveys

Although freshwater mussels were believed to be abundant in most streams and natural pond systems historically, survey data on their historic and current range, abundance, and species richness is sparse within the state of Delaware. For historical context and an understanding of reference conditions, we can look at past surveys in Pennsylvania, which shares some waterways with Delaware. For example, a comprehensive survey was completed in the early 1900s across Pennsylvania (Ortmann 1919), showing that virtually every surveyed stream contained multiple mussel species at numerous survey points. Ortmann surveyed qualitatively (presence/absence) but provided anecdotal observations of typical habitat requirements and densities of different mussel species.

Within Delaware, we have had little success in acquiring historic mussel survey data, necessitating new surveys which to date have also been qualitative (except for one quantitative survey in the Brandywine River). Surveys comprise one of the main facets of the FMRP because places where remnant mussel populations still exists can then be flagged for conservation and protection, and waterways devoid of mussels can be assessed for their restoration promise.

Mussel Reintroductions

Freshwater mussel populations are often lost in a system due to degraded environmental conditions (e.g. lack of fish passage preventing new reproduction, degraded water quality, metal toxicity, and erosion of streambeds). Before investing in mussel restoration in such places, it is prudent to first test whether candidate restoration areas can sustain the growth and survival of adult or juvenile mussels. PDE has developed several approaches for testing and comparing the readiness of candidate streams and ponds for mussel restoration.

One tactic simply reintroduces mussels that are tagged and then monitors and compares whether they survive and stay in place in the recipient waters. Such reintroductions can be completed with either adult mussels that are relocated from reference populations, or juvenile mussels that are propagated from native broodstock. The mussel species that is chosen is based on the likelihood that it existed previously in the targeted waterway and would be hardy enough to survive there. For reintroduction studies, PDE has to date focused on common mussel species that provide the greatest ecosystem services. Small scale test reintroductions can provide empirical data to help decide whether it is appropriate to move towards larger scale restoration efforts in candidate streams.

For the present study, we completed mussel reintroduction studies with the eastern elliptio collected from Blairs Pond which were tagged and reintroduced into the St. Jones River, Waples Pond, and back into Blairs Pond. Mussels were free-released and monitored twice over the project duration for bed retention rates and changes in shell length. This release technique is a relatively low-cost way of providing unique data on whether mussels are able to persist in a given stream or if physical conditions uproot mussels from their reintroduction beds. This method also allows mussels to burrow and move around to seek optimal habitat.

Cage Assessments

Though mussel reintroductions can help determine whether mussels may grow, survive, and remain in streams, those metrics represent acute responses which could take years to be fully discernible. More subtle differences in mussel performance can be obtained by assessing chronic fitness responses, such as their condition index and proximate biochemical composition of their tissues.

Tissue condition metrics typically vary seasonally, similar to marine bivalves (Bayne 1976, Zandee et al. 1980, Okumus and Stirling 1998). Tissue biochemistry may suggest whether

or not mussels are able to meet their seasonally shifting nutritional demands, and suboptimal fitness is typically deduced by deviations in seasonal condition relative to control mussels in reference streams. For examples, bivalves typically build up carbohydrate reserves in summer and fall to furnish an energy reserve for overwintering, and late winter to spring biochemistry typically reflects reproductive conditioning for gametogenesis.

For this study, mussels were collected from reference streams, placed in cages in representative candidate restoration streams using established protocols, and then were monitored for condition index, tissue proteins, and tissue carbohydrates for a one year period, in comparison to mussels in the source stream (Gray and Kreeger 2014). The disadvantages of cage assessments include some additional lab work (cost) plus the need to sacrifice the study organisms; however, fitness data are more sensitive to chronic differences, thereby providing the most sensitive gauge of the health of mussel populations in study streams.

Physiology Experiments

The water quality benefits of freshwater mussel populations depend on the collective filtration and particle processing rates of the total population that comprises a water body. Thus, ecosystem service studies depend on detailed knowledge of the population biomass (sizes, densities, species, extent) as well as detailed knowledge of the physiological processing rates (seston composition, clearance rates, defecation rates, etc). Population demographics are highly variable spatially, whereas physiological rates are highly variable with changing food conditions and water temperatures.

To begin to develop models of the water quality benefits of Delaware's freshwater mussel species, in this study PDE used established methods to directly measure the seasonal clearance rates, defecation rates, absorption efficiencies and seston quantities and qualities for two mussel species from four different source locations. Physiology experiments were performed under simulated natural conditions to ensure that data were representative of a mussel's physiology in nature. This was accomplished by collecting mussels from streams along with ambient water and performing experiments at ambient temperature.

For each study, different sizes of mussels were assessed, providing the first ever robust physiological data set for representative mussel species in the state of Delaware. Future studies will be able to use these data to model studied populations where mussel demographics are quantitatively understood, and for demonstration purposes we show water quality benefits for one quantitatively assessed Delaware population (Brandywine River).

Methods

Qualitative Mussel Surveys

To investigate the current status of freshwater mussels in Kent and Sussex counties, trained PDE scientists performed a total of 13 qualitative mussel surveys in five distinct stream systems. Surveys varied in effort based on accessibility and suitable survey area. In flowing waters, surveyors spent equal time wading along both banks of rivers as well as in the center when possible. For surveys in lentic systems, kayaks were used and surveyors paddled along the perimeter of ponds and lakes scanning for mussels. To aid in the detection of mussels, surveyors utilized metal hand scoops and a long handle scoop to sift through sediments. Scoops were used sparingly and care was exercised not to cause significant disturbance to the benthic habitat. Surveys were timed in order to calculate survey effort in units of person hours (survey time * number of surveyors).

Table 2 summarizes the major waterways surveyed and their respective size (stream length and watershed acreage). Survey areas, presented in Figure 2, were spread across stream reaches and targeted key areas where evidence of freshwater mussels was most likely to be detected (e.g. shells tend to deposit near outfalls of impoundments).

Table 2. Summary of study locations. Lengths include tributaries surveyed.

Waterway	Length (miles)	Watershed Area (Acres)
St. Jones River	15	41,038
Murderkill River	28	44,772
Mispillion River	23	44,026
Prime Hook Creek	20	20,111
Broadkill River	21	33,660

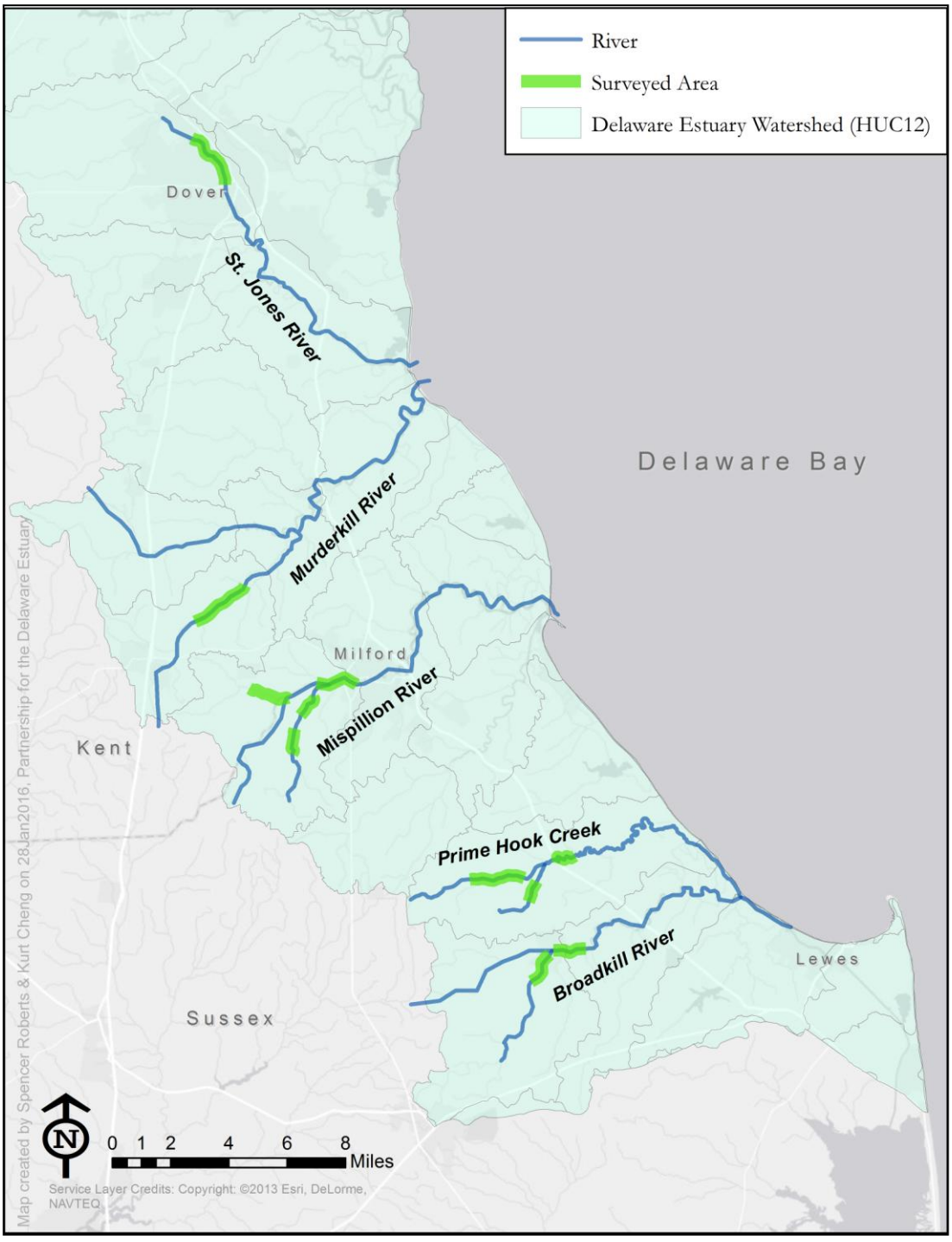

Figure 2. Qualitative freshwater mussel survey locations in southern Delaware.

When surveyors found live specimens, mussels were identified, measured for shell length, and promptly returned to the streambed. Shells found were identified, stored, and later archived for future reference. Single shells were considered to be evidence of previous extant mussels in the system that had either existed in the past, or washed down from upstream. A pair of shells that were still attached at the hinge was considered strong evidence of current extant mussels nearby as this likely represented recent mortality.

Mussel Reintroductions

Our region currently lacks the capacity for providing hatchery propagated mussels at a large scale; therefore freshwater mussels were collected from extant mussel populations robust in mussel abundance. The source population consisted of the eastern elliptio mussel, *Elliptio complanata*, from Blairs Pond. Prior to collection, PDE scientists applied and received the necessary collecting permit from the Delaware Department of Natural Resources and Environmental Control's Division of Fish and Wildlife (Permit #2015-FSC-010).

A total of 90 elliptio mussels were collected for reintroductions. Each mussel was cleaned gently with a brush, patted dry and subsequently tagged with a plastic tag and an electronic tag. Plastic tags were uniquely numbered for visual identification and affixed with cyanoacrylate. The electronic tag was a passive integrated transponder (PIT) which contains a unique code, enabling scientists to identify mussels electronically. PIT tags were affixed with a marine epoxy that was allowed to dry completely. Each mussel's shell length (longest axis) was measured with digital calipers (Mitutoyo CD-6" CXR, ±0.03 mm) and their PIT tags were recorded with a PIT tag reader (Biomark HPR+).

Mussels were then subdivided into three groups of 30 animals for deployment into restoration streams. The St. Jones River (hereafter St. Jones) and Waples Pond were chosen to serve as candidate restoration streams since suitable mussel was identified through previous qualitative surveys. A total of 30 mussels were deployed on June 17th 2015 into St. Jones, Waples Pond, and back into Blairs Pond. Mussel deployment locations are depicted in Figure 3.

