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Citation	Gray, M. W. & Kreeger, D. (2014). Monitoring fitness of caged mussels (Elliptio complanata) to assess and prioritize streams for restoration. Aquatic Conservation: Marine and Freshwater Ecosystems, 24(2), 218-230. doi:10.1002/aqc.2395
DOI	10.1002/aqc.2395
Publisher	John Wiley & Sons, Inc.
Version	Accepted Manuscript
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Monitoring fitness of caged mussels (*Elliptio complanata*) to assess and prioritize streams for restoration

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Abstract:

1. Freshwater mussels (Order: Unionidae) are among the most imperiled aquatic organisms in North America. Conservationists and resource managers within the United States are increasingly advocating restoration of these animals to preserve biodiversity and boost ecosystem services in the nation's waterways.

2. Historically, restoration methods have yielded less than optimal survival rates due in part to an inability to identify suitable habitat for these organisms. Through the use of caged mussels as bioindicators, a method was developed to test prospective restoration sites for their ability to support mussel fitness prior to beginning actual restoration so that investments are strategic.

3. Mussels (*Elliptio complanata)* from a healthy population were caged and deployed to candidate streams. Their survivorship, condition, and proximate biochemical composition (protein, carbohydrate, lipid) was then monitored for one year. Streams that supported mussel fitness as well as or better than their source stream were considered to be suitable for restoration.

4. Four of five candidate streams were found to support mussel fitness. Additionally, reciprocal transfers between two source populations revealed that the seasonal patterns of tissue biochemical composition respond to ambient stream conditions, indicating that this species is diagnostic as a bioindicator of stream quality and habitat suitability.

Keywords: Restoration, Physiology, Invertebrates, Reintroduction, River, Stream.

Introduction:

The precipitous decline of freshwater mussels (Unionidae) in North America is well documented (Master, 1990; Neves *et al*., 1997; Lydeard *et al*. 2004). The decline of species diversity, distribution, and population abundance has been largely attributed to overharvesting, construction of dams, habitat degradation, pollution, and introduction of invasive species (Bogan, 1993; Vaughn and Taylor, 1999). Due to their catastrophic decline as well as the recognition of their cultural and ecological significance, more aggressive action for the restoration of freshwater mussels has been recommended (Strayer *et al*., 2004, Geist 2010). Additionally, unionid restoration has been promoted as a tactic to remediate water quality and build resilience of aquatic systems for changing conditions (Kreeger, 2005a).

Freshwater mussel restoration usually occurs in one of three forms: 1) seeding streams with hatchery-reared juvenile mussels, 2) reintroducing reproductive adults to their historic range from extant populations, or 3) removing dams or other impediments to mussel dispersal via fish hosts for their larvae. Each of these restoration tactics has advantages and disadvantages, but all approaches can be costly. Additionally, restoration by relocating portions of existing populations, a common management tool, has historically been met with moderate to low survival rates (Cope and Waller 1995; Cope et al. 2003). Given the precious nature of these resources (e.g. listed species, glochidia, restoration funds, etc.), any improvement in the identification of suitable habitat would greatly improve restoration outcomes.

In many cases, streams that once held vibrant mussel assemblages have seen sufficient

improvement in water quality or habitat conditions to possibly again sustain these animals, but other streams have not. Therefore, prior to investment in mussel reintroduction, it is prudent to first test whether candidate recipient streams are capable of sustaining mussel populations. The objectives of this study were to examine the use of freshwater mussels as bioindicators of environmental quality while simultaneously evaluating several streams in Southeastern Pennsylvania for suitability prior to restoration.

Approach

As a straightforward first step to screening multiple candidate restoration streams for mussel reintroduction, survival and sublethal indicators of mussel fitness were monitored in caged animals held in candidate streams. Mussel condition index and proximate biochemical composition of mussel tissues were monitored seasonally for a one-year period and contrasted among recipient streams, source streams, and uncaged controls. Significant deviations in expected seasonal patterns of condition and tissue biochemistry, as evidenced by caged and uncaged animals in healthy populations in source streams (hereafter referred to as “source” mussels) were interpreted as suboptimal.

Similar to marine bivalves, the biochemical composition of tissues in healthy, reproductively active freshwater mussels cycle through predictable seasonal patterns (Figure 1; Bayne, 1976; Zandee *et al*., 1980; Okumus and Stirling, 1998). For example, in late winter to spring, high tissue protein content can be associated with gametogenesis, whereas, tissue protein content and condition index following spawning and larvae release is expected to be reduced (Okumus and Stirling, 1998). Therefore, any depression of proteins (and possibly also lipids) during reproductive development indicates that the

animals may not be as reproductively active as animals that have robust seasonal buildup of essential biochemical constituents.

The utility of these tissue metrics as sublethal stress indicators depends on the seasonal context whereby stress must be inferred from deviations in expected normal seasonal patterns observed in animals in healthy populations. Hence, environmentally stressed animals are expected to have either significant shifts in the timing of these cycles, or they will fail to show the seasonal variation normally associated with successful reproduction (e.g. consistently lean with low condition, protein, lipid, and carbohydrate content). The fitness and biochemical composition of mussels transferred to a new stream eventually reflects the prevailing environmental conditions found in the new habitat (Widdows *et al*., 1990), but it is important to allow sufficient time for this to occur before interpreting results.

Elliptio complanata (Lightfoot, 1786) was historically abundant throughout much of Southeastern Pennsylvania (Ortmann, 1919); however, many populations have since been extirpated. A caging experiment with *E. complanata* was conducted to document the normal seasonal cycles of condition and tissue biochemistry in two streams that currently still harbor *E. complanata* as well as in five candidate restoration streams. Fitness parameters of mussels held in candidate streams that consistently fell below those of the source population mussels at crucial times of the year indicated the candidate stream was less suitable. Additionally, mussels that did not display seasonal variation in biochemical composition were considered less suitable as this would be an indication of abnormal mussel physiological cycling and basal metabolic behavior.

Reciprocal transfers of caged adult mussels were made between two source populations of *E. complanata* in addition to transfers to candidate streams (Table 1). Monitoring of these reciprocal transfers between source populations established seasonal cycles for condition and tissue biochemistry in healthy populations and also examined whether they were similar between streams in these tissue metrics.

Candidate streams were scored on performance criteria relative to source mussels and then ranked (see Methods for scoring criteria). Therefore, a weight-of-evidence approach was used to rank the candidate restoration streams in terms of their readiness for mussel restoration, while also contrasting fitness between the two source populations.

Methods:

Study Streams and Caging

The main supply of *E. complanata* for deploying to candidate streams was from Brandywine Creek (BC) in southeastern PA. Kreeger (2005b) reported that approximately 500,000 adult mussels inhabit a 9.65 km reach between Chadds Ford, PA and the Pennsylvania-Delaware state border. The physiological cycles of this species have been studied for the past 10 years, providing intermittent data on expected seasonal patterns of condition and tissue biochemistry. Mussels were also collected from Ridley Creek (RC) within RC State Park for use in the reciprocal transfer study. Candidate restoration streams were West Branch Brandywine Creek (WB), Red Clay Creek (RCL), East Branch White Clay Creek (EBC), Middle Branch White Clay Creek (MWC), and Chester Creek (CC) (Figure 2). All of these sites were reported to once hold diverse species of native unionid mussels (Ortmann, 1919), but decades of recent surveys failed to detect any live unionids therein (Thomas *et al*., 2011).

Populations of *E. complanata* in Brandywine and Ridley Creeks were identified through stream surveys in 2005, and these served as the two source populations in the present study; however, the Brandywine population was larger than in Ridley Creek and therefore it supplied all mussels deployed to candidate streams. In October 2007, 567 mussels were collected in by hand while snorkeling/swimming, cleaned, measured for shell length, and assigned a 6 mm plastic tag with a unique number which was glued to the exterior of the shell with superglue (Krazy Glue) and coated with acrylic nail polish. Mussels were held out of the water for a total approximate time of 40min. Mussels were subdivided and taken to five candidate restoration streams in coolers containing fresh stream water. For each study stream, at least 14 adult mussels of similar size variation per group were added to each of 4 replicate cages per recipient stream.