To track mussel retention and growth over the study, reintroduced mussels were monitored with a PIT tag reader twice, four months (October 13th 2015) and one year (June 14th 2016) post deployment. Mussel surveyors used a handheld antenna connected to a PIT tag reader to scan for tagged mussels in streambeds. The antenna's range of detection (eight inches) provided adequate data however, because retention surveys only provide data if the PIT tag was pinged, data from these surveys are conservative by design. Bed retention recorded in

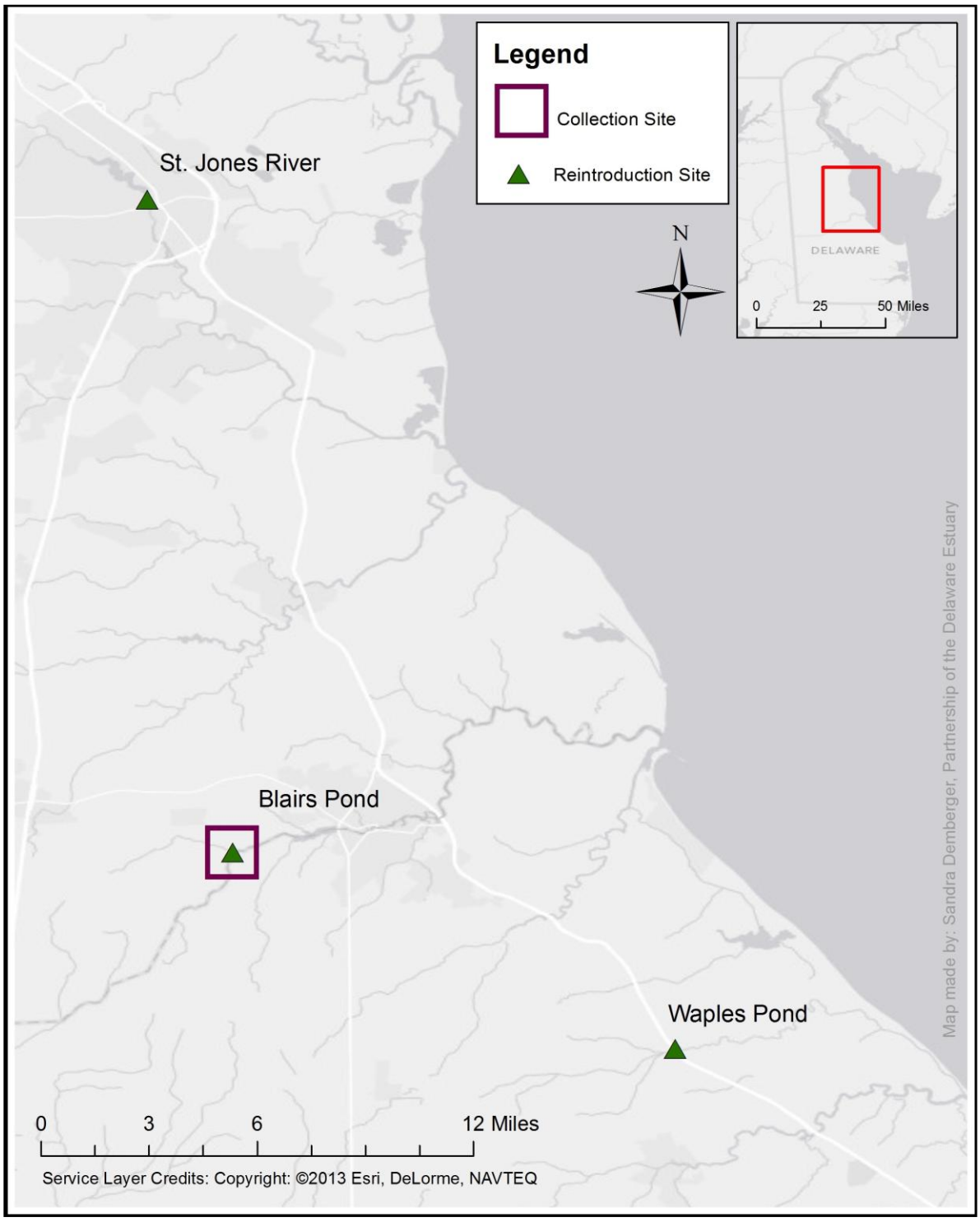

Figure 3. Freshwater mussel reintroduction sites in southern Delaware.

the field potentially underestimates true bed retention due to a variety of factors. The monitoring survey will not record mussels that have moved away from the reintroduction bed but are still present in the stream reach. Field personnel perform multiple passes within each reintroduction bed however, logistical considerations for thoroughly monitoring multiple reaches up to 10 meters long also add to the conservative nature of monitoring surveys. Mussels were measured after one year of deployment to determine if mussels exhibited positive shell growth.

Caging Assessments

The source populations for caging assessments included elliptio mussels from Blairs Pond as well as from Browns Branch in Kent County. Mussels were tagged with a plastic tag according to the same methodology described above. A total of 108 mussels were tagged from Blairs Pond and 54 from Browns Branch. Along with tagged mussels, eight mussels from each source population were collected for seasonal baseline mussel condition during the initial collection and three subsequent seasonal collections for a total of 36 over the study period (only 35 were collected from Blairs Pond). Collections fell within PDE's Delaware scientific collection permit (Permit # 2015-FSC-010).

Mussels were subdivided into groups of nine individuals and placed into cages within restoration streams as well as source streams. Reciprocal caging of mussels controlled for any handling stress or effects due to the caging technique. See Figure 4 for an overview of collection and deployment sites.

Cages were constructed from dishwasher trays and plastic mesh held together with zip ties. Cages were suspended off the bottom of Blairs Pond, St. Jones, and Waples Pond using wooden stakes and twine to avoid detrimental effects of litter buildup blocking flow of water in cages. In Browns Branch, cages were buried halfway into the streambed and secured with rebar due to water levels potentially exposing mussels during low flow. All cages were tethered with a rope to avoid loss during storm events. This caging technique has been applied previously in the Delaware Estuary with success (Gray and Kreeger 2014). A breakdown of the number of mussels from each source stream and the deploy stream is presented in Table 3.

Figure 4. Collection sites and deployment sites for cage assessments in southern Delaware. Inset provides regional context.

Table 3. Breakdown of the number of mussels that were collected and deployed in cages at each site. Nine mussels were placed into each cage.

Source Site	Deploy Site	Deploy Latitude	Deploy Longitude	# Cages	# Mussels
Blairs Pond	Blairs Pond	38.90444 N	-75.48675 W	3	27
	St. Jones	39.16414 N	-75.51973 W	3	27
	Waples Pond	38.82317 N	-75.30698 W	3	27
	Browns Branch	38.94762 N	-75.52772 W	3	27
Browns Branch	Browns Branch	38.94762 N	-75.52772 W	3	27
	Blairs Pond	38.90444 N	-75.48675 W	3	27

Caged mussels were monitored seasonally and a subset of nine mussels was collected from each study stream. Once collected, mussels were patted dry and their total wet weights (TWW) were recorded as well as shell lengths (SL). Tissues were then excised from mussel shells, frozen, and later freeze dried for dry tissue weight (DTW) immediately after collection (Fig. 5). Subsamples of dried tissues were placed in a muffle furnace for two days at 450 °C for ash-free dry tissue weight (AFDTW). Shells were dried in a drying oven at 60 °C for two days and weighed for dry shell weight (DSW). Tissues were ground by mortar and pestle and homogenized subsamples were used for condition index and tissue biochemical composition (protein and carbohydrate

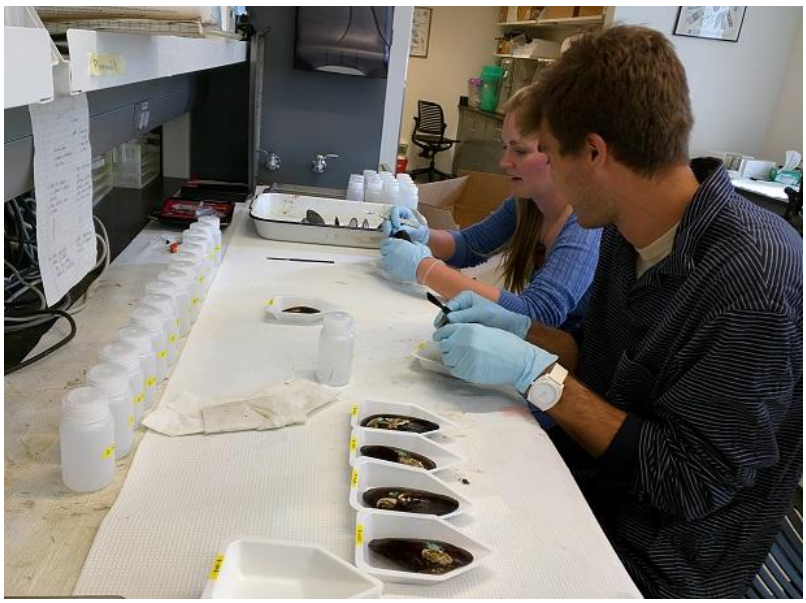

Figure 5. Spencer Roberts and LeeAnn Haaf excising tissues from mussel shells for further analyses.

content) at the time of their harvest. Methods for proximate biochemical analysis of seston and mussel tissue (see below) were adapted as per Kreeger et al. (1997).

Condition Index

Condition index, which is a typical proxy for the meat content of a bivalve, relates the dry weight to the interior shell volume of a mussel (Hopkins 1949). Condition index of mussels was calculated according to the formula described by Crosby and Gale (1990) and modified by Kreeger (1993):

$$CI = \frac{AFDTW * 1000}{TWW - DSW}$$

Protein Content

Protein assays were carried out using the Thermo Scientific bicinchonic acid (BCA) test kit (Pierce #23225X). Subsamples of 8-10 mg of homogenized tissue were weighed out and transferred to test tubes. Each test tube received 4 mL of 0.1M NaOH and sonicated for eight bursts at 50% power (Branson Sonifier M-250). Tubes were then incubated for 45 minutes at 60 °C. Tubes were then vortexed and centrifuged for 15 minutes. Following the BCA kit protocol, reagents and subsamples of sample test tubes were added to microplates along with a standard series and read at 562 nm using a Thermomax microplate reader (Molecular Devices) to determine protein content via colorimetric analysis. Protein content was expressed as a percentage of mussel dry tissue weight.

Carbohydrate Content

Carbohydrate content of mussel tissues was analyzed following the protocol described by Dubois et. al. (1956). A subsample of 1-2 mg of homogenized mussel tissue was weighed out for each mussel and were transferred to test tubes where they were treated with 1mL de-ionized water and 1 mL 5% phenol. Tubes were vortexed and 5 mL 95% sulfuric acid was added and let sit to cool. Tubes were centrifuged and supernatant of each tube was spotted on microplates along with a standard series created from cold soluble potato starch. Plates were read on a Thermomax microplate reader at 490 nm to determine carbohydrate content via colorimetric analysis. Carbohydrate content was expressed as a percentage of mussel dry tissue weight.

Mussel Physiology Experiments

Physiology experiments were performed three times (October 27th 2015, April 18th 2016, and August 2nd 2016) to capture variation in water temperature (10-13 °C, 15-20°C, 25-28 °C respectively) and seston composition (food quality and food quantity). Mussels were collected from four sites including the Brandywine River, Blairs Pond, Browns Branch, and Ingrams Pond. A total of 10 eastern elliptio mussels were collected from each site except for Ingrams Pond where 10 eastern lance mussels (*Elliptio fisheriana*) were collected for each experiment. Proper permits were acquired prior to collection for each year (Delaware DNREC Permit # 2015-FSC-010, 2016-FSC-060; National Park Service Permit FRST-2016-SCI-0001). Ambient water was collected concurrently at each site and transported to an outdoor laboratory in Millsboro, DE. Water was filtered to 53 µm to remove large particulates and water was used for both feeding trials as well as seston analyses.

Seston Analyses

Filtered ambient water from each of the four study sites was filtered onto pre-weighed glass fiber filters for seston composition including particulate matter, organic matter, protein content, and carbohydrate content, as per Kreeger et al. (1997). Filters were frozen prior to processing in the laboratory. Particulate matter was assess gravimetrically by drying filters in a drying oven for two days at 60 °C after which they were weighed on an analytical balance (VWR, 0.01mg). Organic matter of seston was measured through loss-on-ignition. Dried filters were placed in a muffle furnace for two days at 450 °C and weighed.

Protein assays of seston filters were carried out using the Thermo Scientific bicinchonic acid (BCA) test kit (Pierce #23225X). Filters were transferred to test tubes and each test tube received 4 mL of 0.1M NaOH. Tube contents were sonicated for eight bursts at 50% power (Branson Sonifier M-250) and incubated for 45 minutes at 60 °C. Tubes were vortexed and centrifuged for 15 minutes. Following the BCA kit protocol, reagents and subsamples of sample test tubes were added to microplates along with a standard series and read at 562 nm using a Thermomax microplate reader (Molecular Devices) to determine protein content via colorimetric analysis. Protein content was expressed as the percent protein concentration of the average particulate organic matter of seston for each stream.

Carbohydrate assays of seston filters followed the protocol described by Dubois et. al. (1956). Seston filters were transferred to test tubes and were treated with 1mL de-ionized water and 1 mL 5% phenol. Tubes were vortexed and 5 mL 95% sulfuric acid was added and let sit to cool. Tubes were centrifuged and supernatant of each tube was spotted on microplates along with a standard series created from cold soluble potato starch. Plates were

read on a Thermomax microplate reader at 490 nm to determine carbohydrate content via colorimetric analysis. Carbohydrate content was calculated as the percent carbohydrate concentration of the average particulate organic matter of seston for each stream.

Feeding Trials

Methods for feeding trials and quantification of key physiological rate functions of freshwater mussels followed the protocols developed by Kreeger for marine and freshwater bivalves (e.g. Kreeger 1993, Kreeger and Newell 1996). The most comprehensive description of the methods, as applied to freshwater unionid mussels, can be found in Kreeger (2011).