To account for handling and caging effects, mussels from the source streams were similarly caged within their native streams and subsequently monitored in comparison to uncaged mussels. Caged and uncaged mussels were monitored for condition index and proximate biochemical composition in all streams at the start of the experiment and then seasonally as described below. Mortality was also assessed for caged mussels. Travel time from any one creek to another was less than 1 hour. Mussels were deployed in cages consisting of a $0.61m^2$ industrial dishwashing tray (Kitchen Equipment Co.) covered with black plastic netting. The netting was rigid with 20mm square holes that permitted ample flow through the cages. The net covered the top, sides, and bottom of the cage to exclude predators and prevent mussels from escaping. Cages were numbered and deployed into the stream bottom in similar substrates and habitats to those that in which they were collected from in source

streams (mixed cobble, gravel, sand, and silt substrates with unobstructed flows). The bottom of the cage was placed approximately 10 cm below the substrate layer and anchored with 0.3- 0.76m rebar on corners. Trays were then half filled with ambient local silt, sand, gravel and a few large rocks to provide mussels with substrate and to help anchor cages. Rocks placed along the exterior of cages aided in armoring them against debris.

Baseline sampling of uncaged mussels from Brandywine Creek and Ridley Creek occurred in October, 2007. Subsequent sampling of caged mussels deployed in candidate streams and caged and uncaged mussels within source streams occurred on Dec 2007, March 2008, June 2008, and October 2008. During sampling, 12 mussels per stream (3 mussels per cage x 4 cages per stream) and >6 uncaged source mussels were collected and brought back to the laboratory in coolers filled with stream water. Once in the laboratory, excess mud and sediment were gently cleaned from shells from mussels. Mussels were dabbed dry and total wet weight (TWW) and shell height of each animal was recorded. Tissue from each animal was excised and freeze dried to eventually determine their dry tissue weight and dry shell weight. Dry tissue was then ground by hand with a mortar and pestle into a fine, homogeneous powder. Subsamples of homogenized tissue samples from each mussel were used to determine their condition index and proximate biochemical composition (i.e. protein, lipid, and carbohydrate content) at the time of sampling.

Condition Index:

Condition index is a common index for bivalves that estimates meat fatness by comparing the dry weight of the animal tissue to the interior shell volume (Hopkins, 1949).

Condition index (CI) was obtained using the total wet weight (TWW), dry shell weight

(DSW), and ash free dry tissue weight (AFDTW) using the gravimetric method of Crosby and Gale (1990), modified using ash-free dry weight as per Kreeger (1993)

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

Tissue Protein Content:

Approximately 5-10 mg subsamples of homogenized tissue were weighed into pre-weighed test tubes. Each was then treated with 4 mL of 0.1 M NaOH, homogenized for 10 seconds (Tekmar M-1574), and then sonified (Branson Sonifier M-250) at half power (setting 5; 50 %) for 8 bursts. Tubes were then loosely capped and placed in an oven at 60°C for 45 minutes. After incubation, samples were vortexed, allowed to cool for 15 minutes, centrifuged (1500 x g, 10min) and the protein concentration of the supernatant determined spectrophotometrically using a microplate with a 580nm filter (Molecular Devices *Thermomax*) and a BCA protein assay kit (Thermo Scientific).

Tissue Carbohydrate Content:

Subsamples of homogenate from each mussel were quantified, treated with 1 mL of laboratory pure water ((LPW); deionized and distilled water), vortexed for 10 seconds, 1 mL of 5% phenol added to the sample, and was vortexed again for an additional 10 seconds. Finally, 5 mL of concentrated sulfuric acid was slowly added to the sample, allowed to cool for 15 minutes, carefully vortexed, and allowed to sit for 10 min so that the reaction could be completed. Following centrifugation at 1500 x g for 20 minutes, supernatant was withdrawn for spectrophotometric determination of carbohydrate using the procedure described by Dubois *et al*. (1956). Carbohydrate concentrations were

standardized with cold soluble starch (Sigma Chem., Co. Cat # 9765), and the weight of carbohydrate in the tissue subsample was then divided into the dry subsample weight to calculate the carbohydrate content of that mussel (% dry weight/ weight).

Tissue Lipid Content:

Lipid concentrations in tissues were determined gravimetrically following methods from Folch *et al*. (1957), modified for bivalves by (Kreeger and Langdon, 1993). Subsamples of approximately 10 mg of equivalent dry weight of tissue homogenate were added to a tissue vial grinder (10 mL Potter- Elvehjem with PTFE pestle, Wheaton #358039) and ground by hand in 2 mL 2:1 chloroform/methanol (C/M) solution. Using 2 mL more of 2:1 C/M as a wash, all material was transferred back to a test tube and centrifuged (International Equipment Co. M- CL) (1000 x g, 5 min). Each supernatant was then transferred to a 7 mL conical borosilicate glass test tube. An addition of 20% (final v/v) of 0.88% KCl solution was added to each tube and then centrifuged again (1000 x g, 2 min). The lower, lipid-containing chloroform layer was carefully transferred to a pre-weighed 7 mL glass test tube and allowed to dry completely. Tubes were reweighed after drying and amounts of lipid in the original sample were calculated after applying a correction factor for any percent loss in standards. Neutral lipids and 3-Hexadecanone lipid standards (Sigma-Aldrich S543934) were used to determine lipid extraction efficiency.

Stream Suitability Scoring:

The five candidate streams were assigned a simple numerical score according to five criteria, with weighted emphasis on the first three metrics as follows. The scoring approach was based on best scientific judgment of the relative importance of the five metrics as

fitness indicators. Benchmarks for each metric (e.g. condition index >50 in the autumn after 1 year) are based upon values obtained from Ridley Creek mussels which were found to be the most fit population.

1) Survivorship. A stream was assigned 2 points if it was not significantly different from mussels in Ridley Creek, which was considered the most fit source population. If survivorship was significantly lower, then it was scored as 0.

2) Condition Index (CI). Streams that supported mussel condition index >50 in the autumn after 1 year (as per Ridley Creek mussels) were scored 2 points, whereas streams with mussel CI 40-50 were given 1 point, and streams with mussel CI<40 were assigned 0 points.

3) Carbohydrate Content. Streams having mussels with >40% carbohydrates in their tissues sustained throughout the spring to autumn period (as per Ridley Creek mussels) were assigned 2 points. Streams supporting >40% carbohydrate content in only 2 of these 3 seasons were assigned 1 point. Carbohydrate contents that did not exceed 40% in at least 2 of these 3 seasons were assigned 0 points.

4) Protein Content. If protein contents in mussel tissues were greater in autumn and winter than in spring and summer, then streams were assigned 1 point (as per Ridley Creek mussels). If protein did not vary significantly during the year or if the protein peak timing was offset, then the stream was assigned 0 points.

5) Lipid Content. Streams were scored with 1 point if lipid content peaked in winter (as per Ridley Creek mussels) and 0 points if not.

Analysis:

For statistical analysis, all percentage data (e.g. survival %, protein % content) were transformed by arcsine square root and then output means and variabilities were back transformed for presentation. Statistical comparisons among streams and seasons were discerned using ANOVA and Fisher's LSD at 95% confidence intervals to determine significantly different means. Statgraphics v5.0 software was used for statistical analysis. For more detailed statistics on condition index and biochemical constituents, including p-values from all ANOVAs and their respective F-Statistics and degrees of freedom, see Supplementary Tables 2-9.

Results:

Comparison of Candidate Restoration Streams with Brandywine mussels

Survivorship of translocated Brandywine *E. complanata* held in most streams was high (>90%) and not significantly different among all streams with the exception of West Branch Brandywine (WB), which had significantly lower survivorship than all others (53%, p-value = 0.03 $F_{6, 415} = 2.54$). Although the low survivorship in WBB was significant early in the study, statistical differences toward the end of the study were unable to be assessed because flooding and human disturbance led to the eventual loss of 3 of the 4 cages initially deployed. Therefore, data from this site were less informative than other sites during the spring 2008 to autumn 2008 period.

Mean condition index ranged from 32 to 66 among all streams and sampling times during the study (Supplementary material, Table 2; Figure 3). Mussels held in MWC, RCL, RC, and EBC had a significantly greater ($p<.05$, 1-way ANOVA, Fisher's LSD) condition index than BC mussels (66, 61, 55, 52, and 37, respectively) at the final sampling period, which

was the important autumn conditioning period. Mussels held in CC and WB were similar (Fisher's LSD) to Brandywine source mussels at that time. At other times there was no significant difference in the condition index of mussels held in different streams.

Protein content (% w/dry tissue weight [DTW], hereafter (% w/DTW)) varied significantly among translocated Brandywine mussels held among streams (mean range, 21% to 41%) and the greatest protein content was found in autumn. Caged mussels in all candidate streams held their protein content at least as well as mussels in Brandywine Creek (source stream), although the seasonal timing of high and low protein content appeared to shift as animals started to adapt to the ambient conditions of each stream (Figure 3). Protein contents of mussels from all streams were similar during winter. Due to high variability among animals, protein contents were not significantly different among streams in winter and spring when protein may be most important, however, it was interesting to note that mussels in both source streams and MWC had greatest seasonal protein in winter, whereas, two of the candidate restoration streams had lowest seasonal protein contents in winter (Supplementary material, Table 3).