In summary, individual freshwater mussels were collected from the field and then maintained at ambient temperature and held in ambient water from their collection sites, containing ambient seston. For assessment of seasonal ecosystem services, such as filtration rates of suspended particles, it is critically important that the study animals be exposed to simulated natural conditions and diets during the feeding trials to avoid unnatural physiological responses. For a limited time period, ranging from 2 to 4 hours depending on ambient temperature, each mussel was placed in an individual feeding chamber and allowed to filter ambient particles. Additional chambers without live mussels constituted controls.

Clearance rates were then determined by tracking the depletion of particles in the feeding chambers over the experimental period, via periodic subsampling and subsequent particle analysis of fixed samples of the water from the chambers. Clearance rates by live mussels were normalized for ambient particle settlement or other biological processes by subtraction of any changes in particle concentrations in control chambers, per water type. Particle filtration was then calculated by contrasting volumetric clearance rates with separate detailed analysis of the weight and biochemical composition of the seston that the mussels fed on. At the end of the feeding trial, feces were collected and analyzed for their organic contents, ammonia concentrations were determined in the overlying water, and mussel biometrics were assessed for calculation of condition index and tissue weight, which were then used for allometric scaling of physiological rate functions to mussel body sizes. See Kreeger (2011) for a full description of methods and principles.

Twelve 1 liter tri-pour beakers were used for each of the four study streams. All beakers were filled with 800 mL of filtered ambient water from respective streams and mussels were added to eight of the twelve beakers. Four of the beakers served as the replicate controls, per water type/stream. Mussels were left to feed for at least two hours, and the total feeding time per animal was recorded. Prior to adding mussels and at four 30-minute intervals after

mussels were put in beakers, 10 mL water samples were taken from each beaker and preserved. Water was well mixed to ensure uniform particle distributions. Upon completion of feeding trials, mussels were extracted from beakers, patted dry, and weighed for total wet weight (TWW). Tissues were excised from shells, frozen, and later freeze dried for analysis of dry tissue weight (DTW). Shells were dried at 60 °C for two days and weighed to determine dry shell weight (DSW).

Feces produced by each mussel were collected from each beaker onto pre-weighed glass fiber filters, which were initially frozen and later dried (> 2 days at 60°C), weighed, combusted (2 days at 450°C), and re-weighed to for determine organic content via standard loss on ignition methods. At the end of each trial, 50 ml samples of overlying water from each chamber were also passed through a 0.2 µm membrane filter, and a 20 ml aliquot of the filtrate was then frozen for potential later analysis of ammonia-nitrogen production. Analysis of fecal and ammonia production provide additional information regarding the fate of filtered matter, however those metrics were beyond the scope of this study and the samples/data have been stored for potential future analysis.

Physiological Rate Calculations

To measure the clearance rates (volume of water processed per unit time) of mussels, water samples taken from feeding trials were diluted with 10 mL of a filtered (0.22 µm) electrolyte solution (Isoton II diluent, Beckman Coulter) and analyzed for particle concentration and particle size distribution (2-63 µm) using a Multisizer II (Beckman Coulter). The change in particle concentration per chamber during the feeding trial was determined by fitting a regression equation of the logarithm of the concentration from the (up to) five samples taken. The regression equation was then used to estimate the initial particle counts (Ci) and final particle counts (Cf). Control beaker settling rates (SR) were calculated per water type as described by Coughlan (1969) with time (t) in minutes and volume (V) in milliliters:

$$SR = \left(\frac{\ln(Ci) - \ln(Cf)}{t}\right)V$$

Settling rates of beakers for seasonal physiology experiments were averaged within season and study stream and used to calculate Clearance Rates (CR) of mussels using the following formula described by Coughlan (1969):

$$CR = \left(\left(\frac{\ln(Ci) - \ln(Cf)}{t}\right)V\right) - SR$$

Clearance rates were scaled with mussel dry tissue weight by finding the slope of the

relationship between the mussel dry tissue weight and clearance rate which was determined by season and stream using the formula:

$$M = \frac{Y - B}{X}$$

Where Y represents the natural log of mussel clearance rates, X represents the natural log of mussel dry tissue weights, and B represents the y-intercept of the relationship of the natural log of clearance rate to the natural log of dry tissue weight.

Using M in combination with the seasonal average of dry tissue weight of mussels (ADTW) per stream, clearance rates of individual mussels in each of the four study streams were adjusted according to individual clam Dry Tissue Weight (DTW) in each stream during each season. The Weight-specific Clearance Rate (WCR) was calculated by scaling CR using the following allometric formula:

$$WCR = e^{CR+\left(M*(\ln(ADTW)-DTW)\right)}$$

WCR was expressed as L/hr [g DTW]$^{-1}$.

Seasonal filtration rates were calculated by multiplying the seasonal WCR of mussels by the particulate matter concentration (mg/L) of seston during respective seasons. Filtration rates were expressed as mg/hr [g DTW]$^{-1}$.

Water Quality Monitoring

Water quality data were routinely taken during mussel activities using a YSI Pro + sonde. Water quality parameters measured included dissolved oxygen (mg/L and % saturation), water temperature (°C), pH, and conductivity (uS/cm). Field scientists fully submerged probes, avoided contact with the stream bottom and gently circulated probes for accurate dissolved oxygen reading in lentic systems. The YSI sonde was calibrated prior to use for each field day.

Statistical Analyses

Statistical analyses were performed in the statistical software R (R Core Team 2013). Within R, the package “ggplot2” was used to generate graphical representations of data (Wickham 2009). Proportional were transformed by arcsine square root for statistical analyses (Sokal and Rohlf 2012).

Results

Qualitative Mussel Surveys

Live specimens of native species of freshwater mussels, as well as shells, were found during many surveys in southern Delaware. Species found included the eastern elliptio (*Elliptio complanata)*, the eastern floater (*Pyganodon cataracta*), and possibly the eastern lampmussel (*Lampsilis radiata*) although the shells suspected to be *L. radiata* were considerably damaged making identification uncertain. Most sites that did not have freshwater mussels were judged to contain suitable mussel habitat based on course physical characteristics. The majority of survey locations supported healthy populations of fish, turtles, and other wildlife. Survey results are summarized in Table 4 and discussed in detail below.

Table 4. Summary of qualitative survey efforts at each study location.

Location	Sub-location	Survey Effort (Person Hours)	Mussel Observations	
			Live	Shells
St. Jones River	St. Jones River	1.0	×	×
	Silver Lake	4.0	×	✓
Prime Hook Creek	Waples Pond	2.7	×	✓
	Reynolds Pond	2.7	✓	✓
	Sowbridge Branch	0.1	×	✓
	Ingram Branch	0.2	×	×
Mispillion River	Blairs Pond	2.7	✓	✓
	Abbotts Pond	2.7	×	✓
	Johnson Branch	0.3	×	×
	Haven Lake	1.0	✓	✓
Murderkill River	Browns Branch	1.0	✓	✓
Broadkill River	Broadkill River	1.3	×	×
	Wagamons Pond	2.7	✓	✓

St. Jones River

The St. Jones River system was assessed for evidence of freshwater mussels on two survey days. Silver Lake (Dover, DE) as well as the St. Jones River just downstream of Silver Lake comprised two of the survey areas. Surveyors used kayaks to scan the perimeter of Silver Lake, from the Silver Lake dam to the Route 13 Bridge, for any evidence of freshwater mussels. Though much of the perimeter was hardened shoreline, eastern floater shells (single shells and paired shells) were found in shallow sandy areas as well as two partial single shells that were suspected to be *Lampsilis radiata*. As surveys only encompassed shallow areas around the perimeter of the lake, it is possible that live mussels exist in deeper areas of the lake. Turbidity was a major factor that prevented deeper detections.

Within the downstream waters of Silver Lake, no evidence of extant freshwater mussels was detected. However, adequate mussel habitat appeared to occur in that area, which contained moderate flowing water, adequate benthic substrate for burrowing, and sufficient water depth. The St. Jones River supported substantial aquatic vegetation (*Nuphar lutea*) as well as diverse wildlife, including small schooling fish, larger fish, and turtles. Stream banks had significant riparian cover, although areas of stormwater runoff and erosion were also noted.

Prime Hook Creek

To assess the waters of Prime Hook Creek for evidence of freshwater mussels, surveyors investigated four areas that represented both lentic and lotic environments. A kayak survey performed in Waples Pond yielded one single eastern floater shell, suggesting that eastern floaters once existed in the pond and may still currently exist there. Small turtles were also found in the pond along with substantial riparian cover. Water depths were 2-3 feet deep along the perimeter to over 6 feet deep near the center of the pond.

A qualitative survey of Reynolds Pond yielded one live eastern floater as well as multiple eastern floater shells. Live mussels and shells were found in bottom types characterized as sandy and contained considerable decaying organic matter. Turtles and large fish were also observed during the survey.

Examination of the outfall of Reynolds Pond (Sowbridge Branch) yielded more evidence for eastern floaters in that system. A total of three paired shells were found suggesting that those animals had recently died, further supporting that extant eastern floaters still exist in

that watershed area.

Surveyors attempted to survey the Ingram Branch (south of Waples Pond), however, survey efforts were constrained by unsafe wading (stream bottom) conditions. A brief survey did not yield evidence for freshwater mussels, but further investigation using other tactics is warranted.

Mispillion River

Qualitative surveys within the Mispillion River system were performed in a series of ponds, including Blairs Pond, Abbotts Pond, Haven Lake, and Johnson Branch. Along the perimeter of Blairs Pond, both live specimens and shells of the eastern elliptio and eastern floater were found in dense aggregates. Along with mussels, multiple species of turtles and fish, including fish nests, were observed.

In Abbotts Pond, a single eastern floater shell was found during the qualitative survey. Surveying techniques were inefficient because much of the shallow areas were covered in dense algal mats. Tactile detections were used but are typically less efficient than visual detections. While surveying, scientists also noted a beaver lodge as well as multiple turtles, catfish, and egrets utilizing the pond.

A survey performed in Haven Lake along the perimeter between Lednum and Copper Branches yielded similar results to that of Blairs Pond with detections of live eastern elliptio mussels and one single shell of the eastern floater. One suspected shell of the eastern lampmussel was found, although that specimen was a damaged partial shell, and more investigation is warranted. Fish nests were also observed in the pond. Shell quantities were not as abundant as in Blairs Pond.

Within Johnson Branch, no mussels were found. The stream bottom was characterized by unstable sediments and high amounts of organic matter. Swamp-like conditions prevented effective qualitative surveying, and additional tactics would be needed to survey more effectively. Surveyors noted many live Asian clams (*Corbicula fluminea*) in this system, representing the only bivalve species observed.

Murderkill River

Within the Murderkill River system, surveyors discovered a significant population of eastern elliptio mussels within Browns Branch. A few shells were found in addition to over a dozen live mussels. Live mussels had a notable amount caddisfly cases attached to the

posterior end of their shells (i.e., above the substrate). Mussels were mainly found in sand, both in deeper areas along areas of strong stream flow and near the stream banks. Surveyors noted that spatial distribution of mussels was patchy. Small fish were also observed in addition to macro invertebrates.

Broadkill River

While surveying the Broadkill River system, a live specimen as well as shells of the eastern floater were found in Wagamons Pond. Surveyors also noted the presence of extensive *C. fluminea* within shallow areas of the pond. Other signs of wildlife observed along the pond include a beaver lodge, turtles, fish, and an egret. Some sections of the pond's perimeter were covered with dense algal mats, whereas the areas where mussels were found were free of these mats. Although it is possible that the algal mats obscured mussel detection, the absence of mussels in tactile sampling under the mats suggested that such algal mats might degrade habitat suitability for mussels.

The main stem Broadkill River downstream of Wagamons Pond was also surveyed. Because the area was tidally influenced, surveyors kayaked along the river banks during ebb tide to maximize likelihood of mussel detections. Turtles and fish were noted, however no freshwater mussel specimens were found. Areas seemed to have adequate freshwater mussel habitat, and mussels still might exist deeper than where surveyed.

Mussel Reintroductions

Mussels that were collected from Blair's Pond and relocated to reintroduce mussels into St. Jones and Waples Pond, as well as "handling stress" controls of mussels put back into Blair's Pond, demonstrated high and consistent retention in the planted locations over the course of one year. Four months after deployment, mussel retention ranged from 77-93% in all study areas. After one year of deployment, the percentage retained remained the same for Blairs Pond (90%) and St. Jones (93%), and increased in Waples Pond (83%). No mortalities were found during the study. Bed retention data are presented in Table 5.

Growth was assessed after one year of deployment of reintroduced mussels. A total of 9, 15, and 18 mussels were measured from Blairs Pond, St. Jones, and Waples Pond respectively. Average change in shell length was below 1 mm (<1% of total shell length). Mussel shell length data are presented in Table 6.

Table 5. Reintroduced mussel bed retention and shell length change data summarized by deployment site.

Deployment Site	# Mussels Deployed	Recovery 1 (4 Months)		Recovery 2 (12 Months)	
		# Recovered	Bed Retention (%)	# Recovered	Bed Retention (%)
Blairs Pond	30	27	90	27	90
St. Jones	30	28	93	28	93
Waples Pond	30	23	77	25	83

Table 6. Shell Length (SL) change data of reintroduced mussels measured one year post deployment, summarized by deployment site. N = sample size, Ti = initial measurement, Tf = final measurement, SEM = standard error of the mean.

Deployment Site	N	T_i Mean SL (mm)	T_f Mean SL (mm)	Mean SL Change (mm)	SEM	% Change
Blairs Pond	9	105.8	106.3	0.57	0.001	0.54
St. Jones	15	96.0	96.0	-0.02	0.001	-0.03
Waples Pond	18	98.9	99.7	0.80	0.002	0.81

Restoration Readiness Assessments

Key biological metrics that help assess and track changes in mussel health, including condition index, tissue protein content and tissue carbohydrate content, were assessed and compared among mussels held in cages in candidate mussel restoration sites during each of three seasons (fall, winter, spring). Fitness is primarily gauged by examining whether the normal seasonal shifts in tissue metrics, as determined in mussel source streams (i.e., reference locations), is supported in the candidate restoration streams and ponds (i.e., experimental locations). Healthy mussel populations typically have pronounced seasonal variation in tissue composition, reflecting sequestration of energy and protein reserves for reproduction and overwintering, followed by depletion. In contrast, impaired mussels typically have more modest nutritional reserves and "flat line" seasonal patterns in energy and protein contents. Since the final monitoring period was nearly a year after deployment in summer 2015, it can also be instructive to compare the final mussel condition to the initial condition. For this report, fitness was simply assessed as the annual average condition index, tissue protein content, and tissue carbohydrate content; however, more robust seasonal statistical analyses are planned.

Since mussel fitness can also vary with age and body size, shell lengths of each reference and experimental treatment group were statistically compared to confirm that they were similar at the start of the transplant study. Differences in shell length and each fitness metric were also summarized and statistically compared between the two mussel source locations (Blair's Pond vs. Browns Branch). This frame of reference is also needed because in previous studies (Gray and Kreeger 2014) mussel fitness has been found to sometimes vary even among source streams.

Shell Length

Average shell lengths of transplant groups of mussels sourced from Blairs Pond (range: 95.07 – 107.14 mm) were greater on average than mussels from Browns Branch (range: 83.07 - 92.50 mm). A summary of mussel shell lengths for mussels sourced from Blairs Pond and Browns Branch are reported in Tables 7 & 8 respectively. This size difference reflects survey data, and suggests that growing conditions in the pond may be more supportive of larger sized mussels, compared to the stream.

Blairs Pond

Shell lengths of mussels from Blairs Pond were compared among mussels caged in each

deployment site using a 3-way ANOVA with main factors of season, site, and type (caged vs. uncaged animals). No differences in mean shell length of mussels were found among season (p=0.35), site (p=0.76) or between types (p=0.96). This indicates that fitness metrics can be compared among these factors without concern regarding possible interactions from

Table 7. Summary of shell lengths (mm) of mussels sourced from Blairs Pond used in cage assessments broken down by deploy site and season. SEM = standard error of the mean; N=sample size.

	Blairs Pond (reference)			**Blairs Pond (cage)**			**Waples Pond (cage)**			**St. Jones (cage)**			**Browns Branch (cage)**		
	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
Fall	100.75	4.18	8	100.21	3.40	9	101.02	1.96	9	98.39	2.66	9	101.91	3.61	9
Winter	95.07	2.81	7	102.30	2.69	9	102.35	3.36	9	97.64	1.55	9	99.11	2.02	9
Spring	107.14	5.79	8	99.53	2.24	9	103.39	2.82	9	103.33	4.15	9	98.47	3.00	7

Table 8. Summary of shell lengths (mm) of mussels sourced from Browns Branch used in cage assessments broken down by deploy site and season. SEM = standard error of the mean; N=sample size.

	Browns Branch (reference)			**Browns Branch (cage)**			**Blairs Pond (cage)**		
	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
Fall	83.07	3..01	8	92.50	0.98	9	85.95	2.83	9
Winter	86.03	2.92	8	86.93	3.72	6	87.44	1.68	9
Spring	90.82	2.41	8	83.12	2.37	6	89.60	1.84	6

body size effects.

Browns Branch

Shell lengths of mussels from Browns Branch were also compared among mussels caged in each deployment site using a 3-way ANOVA by season, deploy site, and type (caged vs. reference). No differences in mean shell length of mussels were found among season (p=0.81), site (p=0.72) or between type (p=0.47). Similar to Blair's sourced mussels, this indicates that fitness metrics can be compared among factors without concern regarding

body size effects.

Condition Index

Blairs Pond

Caged mussels from Blairs Pond and relocated in different groups exhibited annual average condition indices ranging from 76.89 – 82.92, whereas uncaged mussels from Blairs Pond exhibited condition indices ranging from 73.74 – 85.52. A 1-way ANOVA found no difference between caged and reference mussels within Blairs Pond ($p=0.88$), indicating that the caging protocol had no effect on mussel condition index.

However, mussel condition did vary significantly among deployment sites ($p<0.001$), but not by season ($p=0.95$) (2-way ANOVA). A post-hoc Tukey test found that all mussels placed into Browns Branch (annual average condition index 58.55 – 64.37) had significantly lower condition indices than Blairs-sourced mussels relocated into Blairs Pond (annual average condition index 76.89 – 82.92; $p<0.001$), St. Jones (annual average condition index 72.77-80.07; $p<0.001$), and Waples Pond (annual average condition index 82.94-96.76; $p<0.001$). These results suggest that Brown's Branch, despite having an extant population of one mussel species, is not supportive of the same mussel fitness as all other locations, including candidate restoration sites where no extant mussel populations have been found.

Additionally, Blairs-sourced mussels that were relocated to Waples Pond had greater condition indices than mussels caged in both Blairs Pond ($p<0.05$) and St. Jones ($p<0.005$). The final condition index of Blairs-sourced mussels in Blairs Pond and St. Jones did not differ significantly ($p=0.86$). Annual average condition indices for mussels sourced from Blairs Pond are depicted in Figure 6.

Browns Branch

The annual average condition index for Browns-sourced mussels that were caged and held in Browns Branch ranged from 51.43 – 63.21, whereas uncaged reference mussels from Browns Branch ranged from 57.38 – 68.76 in annual average condition index. A 1-way ANOVA determined that caging had a significant effect ($p<0.05$) with slightly lower condition indices observed for caged mussels than uncaged mussels. In all previous studies using the same caging protocol, a caging effect was not found, similar to the Blairs results here (see above). This result is likely anomalous because Browns Branch is a very small stream, placement of cages in unstable sediments was difficult, and some cages appeared to

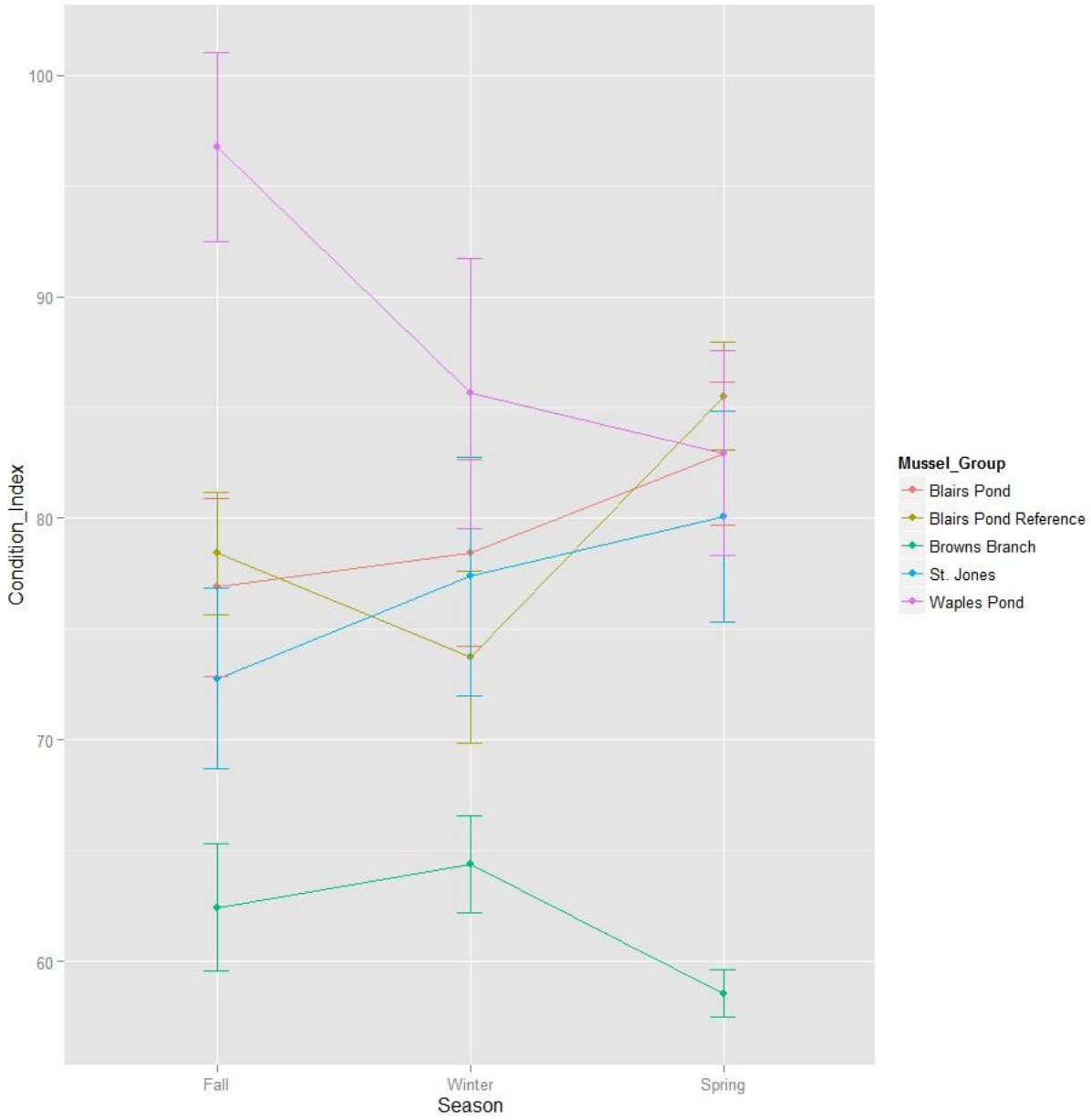

Figure 6. Seasonal changes in average condition index of Blairs Pond mussels deployed at each site. Reference (uncaged) mussels from Blairs Pond are included. Error bars represent standard errors of the mean.