Carbohydrate contents of mussels from all streams, except WB, varied significantly ($p < 0.05$, 1-way ANOVA) over the study period (Figure 3). Mean carbohydrate content of mussels from all streams ranged from 24% to 57% throughout the study (Supplementary material, Table 4). Carbohydrate content of mussels varied most significantly among streams during the summer when, WB mussels were significantly lower ($p < 0.05$, 1-way ANOVA, Fisher's LSD) in carbohydrate content than all other streams. *E. complanata* held in MWC peaked in carbohydrate content during the summer and were significantly greater

(Fisher's LSD) than mussels sampled from EBC and WB (54%, 45%, and 22%, respectively). In autumn (2008), mussels held in MWC were significantly greater ($p=0.04$, 1-way ANOVA, Fisher's LSD) than mussels held in WB and BC mussels during the final sampling period (49%, 57%, 26%, and 33%, respectively). Late summer to autumn was considered the most important time for diagnosing ecological fitness in terms of carbohydrate content. Mussels in source streams tended to have carbohydrates contents >40% from spring to autumn. In comparison, 4 of the 5 candidate restoration streams were similar, also >40% carbohydrates during that period. However, WB failed to ever show carbohydrate >40%.

Lipid content of mussels was low and relatively unchanging overall (varied 5-16%) (Supplementary material, Table 5). No significant differences were found among streams at any time, and all followed the same seasonal pattern (Figure 3). Seasonality was characterized by a winter peak and remained low thereafter. More study of protein and lipid demands of freshwater mussels is needed to provide greater understanding of their role in judging mussel fitness and stream suitability for restoration

Reciprocal Transfers and Comparisons to Uncaged Mussels

At no time during the study was the condition index or proximate biochemical composition of caged source mussels held in their native stream significantly different from that of uncaged source mussels, suggesting that caging and handling stress was negligible. Condition index varied significantly among Brandywine source mussels, transferred Brandywine and Ridley mussels, but not Ridley source mussels that remained in RC throughout the entire study (Figure 4). Both source populations were found to be similar

for condition index during the summer. Ridley Creek source mussels had greater condition than that of Brandywine source mussels at all times except summer. Reciprocally transferred populations were similar in condition index to that of the source mussels of the recipient stream in the summer sampling period. At the end of the study (autumn 2008), all mussels held in Ridley Creek, regardless of origin, were significantly greater in condition index than Brandywine source mussels, while transferred Ridley mussels were similar to both source populations (p-value =0.01, 1-way ANOVA, Fisher's LSD). By the end of the study, mussels reciprocally transferred between source streams (i.e. Ridley transferred to Brandywine and vice versa) had a condition index similar to mussels native to those streams, suggesting that mussel physiology and fitness responded to new stream conditions (p-value =0.01, 1-way ANOVA, Fisher's LSD).

Protein content of source mussels and all reciprocally transferred mussels varied significantly throughout the study, except for Brandywine mussels transferred to Ridley Creek (p-value =0.07, 1-way ANOVA, Fisher's LSD). Ridley source mussels were initially significantly greater in terms of protein content during the autumn of 2007 (p-value =0.01, 1-way ANOVA, Fisher's LSD). All mussels held in Ridley Creek were similar in protein content by the winter and spring sampling periods. Ridley Creek mussels transferred to Brandywine were significantly greater in protein content than all other source mussels at the end of the study in autumn 2008.

Carbohydrate content of both source populations and Brandywine mussels transferred to Ridley Creek varied significantly over the course of the study (Supplementary material, Table 8, Figure 4). However, Brandywine mussels transferred to Ridley Creek did not vary

over time but were significantly greater than all other treatments by the autumn of 2008. Ridley mussels transferred to Brandywine Creek declined significantly from spring to the autumn of the 2008. By the end of the study, these mussels contained the lowest carbohydrate content of any group, but were still similar to Brandywine mussels. The low carbohydrate content of transferred Ridley might also explain the high protein content found in these mussels during this same time period. Since percentage content reflect the interplay among biochemical constituents (protein, lipid, carbohydrates, ash), a high protein level can result simply from a drop in some other constituent, for example.

Lipid content of all source mussels tended to decrease over the yearlong study period, but varied among mussels at most study sites between 8-16% w/dtw. There were no significant differences in lipid content among any treatments at any point in the study.