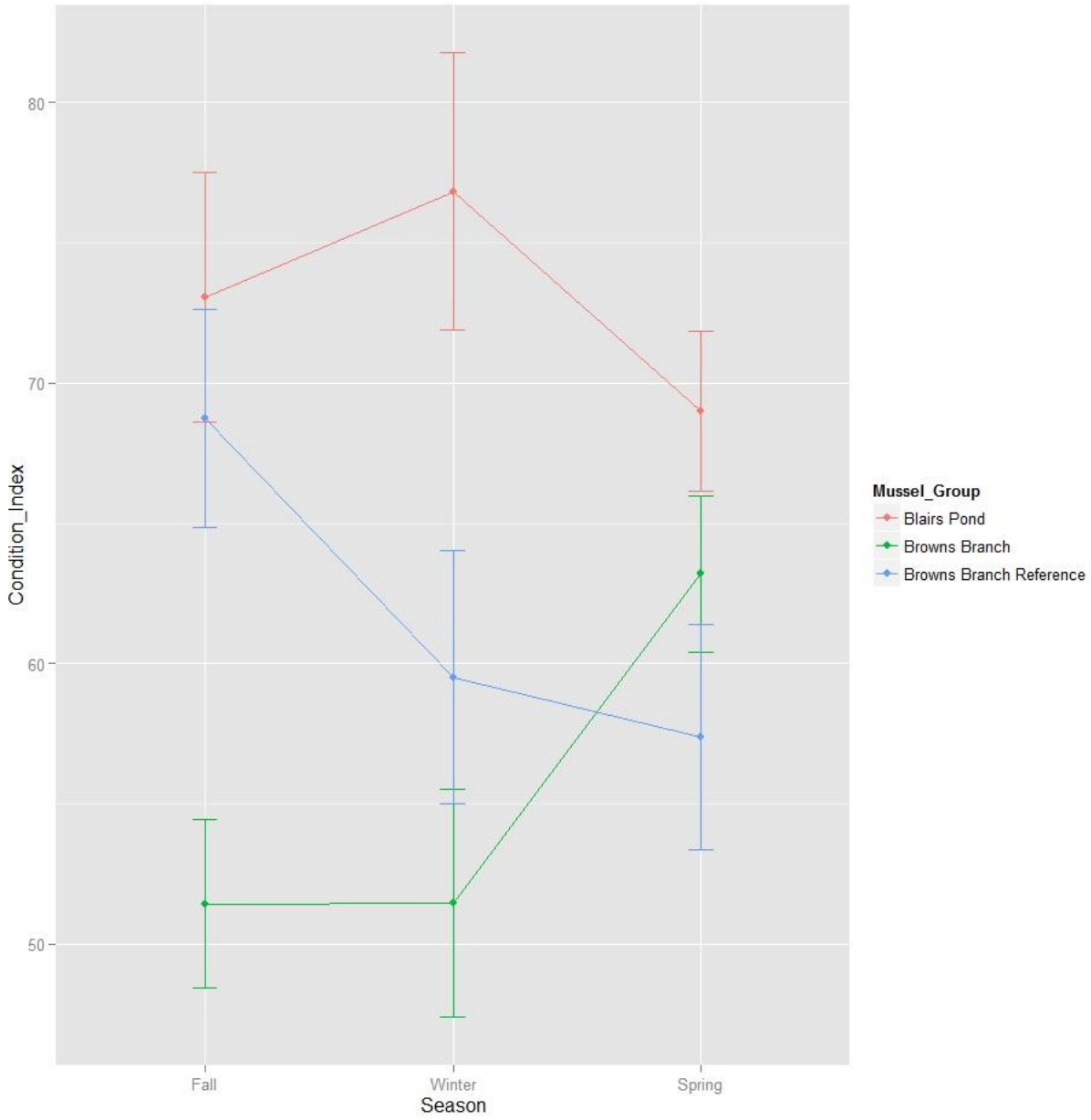

Figure 7. Seasonal changes in average condition index of Browns Branch mussels deployed at each site. Reference (uncaged) mussels from Browns Branch are included. Error bars represent standard errors of the mean.

be impacted by either high stream flow events and/or physical disturbance by people.

Condition index was compared among caged mussel groups that were Browns-sourced using a 2-way ANOVA with main effects of season and site. As found for Blairs-sourced mussels (see above), condition index of Browns-sourced mussels was found to be significantly different among deployment sites ($p<0.001$) but not seasons ($p=0.62$). A post-hoc Tukey test revealed that mussels had significantly greater condition indices in Blairs Pond (annual average 69.00 – 76.84) than in Browns Branch (annual average 51.43 – 63.21) during fall ($p<0.005$) and winter ($p<0.005$), but not during spring ($p=0.94$). Annual average condition indices for mussels sourced from Browns Branch are depicted in Figure 7.

Tissue Protein Content

Blairs Pond

The percentage of protein content of mussel tissues ranged from 32.24 – 33.07 and 34.00 – 37.61 for caged and reference mussels from Blairs Pond, respectively. A 1-way ANOVA found that reference (uncaged) mussels had greater tissue protein content than caged mussels ($p<0.05$), suggesting that caging might have affected protein content; however, this result needs to be studied further (e.g., by season, and between initial and final).

Comparing protein contents of mussels among caged treatment groups held in different locations, they ranged from 32.24 - 33.07 in Blairs Pond, 29.22 – 32.88 in Browns Branch, 27.33 – 39.01 in St. Jones, and 31.73 – 35.09 in Waples Pond. A 2-way ANOVA indicated that protein contents differed significantly among sites ($p<0.01$), but not seasons ($p=0.88$); however, there was a significant interaction between season and site ($p<0.001$). Figure 8 depicts the annual average tissue protein content of mussels sourced from Blairs Pond.

Browns Branch

For caged and reference mussels collected from Browns Branch, annual tissue protein content ranged between 33.30 – 36.30 and 30.85 – 39.16 percent, respectively. A 1-way ANOVA found no difference in tissue protein content between caged and reference mussels ($p=0.75$), indicating that caging did not influence protein content in Browns mussels.

Comparing among caged mussel groups, tissue protein content did not vary by season ($p=0.10$) but was significantly greater in mussels deployed to Blairs Pond (38.44 – 42.17) compared with mussels caged and held in Browns Branch (33.30 – 36.30; $p<0.001$; 2-way ANOVA). Annual average tissue protein contents of mussels sourced from Browns Branch are presented in Figure 9.

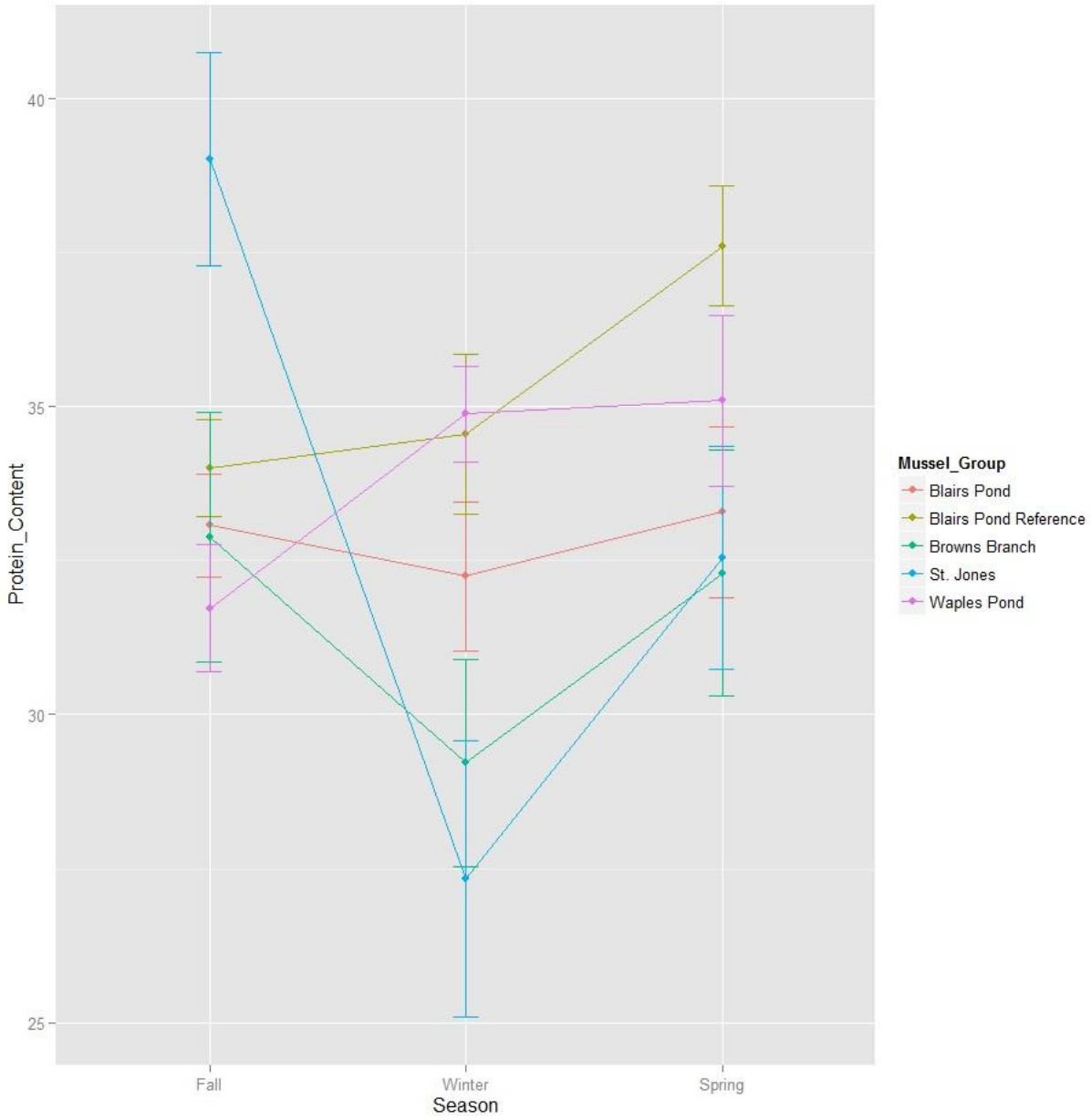

Figure 8. Seasonal changes in average tissue protein content of Blairs Pond mussels deployed at each site. Reference (uncaged) mussels from Blairs Pond are included. Error bars represent standard errors of the mean.

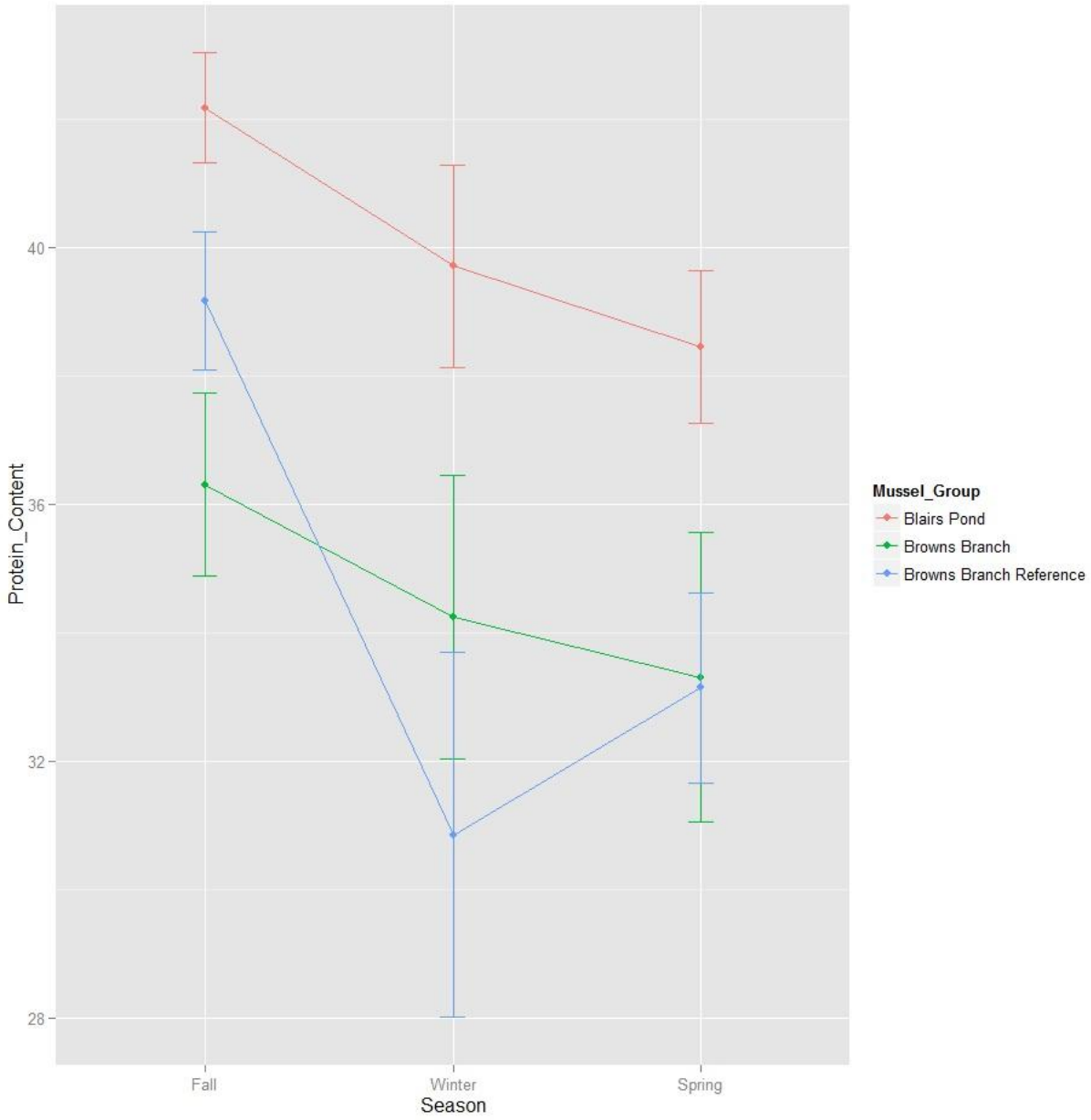

Figure 9. Seasonal changes in average tissue protein content of Browns Branch mussels deployed at each site. Reference (uncaged) mussels from Browns Branch are included. Error bars represent standard errors of the mean.

Tissue Carbohydrate Content

Blairs Pond

Caged and reference mussels from Blairs Pond had similar percentages of annual tissue carbohydrate content (range 25.03 – 46.86, 29.59 – 44.21, respectively), tested via 1-way ANOVA ($p=0.75$).