Discussion:

Relocation of adult freshwater mussels and reintroduction of juvenile mussels to restored habitat has been identified as a key tool in the reestablishment of populations (Jenkinson, 1985; Hubbs *et al.*, 1991; Layzer and Gordon 1993 Haag and Williams, 2013). Despite moderate improvements in relocation efforts (e.g. Cope *et al*., 2003), the historically high mortality rates associated with these activities has prompted some to consider it a last resort conservation strategy (e.g. Cosgrove and Hastie, 2001; Haag and Williams, 2013). Surprisingly, when relocation efforts have been performed, outcomes are rarely monitored due to a lack of tactics to assess performance of translocated mussels (Gum *et al.*, 2011). Lack of suitability measures has forced many restoration programs to shift focus on stock augmentation (Haag and Williams, 2013). Indeed, indentifying habitat suitability has now

become a major impediment for restoration of these declining taxa.

Previous measures of suitability have relied on survivorship data of relocated mussels collected over short periods of time (1-3 years) (Havlik, 1997; Dunn *et al*., 2000; Cope *et al*., 2003). However, due to the long-lived nature of these organisms, it is difficult to determine the success of any conservation strategy for freshwater mussels by simply measuring survivorship in the short term. In this study, sublethal measures of freshwater mussel condition and tissue biochemistry were more diagnostic of rearing conditions in targeted streams for restoration in comparison to survivorship alone. Indeed, except for one location where mortality was high, survival of caged mussels held for one year in seven streams was near 100% and indistinguishable.

In contrast to survivorship (an indicator of acute stress), the autumn condition index after one year (an indicator of chronic stress) differed significantly among various candidate restoration streams. Importantly, the condition of mussels held in some candidate streams (3 of 5) was higher in the autumn of 2008 than mussels left in Brandywine creek, whereas, 1 candidate stream was clearly suboptimal for mussels. This was thought to be physiologically significant as high condition in autumn indicated the accumulation of energy stores which would be needed throughout the unproductive winter months (Gabbott, 1983; Beninger and Lucas, 1984).

Monitoring of the proximate biochemical composition of mussel tissues yielded additional information to discern subtle differences among sites and times in mussel fitness, and hence habitat suitability, among the studied streams. There are several different ways to express proximate biochemical composition. In nutritional studies that seek to quantify seasonal

dietary needs, tissue biochemical components can be calculated as absolute concentrations (e.g., protein concentration, mg per animal). This information is useful to examine times when animals accumulate or use up macromolecules over time. Proximate biochemical components can also be expressed as relative percentages of total dry tissue mass (e.g. protein content, % w/w), which is a useful approach for comparing seasonal and spatial differences in physiological status among treatment populations. Here, the biochemical composition of animals in each stream was reported as relative percentage differences to facilitate comparisons among mussels that varied in size and were held in different streams.

Interpretation of tissue biochemistry data focused on the proportion of important constituents during critical periods when the constituent was thought to be needed most, such as for reproduction or growth (protein, lipid) or maintenance of energy balance and storage (carbohydrate). Care must be taken in interpreting these data, since suboptimal health must be inferred by deviations from normal cycling patterns rather than simple high or low values. Hence, it is critical to identify reference conditions, which we determined here to be the seasonal patterns of condition and tissue biochemistry of mussels from the Ridley Creek source population, which was deemed to be healthier than the Brandywine source population based upon numerous observations (e.g. presence of diverse size range including juveniles, less shell erosion, fatter and more biochemically enriched tissues).

Carbohydrate stores are critical for sustaining mussels through winter and expected to be high in autumn (Gabbot, 1983; Beninger and Luca, 1984). However, significantly lower carbohydrate content and condition index in mussels transferred among candidates were found during critical time periods; namely, mussels transferred to WB suffered reduced

carbohydrate content and condition index during the autumn sampling periods prior to when these mussels would have undergone overwintering indicating poor suitability. Although our biochemical data for this stream was statistically invalid after the winter sampling due to lack of replication, macroinvertebrate surveys from this stream supports our assertion that WB was impacted from 2007-2008 (USGS, 2012). Furthermore, macroinvertebrate surveys from all other candidate streams reported these streams to be less impacted or non-impacted during the study period (USGS, 2012).