For caged mussels deployed at each site, percent tissue carbohydrate content varied significantly by season ($p<0.001$), site ($p<0.001$) and to a lesser extent, by the interaction of season and deploy site ($p<0.01$; 2-way ANOVA). A post-hoc Tukey test found that mussels deployed into Browns Branch contained significantly lower tissue carbohydrate content (21.07 – 27.37) than mussels deployed in Blairs Pond (25.03 – 46.86; $p<0.001$), St. Jones (26.15 – 41.10; $p<0.005$), and Waples Pond (33.56 – 44.82; $p<0.001$; post-hoc Tukey test). Additionally, mussels collected in the spring contained greater carbohydrate than mussels collected during winter ($p<0.005$) and fall ($p<0.001$) with no significant difference found between fall and winter ($p=0.58$). The annual carbohydrate contents of mussels at each deploy site are represented in Figure 10.

Browns Branch

A cage effect was found for tissue carbohydrate content, comparing caged (annual range 10.22 – 29.54) and reference mussels (annual range 23.71 – 27.82) sourced from Browns Branch ($p<0.005$; 1-way ANOVA). The high range reflects considerable variability, and further study is warranted to determine if caging might have impacted mussel carbohydrate accumulation. In the small stream system, cages can become clogged with leaf litter, for example, which might lead to suboptimal nutrition as a result of clogged cages rather than the impact of the cage itself.

Significant differences in carbohydrate content were detected between Browns-sourced mussels that were deployed in cages in Browns Branch versus Blairs Pond ($p<0.001$; 2-way ANOVA) as well as by season ($p<0.001$). A post-hoc Tukey test determined that mussel carbohydrate content was greatest in mussels collected in spring, compared to fall ($p<0.001$) and winter ($p<0.001$), with no difference found between collections in fall versus winter ($p=0.99$). Additionally, mussels deployed in Blairs Pond (18.06 – 40.23) contained significantly more carbohydrate than mussels deployed in Browns Branch (10.22 – 29.54; $p<0.001$). The annual carbohydrate contents of mussels at each deploy site are represented in Figure 11.

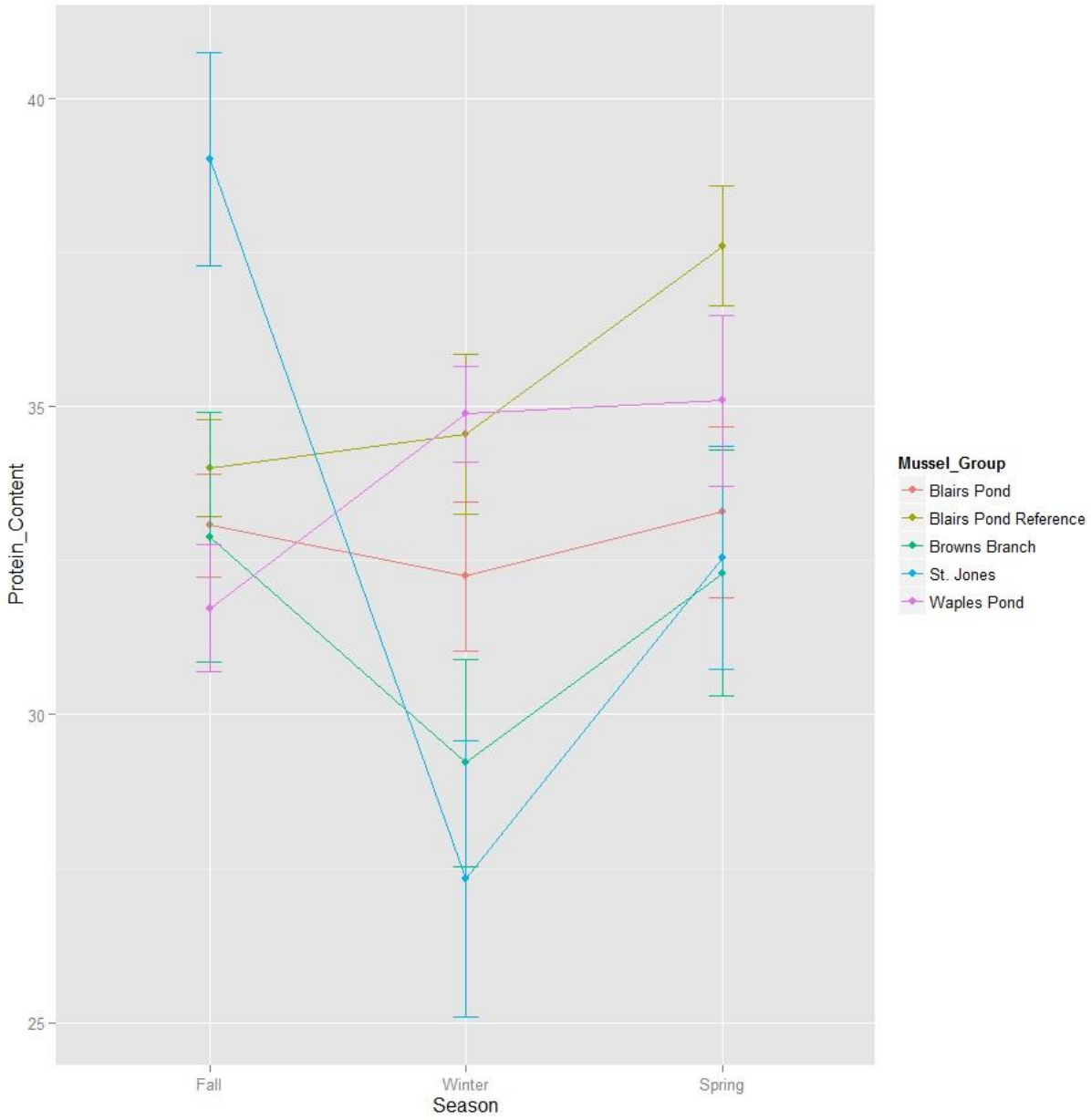

Figure 10. Seasonal changes in average tissue carbohydrate content of Blairs Pond mussels deployed at each site. Reference (uncaged) mussels from Blairs Pond are included. Error bars represent standard errors of the mean.

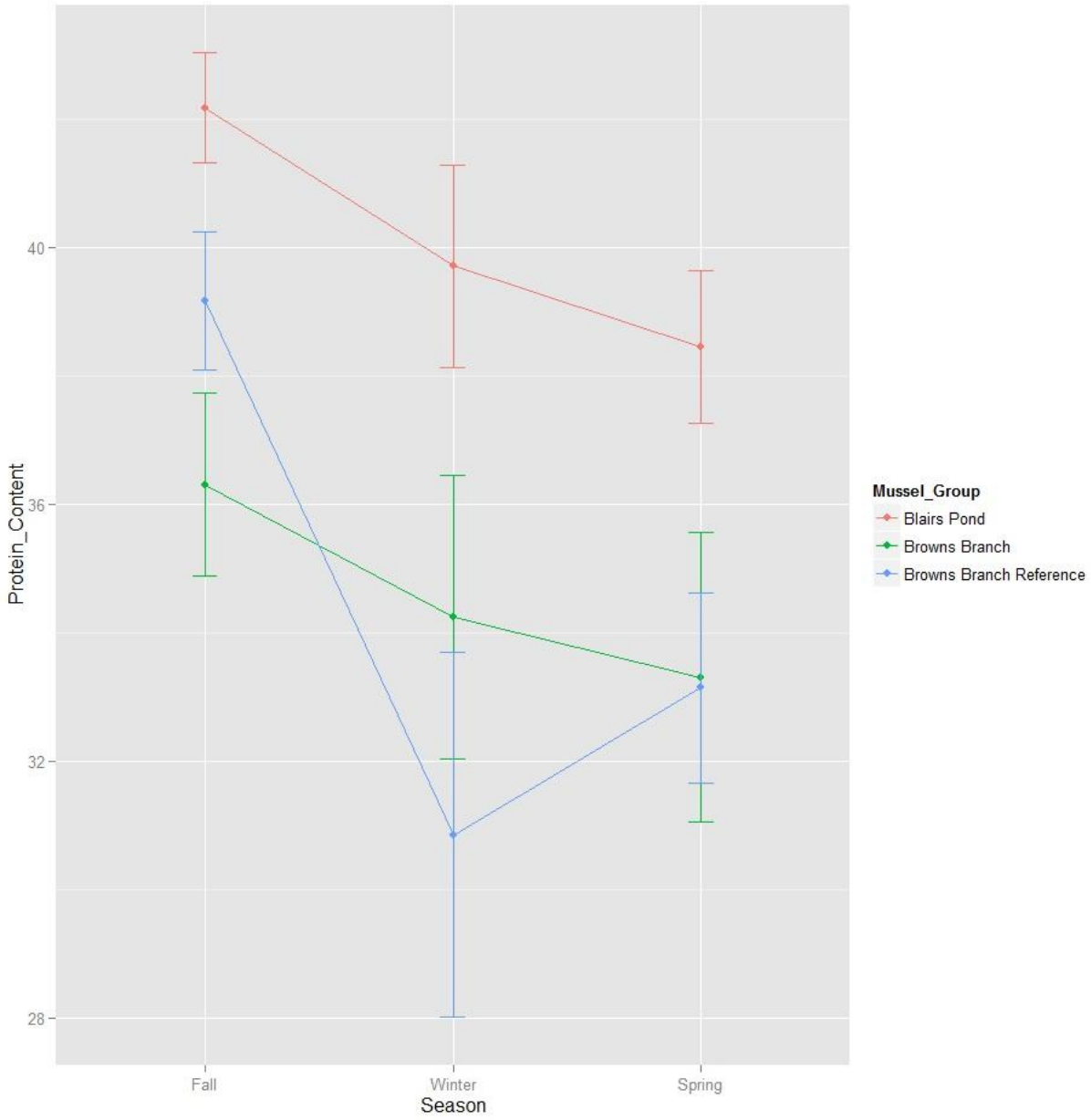

Figure 11. Seasonal changes in average tissue carbohydrate content of Browns Branch mussels deployed at each site. Reference (uncaged) mussels from Browns Branch are included. Error bars represent standard errors of the mean.

Mussel Physiology Studies

Seston Analyses: Food quantity & quality

Seston analyses includes the particulate matter concentration (PM), particulate organic matter concentration (POM), organic content (percentage of organic matter of PM), protein concentration, protein content (percentage protein of POM), carbohydrate concentration, and carbohydrate content (percentage carbohydrate of POM). The average PM of seston varied significantly by season ($p<0.001$) and site ($p<0.001$), and the interaction of season and site was highly significant as well ($p<0.001$; 2-way ANOVA). A series of 1-way ANOVA tests determined that PM did not vary significantly by site ($p>0.05$) but did vary by season ($p<0.001$). Similarly, the average POM varied significantly by site ($p<0.001$), season ($p<0.001$) as well as their interaction ($p<0.001$; 2-way ANOVA). Other metrics including seston organic content, protein content, and carbohydrate content were tested for differences by site and season via 2-way ANOVA tests and all tests as well as interactions were highly significant ($p<0.001$, all tests).

These test results indicate that bulk seston quantity and quality did not follow consistent seasonal and spatial trends when contrasted among the 3 physiological experiments when seston was fully analyzed, thus seston should be examined discretely among locations and times. This dissection of seston outcomes is provided by discussing results per water body below.

Brandywine River

In the Brandywine River, the average PM was 1.70, 5.26, and 4.31 mg/L in fall, spring, and summer respectively. These PM concentrations were significantly different ($p<0.001$; 1-way ANOVA) with the highest PM concentration in spring compared to summer ($p<0.05$; post-hoc Tukey test) and fall ($p<0.001$, post-hoc Tukey test). Organic content varied significantly by season ($p<0.001$; 1-way ANOVA) and was greatest in fall ($p<0.001$; 77.9%) compared to summer ($p<0.001$; 43.1%) which was greater than organic content in spring ($p<0.005$; 33.6%) as determined by a post-hoc Tukey test.

Protein content also exhibited significant variation among fall, spring, and summer (0.963, 15.2, and 15.3% respectively) as tested by 1-way ANOVA ($p<0.001$). Protein content was significantly lower in fall compared to spring ($p<0.001$) and summer ($p<0.001$) while spring and summer were similar ($p=0.99$) as determined by a post-hoc Tukey test. Carbohydrate content during fall, spring, and summer was 10.2, 13.2, and 8.34% respectively. These

annual carbohydrate content variations were not found to significant (p=0.12; 1-way ANOVA). A summary of all seston composition metrics are presented in Table 9.

Table 9. Summary of seston quantity and quality in Brandywine River over three seasons. SEM = standard error of the mean; N = sample size.

Seston Metric	Fall			Spring			Summer		
	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
Particulate Matter (PM; mg/L)	1.70	0.05	6	5.26	0.5	6	4.31	0.4	6
Particulate Organic Matter (POM; mg/L)	1.33	0.05	6	1.74	0.10	6	1.56	0.12	6
Organic Content (% of PM)	77.9	1.7	6	33.6	1.30	6	43.1	1.5	6
Protein Concentration (mg/L)	0.0130	0.009	6	0.265	0.03	6	0.238	0.04	6
Protein Content (% of POM)	0.963	0.6	6	15.2	1.7	6	15.3	2	6
Carbohydrate Concentration (mg/L)	0.135	0.008	6	0.228	0.015	6	0.165	0.02	6
Carbohydrate Content (% of POM)	10.2	0.6	6	13.2	0.8	6	8.34	1.4	6

Blairs Pond

Water in Blairs Pond contained significantly different PM concentrations ($p<0.001$; 1-way ANOVA) among fall, spring, and summer seasons (1.54, 2.61, 4.31 mg/L respectively). A post-hoc tukey test determined that PM concentration was significantly greater in summer than spring, which in turn was greater than fall ($p<0.001$ all comparisons). The average organic content of seston was significantly different by season ($p<0.001$; 1-way ANOVA) with greater organic content during fall (91.0%) compared to spring ($p<0.001$; 51.8%) and summer ($p<0.001$; 76.2%) with organic content greater in summer than spring ($p<0.001$) as determined via a post-hoc Tukey test.

Similarly, protein content varied significantly by season ($p<0.05$; 1-way ANOVA). A post-hoc Tukey test found protein content was greater in fall (36.3%) compared to summer ($p<0.05$; 25.4%) but similar to spring ($p=0.07$, 26.6%) while summer and spring were similar as well ($p=0.94$). Seasonal variation in carbohydrate content was found to be significant ($p<0.05$; 1-way ANOVA) with significantly more carbohydrate content observed during fall (13.0%) compared to summer ($p<0.05$; 8.34%) as determined via a post-hoc Tukey test. Seasonal seston metrics are summarized for Blairs Pond in Table 10.

Table 10. Summary of seston quantity and quality in Blairs Pond over three seasons. SEM = standard error of the mean; N = sample size.

Seston Metric	Fall			Spring			Summer		
	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
Particulate Matter (PM; mg/L)	1.54	0.04	6	2.61	0.10	6	4.31	0.2	6
Particulate Organic Matter (POM; mg/L)	1.40	0.05	6	1.35	0.04	6	3.27	0.15	6
Organic Content (% of PM)	91.0	1.7	6	51.8	1.2	6	76.2	1.9	6
Protein Concentration (mg/L)	0.508	0.06	6	0.360	0.02	6	0.830	0.06	6
Protein Content (% of POM)	36.3	4	6	26.6	1.5	6	25.4	1.8	6
Carbohydrate Concentration (mg/L)	0.182	0.009	6	0.142	0.02	6	0.273	0.05	6
Carbohydrate Content (% of POM)	13.0	0.6	6	10.6	1.4	6	8.34	1.4	6

Browns Branch

The average PM concentration in Browns Branch was significantly different among seasons ($p<0.001$; 1-way ANOVA), increasing from 0.917 mg/L in fall to 2.81 mg/L in spring and 3.65 mg/L in summer. A post-hoc Tukey test found that PM was greatest in summer compared to spring and greater in spring compared to fall ($p<0.001$ all comparisons). The organic content of seston was significantly different by season ($p<0.001$; 1-way ANOVA). A post-hoc Tukey test found that organic content was greatest in fall (89.3%) compared to spring ($p<0.001$; 32.8%) and summer ($p<0.001$; 36.2%) while organic content was similar between spring and summer ($p=0.63$).

Protein content was statistically different by season ($p<0.001$; 1-way ANOVA) with the lowest content in fall (2.27%) compared to spring ($p<0.001$; 44.3%) and summer ($p<0.001$; 32.5%) while spring and summer were similar ($p=0.07$) as determined via a post-hoc Tukey test. Carbohydrate content varied significantly by season ($p<0.001$; 1-way ANOVA). A post-hoc Tukey test found that carbohydrate content was greater in spring (26.4%) compared to fall ($p<0.001$; 12.8%) and spring ($p<0.001$; 12.7%) which were not different from each other ($p=0.99$). A summary of seasonal seston data for Browns Branch is presented in Table 11.

Table 11. Summary of seston quantity and quality in Browns Branch over three seasons. SEM = standard error of the mean; N = sample size.

Seston Metric	Fall			Spring			Summer		
	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
Particulate Matter (PM; mg/L)	0.917	0.06	6	2.81	0.2	6	3.65	0.4	6
Particulate Organic Matter (POM; mg/L)	0.895	0.09	6	0.938	0.12	6	3.75	0.15	6
Organic Content (% of PM)	89.3	3	4	32.8	2	6	36.2	1.9	6
Protein Concentration (mg/L)	0.0183	0.008	6	0.415	0.04	6	1.22	0.05	6
Protein Content (% of POM)	2.27	1.1	6	44.3	4	6	32.5	1.4	6
Carbohydrate Concentration (mg/L)	0.103	0.005	6	0.248	0.02	6	0.477	0.03	6
Carbohydrate Content (% of POM)	12.8	0.6	6	26.4	2	6	12.7	0.8	6

Ingrams Pond

Seasonal changes in average PM were significantly different in Ingrams Pond ($p<0.001$; 1-way ANOVA). PM was greatest in summer (5.07 mg/L) over spring ($p<0.001$; 3.64 mg/L) and fall ($p<0.001$; 3.15 mg/L) while spring and fall were similar ($p=0.38$) as determined via a post-hoc Tukey test. Accordingly, organic content was significantly different by season ($p<0.001$; 1-way ANOVA). A post-hoc tukey test found that organic content was similar during fall (54.7%) and spring ($p=0.99$; 54.9%) but was significantly greater in summer (90.4%) compared to fall ($p<0.001$) and spring ($p<0.001$).

Seasonal variation in protein content was significant ($p<0.01$; 1-way ANOVA). Average protein content was significantly greater in fall (28.4%) compared to summer ($p<0.005$; 21.5%) but similar to spring ($p=0.18$; 24.9%) while protein content was similar between spring and summer ($p=0.20$). Average carbohydrate content was 33.7, 17.9, and 13.6% during fall, spring, and summer respectively. A 1-way ANOVA found this seasonal variation in carbohydrate content to be significant ($p<0.001$) and a post-hoc Tukey test

determined carbohydrate content was greater in fall compared to spring ($p<0.001$) and greater in spring compared to summer ($p<0.05$). A summary of seasonal seston data for Ingrams Pond is presented in Table 12.

Table 12. Summary of seston quantity and quality in Ingrams Pond over three seasons. SEM = standard error of the mean; N = sample size.

Seston Metric	Fall			Spring			Summer		
	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
Particulate Matter (PM; mg/L)	3.15	0.11	6	3.64	0.12	6	5.07	0.4	6
Particulate Organic Matter (POM; mg/L)	1.72	0.07	6	1.99	0.03	6	4.84	0.2	6
Organic Content (% of PM)	54.7	1.1	6	54.9	1.6	6	90.4	4	4
Protein Concentration (mg/L)	0.490	0.016	6	0.495	0.03	6	1.01	0.06	6
Protein Content (% of POM)	28.4	1.0	6	24.9	1.7	6	21.5	1.2	6
Carbohydrate Concentration (mg/L)	0.580	0.02	6	0.355	0.02	6	0.640	0.06	6
Carbohydrate Content (% of POM)	33.7	1.2	6	17.9	1.1	6	13.6	1.3	6

Physiological Rates

Clearance Rate

Average clearance rate for eastern elliptio mussels in Brandywine River, Blairs Pond, and Browns Branch and eastern lance mussels from Ingrams Pond ranged from 0.52 to 1.5 L/hr DTW^{-1} over three seasons. A comparison of average clearance rates by season and site via a 2-way ANOVA found site not to be significant ($p=0.19$) but season to be significant

($p<0.01$) as well as the interaction of site and season ($p<0.01$).

The average clearance rate for mussels collected from the Brandywine River was significantly different by season ($p<0.01$; 1-way ANOVA). A post-hoc Tukey test determined that clearance rate was greater in summer compared to fall ($p<0.001$) and similar compared to spring ($p=.08$), while spring and fall rates were similar ($p=0.14$). Mussels collected from Blairs Pond exhibited statistically different clearance rates by season ($p<0.001$; 1-way ANOVA). A post-hoc Tukey test found that clearance rate was greatest in spring compared to fall ($p<0.005$) and summer ($p<0.005$) while fall and summer rates were similar ($p=0.94$). Clearance rates for mussels collected from Browns Branch were found to statistically similar ($p=0.21$; 1-way ANOVA) by season. Clearance rates for eastern lance mussels from Ingrams Pond were found to vary significantly with season ($p<0.05$; 1-way ANOVA). Further investigation via a post-hoc Tukey test found that clearance rate was greater in spring compared to fall ($p<0.05$) and similar to summer ($p=0.14$) while fall and summer rates were similar ($p=0.36$). Clearance rates are summarized in Table 13.

Table 13. Summary of seasonal clearance rates (L/hr DTW^{-1}) for mussels from each collection site. SEM = standard error of the mean; N = sample size.

Mussel Site	Fall			Spring			Summer		
	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
Brandywine River	0.73	0.10	8	1.1	0.11	7	1.5	0.16	8
Blairs Pond	0.53	0.10	8	1.0	0.10	7	0.57	0.05	8
Browns Branch	0.90	0.2	5	1.3	0.15	8	1.2	0.11	8
Ingrams Pond	0.61	0.05	7	1.1	0.2	4	0.79	0.05	7

Filtration Rate

Although clearance rates of mussels exhibited modest spatial and seasonal variation (see above), the seston filtration rates were considerably more variable largely because of variation in seston abundance and composition. For example, the average filtration rate for eastern elliptio mussels in Brandywine River, Blairs Pond, and Browns Branch, and eastern lance mussels from Ingrams Pond, ranged from 0.81 to 12 mg/hr g DTW^{-1} over all seasons. A 2-way ANOVA test compared filtration rate by site and season as well as their interaction

and found filtration rate to be similar among sites ($p=0.40$) but different among seasons ($p<0.05$), however with highly significant interactions between the two main effects ($p<0.001$).

Seasonal filtration rates of mussels from Brandywine River were found to be significantly different by season, as determined using a 1-way ANOVA ($p<0.001$). A post-hoc Tukey test found that fall filtration rates were lower than spring ($p<0.001$) and summer ($p<0.001$) while spring and summer rates were similar ($p=0.93$).

Mussels from Blairs Pond were also found to have significantly different filtration rates by season ($p<0.001$; 1-way ANOVA). Further analysis via a post-hoc Tukey test determined that fall filtration rates were lower than spring ($p<0.001$) and summer ($p<0.001$) while spring and summer rates were similar ($p=0.72$).

Filtration rates of mussels from Browns Branch were also found to vary significantly by season via a 1-way ANOVA ($p<0.001$). A post-hoc Tukey test was performed which found that summer filtration rates were greater than spring ($p<0.001$) and fall ($p<0.001$) whereas spring and fall rates were similar ($p=0.07$).

Eastern lance mussels from Ingrams Pond also exhibited significantly different filtration rates among seasons, as determined via a 1-way ANOVA ($p<0.01$). Fall filtration rates for eastern lance mussels were found to be lower than spring ($p<0.01$) and summer ($p<0.01$) while spring and summer rates were similar ($p=0.99$) as determined via a post-hoc Tukey test. Seasonal filtration rates are summarized in Table 14.

Table 14. Summary of seasonal filtration rates (mg/hr DTW^{-1}) for mussels from each collection site. SEM = standard error of the mean; N = sample size.

Mussel Site	Fall			Spring			Summer		
	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
Brandywine River	1.2	0.17	8	5.8	0.6	7	5.5	0.6	8
Blairs Pond	0.81	0.15	8	2.7	0.3	7	2.5	0.2	8
Browns Branch	0.83	0.21	5	3.7	0.41	8	12	1.1	8
Ingrams Pond	1.9	0.17	7	4.0	0.8	4	4.0	0.2	7

Water Quality Monitoring

Water quality data monitored as part of the reintroduction tagging study and the caged mussel fitness study are summarized in Table 15, contrasting among the various study sites (Blairs Pond, Browns Branch, St. Jones, and Waples Pond) and sampling times. Water temperature reflected expected seasonality, with slightly lower temperatures at Browns Branch during most seasons. Dissolved oxygen was consistently above the necessary levels needed to support freshwater mussels, during all sampling events, and the St. Jones location consistently exhibited the lowest oxygen concentrations of all sites. Conductivity was typical of freshwater systems throughout the region, and pH levels were also within typical ranges, with a few sites exhibiting slightly more alkaline conditions.

Table 15. Summary of water quality monitoring data collected at various freshwater mussel study locations in lower Delaware, between June, 2015, and June, 2016.

Date	Site	Temperature (°C)	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	Specific Conductivity (mS/cm)	pH
17-Jun-15	Blairs Pond	29.9	95.7	7.24	0.171	6.89
17-Jun-15	Browns Branch	21.5	92.9	8.20	0.200	7.40
17-Jun-15	St. Jones	27.4	70.2	5.55	0.163	7.39
17-Jun-15	Waples Pond	30.9	160.4	11.94	0.169	9.12
13-Oct-15	Blairs Pond	18.9	114.1	10.59	0.179	7.90
13-Oct-15	Browns Branch	16.4	84.8	8.28	0.223	7.48
13-Oct-15	St. Jones	19.2	59.6	5.50	0.275	7.36
13-Oct-15	Waples Pond	18.1	101.6	9.59	0.187	7.19
29-Feb-16	Blairs Pond	8.3	86.2	10.12	0.171	7.42
29-Feb-16	Browns Branch	9.8	83.3	9.45	0.203	7.53
29-Feb-16	St. Jones	8.5	79.1	9.25	0.182	7.12
29-Feb-16	Waples Pond	8.2	90.0	10.60	0.178	7.70
14-Jun-16	Blairs Pond	24.3	114.6	9.58	0.187	7.68
14-Jun-16	Browns Branch	18.5	93.9	8.79	0.211	6.91
14-Jun-16	St. Jones	25.4	65.3	5.36	0.202	7.09
14-Jun-16	Waples Pond	24.3	109.6	9.18	0.184	8.07

Discussion

Compared to our earlier studies of freshwater mussel status and restoration prospects in piedmont streams of northern Delaware, the collective results of this study suggest that freshwater mussel populations may be in better shape and have brighter short-term restoration prospects in the coastal plain waterways in the southern half of the state.

Mussel Restoration and Protection Needs

Evidence of extant mussel populations was found in most of the surveyed streams and ponds of Sussex and southern Kent County, and at least two species were found in many locations. In contrast, earlier surveys in New Castle and northern Kent County revealed extant mussel populations in only a few locations, and typically just one species (eastern elliptio). These results are consistent with our mussel survey findings in other states of the Delaware River Basin, where mussel presence, species richness, extent, and abundance appear to be lower in Piedmont streams impacted by stormwater, heavy land use, and dams, compared to many coastal plain systems which may be comparatively less impacted by physical and chemical disturbances.

Specifically, qualitative surveys were performed which found evidence of mussels in every system surveyed during qualitative surveys. Additionally, every system surveyed except for the St. Jones River had at least one segment of a stream or pond where live mussels were found. The greatest abundance of mussels was found at Blairs Pond and Browns Branch. Though these mussels were all large enough to be considered adults, there is the potential that these populations are still actively reproducing given the availability of an appropriate fish host. Blairs Pond in particular had two species of mussels as did Haven Lake. Our qualitative surveys suggest that ponds in southern Delaware may serve as refuges for mussels where other streams may have lost their live populations. Additionally, the eastern lance mussel (*Elliptio fisheriana*) was found in Ingrams Pond (thanks to Dr. Marianne Walch of the Delaware Center for the Inland Bays), which was used in physiological experiments. To our knowledge, this accounts for the first record of the eastern lance in Delaware.

Locations where only shells were found may warrant further investigation due to limited surveying efforts associated with depth or accessibility. Mussels can be inherently patchy in distribution (Strayer 2008) and so surveys often miss mussels based on detectability. Relative to surveys performed in northern Delaware (New Castle and Kent Counties) during

2013 and 2014 (Kreeger et al. 2014; Cheng and Kreeger 2015), Sussex County appears to harbor relatively more mussel species in a greater number of streams. New Castle streams such as the Red Clay Creek and White Clay Creek were not found to currently support mussels and few streams in Kent County were found to support extant mussel populations. This may be due in part to northern Delaware having greater development and concomitant impervious surfaces, flooding, and other disturbances to streams. Additionally, lentic environments such as the lakes of the Mispillion River system may serve as refuges for mussels.

For all surveys performed in Delaware to date by PDE scientists, we have found four species including the eastern elliptio, eastern floater, the eastern lance and likely the eastern lampmussel (possibly five due to difficulty identifying live alewife floaters from eastern floaters). These three species (excluding the eastern lance) account for less than half of the seven species still thought to exist in the state. By furthering our understanding of which species of mussels still reside in Delaware waterways, we can identify areas to protect from impacts that may affect mussels (e.g. draining ponds, increased stormwater runoff, other significant hydrological changes).

Mussel Restoration Readiness

Similarly, the restoration prospects for freshwater mussels appeared to be strong in southern Delaware. Mussels collected from two source locations were relocated to several candidate locations where mussels likely once existed but no longer do, based on our survey data. Relocations consisted of either PIT-tagged and free-released mussels or caged mussels. Their survival, movements (tagged) and fitness (caged) were then tracked for one year. Survival was consistently high for all treatment groups. Retention of tagged and released mussels in relocation sites was very high compared with other studies, irrespective of whether they were transplanted to new locations or reciprocal transplants among source locations. Assessment of chronic fitness measures in caged mussels, such as condition index and the proximate biochemical composition within mussel tissues, revealed that the best final fitness was actually achieved in one of the new locations (Waples Pond), supporting slightly better mussel fitness than the best reference (source) site (Blair's Pond).

Interestingly, one of the two mussel source locations (Brown's Branch) was supported the lowest fitness of freshwater mussels, and typical sizes of extant mussels in Brown's are smaller than for the same species living in the other source location (Blair's Pond). Although this might simply reflect diminished food resources in streams compared to ponds,

it was notable that higher fitness was supported in the other stream location (St. Jones), which was a candidate restoration site that appears to not have extant mussels, at least in our surveyed reaches. These differences likely reflect other factors, especially historical constraints, whereby conditions in St. Jones might have been more degraded in the past but natural recolonization by mussels might be currently impeded by blockages of essential fish hosts for mussel reproduction and larval dispersal. In contrast, suboptimal conditions in Brown's Branch could reflect a decline in current conditions, relative to the past. More study, such as a more prolonged monitoring period and enhanced analysis of past conditions, would help to tease apart such differences in current conditions.

These results clearly suggest that efforts to restore freshwater mussels to the studied streams and ponds where they had become extirpated can proceed to the next phase (e.g. release of hatchery propagated juveniles). Although this study did not assess all of the essential factors that determine mussel habitat suitability, it appears that the water quality and food conditions in Waples Pond and the St. Jones River are appropriate for sustaining mussel survival, nutrition, and growth. Based on our anecdotal observations during the more extensive mussel surveys, we suspect that many other similar sites without current mussel populations in southern Delaware would yield similar, favorable outcomes.

This study's results therefore filled important gaps in our current understanding of freshwater mussel distribution, range, species richness, and overall health of mussels in the state by expanding our earlier work to southern Kent and Sussex Counties. Inclusion of the caging assessment and fitness measures also strengthened our analysis of restoration prospects, confirming results of tagging protocols. Although there are many streams and ponds in Delaware that we have not yet surveyed or assessed for restoration readiness, this study's outcomes solidified our understanding of key patterns and differences between piedmont versus coastal plain systems, and between ponds and streams.

No mortalities were observed when translocating mussels for either project (aside from unnatural causes; e.g., some cages with mussels were lost due to vandalism). Relative to other reintroduction efforts in the northern part of the state, mussels in Blairs Pond, Waples Pond, and St. Jones River all demonstrated superb retention rates. Rates were well above the average for Delaware streams and above average for other reintroductions in Pennsylvania (Cheng and Kreeger 2015; Kreeger et al. 2014; Kreeger and Thomas 2014; Kreeger et al. 2015). This may be largely due to Waples Pond and Blairs Pond serving as lentic ecosystems with low flow and likely fewer shifts in benthic structure (e.g. stormwater runoff shifting streambeds). However, ever the St. Jones River, which is a flowing stream, was able to support mussels which had a very high bed retention rate.

Growth was not readily seen in mussels that were reintroduced, though this is typical for mussels of a larger size class. Mussels tend to grow faster during their youth but are generally very slow growing later in life. Mussels that were reintroduced may likely still be growing but have not reached an observable growth yet. For the current project, any changes in shell length were likely due to small differences in surveyors operating calipers. Similar growth results have been observed in other reintroduction studies and those mussels that have been monitored for multiple years post-reintroduction have demonstrated observable changes in shell length.

Cage assessments further supported restoration potential in Waples Pond and St. Jones River. Blairs Pond mussels that were caged in Waples Pond and St. Jones River demonstrated generally similar condition indices relative to their source population mussels. Blairs Pond mussels in Waples Pond demonstrated the greatest condition index for mussels particularly during the fall when conditioning is important for overwintering. Protein content was fairly consistent among Blairs Pond mussels in Waples Pond with increases towards spring while St. Jones River mussels demonstrated stark changes in protein over the three seasons but rebounded in spring. Combined with data from reintroductions, Waples Pond and St. Jones River may be prime candidates for a larger restoration effort as mussels were able to maintain high bed retention rates and exhibited good overall health.

Though no reintroductions were carried out in Browns Branch, caging assessments provided insights on the current health of eastern elliptio mussels. Condition indices were generally greater in mussels that were transplanted from Browns Branch into Blairs Pond suggesting better overall living conditions in Blairs Pond. Similar trends were seen for tissue protein content and to a lesser extent, carbohydrate content. Mussels transplanted into Blairs Pond were able to synthesis more protein over all seasons and contained greater carbohydrate in their tissues than mussels in Browns Branch in the spring. This is compounded with biometric data from mussels transplanted from Blairs Pond into Browns Branch where mussels suffered much lower condition indices and carbohydrate content compared to all other mussels particularly as the seasons progressed. Mussels from Blairs Pond transplanted into Browns Branch had the lowest condition indices and carbohydrate contents of all other mussels by almost half. Though mussels persist in Browns Branch, the current study provides insight on the relative condition between mussels living under habitat conditions in Browns Branch vs. all other study sites. As such, the current population of eastern elliptio mussels in Browns Branch warrants protection and potential further investigation on longer term trends on the overall health of the population.

Mussel Effects on Water Quality

Besides supporting freshwater mussel conservation and restoration more broadly because they are our most imperiled taxon, our physiological results also confirm that future investments in rebuilding populations of freshwater mussels will help to sustain and improve water quality. Weight-specific filtration rates of suspended sediments and particulate nitrogen by two Delaware mussel species were found to be comparable to marine bivalves such as oysters. Filtration rates of suspended particles and associated pollutants depend on both the mussel's water clearance rates and the amount and composition of particles in the water column. Since seston composition was found to vary widely among seasons and places in this study, the simplest means of comparing our results to other studies is to contrast clearance rates. Here, clearance rates varied by species, site and season from 0.53 – 1.5 L h^{-1} g dry mussel $tissue^{-1}$, averaging about one liter per hour per gram. This is very typical of rates found for oysters, marine mussels, and for freshwater mussels studied elsewhere (Kreeger 2015). Clearance rates vary with temperature, but are reasonably consistent among species and locations per unit of tissue biomass. Therefore, models of the water quality benefits of beds of freshwater mussels require only detailed knowledge of the seston composition, seasonal temperature, and population demographics (biomass densities and size class distirbutions) – mussel species and location, per se, is not a main determinant.

To examine the actual water quality benefits of extant mussel beds (e.g. in the reference systems where mussels were collected) would therefore require quantitative surveys of mussel body size and population abundance, which are intensive and were not planned for this study. Quantitative surveys of reference mussel beds in the upper estuary (PA and NJ), compared with physiological data from this study (DE), illustrate the tangible benefits of mussel bed conservation and restoration, however. For example, typical mussel beds in the Delaware River upstream of Philadelphia contain from 4-20 mussels per square meter, and about 100,000 mussels per hectare of suitable habitat. Based on typical concentrations of suspended matter (TSS) and associated nitrogen, such populations have been estimated to remove more than 10 tons of TSS and 400 pounds of nitrogen per hectare per year. In the Brandywine River immediately upstream of DE, current mussel densities are lower, about 1.7 per square meter; however, TSS removal has been estimated to be 26 tons per year in the reach between Chadd's Ford, PA and the Delaware state line.

To similarly illustrate the benefits of freshwater mussels in southern Delaware, data from this report can be used to project typical water cleaning benefits for a hypothetical bed of

1000 mussels, which is far less than is currently living in Brown's Branch or Blair's Pond, based on our anecdotal observations from the qualitative surveys. In those systems, only 1000 typical adult mussels would typically contain about 1.2 kg of dry tissue weight, would clear 2.8 million gallons of water per year, and would filter 173 kg of (dry) total suspended solids per year. Nitrogen removal would depend on the N content of the TSS, which varies widely among streams and ponds. Considering that healthy reference beds of mussels elsewhere typically contain 1,000 – 100,000 mussels per mile, it is clear that investments in mussel conservation and restoration can yield substantial contributions to sustaining and improving water quality. More work is needed to gauge the fate of filtered matter, since a portion of the gross removal will be recycled *in situ*, but we conservatively estimate that at least 25% of filtered matter becomes bound and net removed. The seasonal physiological data from the current study, for two mussel species from various source locations, provide critical empirical information from which we can now model water quality outcomes from future restoration investments.

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