Protein and lipid contents varied seasonally, and among streams, however it is unclear from this study alone how to interpret these differences in terms of fitness due to a lack of literature information on the specific nutritional demands of freshwater mussels. Protein and lipid are important for reproduction and perhaps larval brooding, but little is known about whether and how tissue composition varies in association with changing needs throughout the year and in relation to various reproductive stages. In this study, the greatest protein content was observed in autumn and the greatest lipid content during winter in the Brandywine source population. These findings could reflect gametogenesis in late autumn to winter, consistent with reproductive cycles of freshwater mussels that are short-term brooders (Zale and Neves, 1982; Garner *et al*., 1999), which include *E. complanata*. In marine mussels, higher protein contents and nutritional demands for protein occur in late winter to spring. More studies of reproductive cycling and associated physiological and nutritional processes are needed in order to fully assess whether biochemical composition can help discern mussel fitness. For these reasons, our scoring approach to characterizing suitability among candidate restoration streams assigned a lower weight to these

biochemical constituents, reflecting lower confidence in their utility as fitness indicators.

Reciprocal transfers of mussels between source streams yielded clear evidence of biochemical adaption. By spring (two seasons following deployment), all biochemical constituents of transferred mussels had begun to mirror those of mussels native to the host stream. By the end of the study, other indices, such as condition index or carbohydrate content, indicated full adaptation as reciprocally transferred mussels biochemically resembled host stream mussels. For example, mussels held in Brandywine consistently had lower condition index than mussels held in Ridley, suggesting that RC may be the superior habitat. The condition index and carbohydrate data from reciprocally transferred mussels supports this statement as transferred mussels more closely resemble that of host stream mussels than their own source mussels by the end of the study with respect to these condition index and carbohydrate content (Figure 4).

Hence, reciprocal transfers indicated that *Ellipitio complanata*, like other bivalve species, gradually adapted to their new environments in the recipient streams, with tissue biochemistry and condition shifting to reflect the new conditions. The time required for full adaptation was approximately 6-12 months, depending on the parameter. This result was consistent with the wealth of data showing that suspension-feeding bivalves serve as excellent bioindicators of environmental quality (e.g. Burns and Smith 1981; Boening 1999; Gunther et al. 1999). A surprising result was that the condition of mussels in one of our source streams (Brandywine) was suboptimal, suggesting that the presence of extant mussels does not necessarily indicate the presence of high quality habitat. This result was supported by other observations (Kreeger and Gray unpublished) that the mussel population

of the Brandywine is not successfully reproducing and experiences greater shell erosion, compared to the Ridley reference location. The reasons for these differences are unclear, possibly including variation in fish hosts, benthic habitat conditions, food quantity/quality, or water quality. Hence, streams that still support at least some mussels should not automatically be regarded as reference streams (e.g. Cosgrove and Hastie 2001), especially given the long-lived nature of some unionids (Anthony et al. 2001),

There are many types of physiological, biochemical, and molecular markers that have been developed to diagnose chronic stress in aquatic organisms, but differentiating subtle differences in fitness is often best accomplished by a weight-of-evidence approach that relies on multiple stress indicators (e.g., in this study: condition index, protein content, lipid content, carbohydrate content). Some of the other potential measures of chronic fitness include physiological rate functions (feeding, respiration, assimilation), O:N ratios, scope for growth, and biomarkers. Direct tracking of reproductive status (gametes, brooded larvae) would also determine if a population is reproductively active and, hence, healthier. Molecular biomarkers are also well established for their ability diagnose sublethal effects of unknown and known contaminants and can be short-term predictors of long-term ecological impacts (Bayne *et al*., 1979; Murphy and Kapustka, 1989; Monserrat *et al*., 2007). However, the technical expertise required and cost for making measurements using some biomarkers can be high (Livingstone, 1993). All of these measurements can be time consuming and difficult to widely use, especially during *in situ* monitoring. The method described here is relatively straightforward and comparatively inexpensive, especially if only condition is tracked among caged mussels.

Monitoring caged mussels was a valuable tool for evaluating the suitability of candidate streams for *E. complanata*. Most candidate restoration streams were found to be suitable with regard to water quality and food conditions since they supported the expected seasonal pattern of tissue biochemistry and overall condition as measured in the source population. It is important to note that sustainable mussel populations also require appropriate substrate habitat, stable bottom conditions with minimal erosion, and free passage of fish hosts for larvae during reproduction. Thus, due to the complex life-history traits of *E. complanata*, restoration of sustainable mussel populations could still be hampered by lack of fish hosts or other factors even though this fitness assessment indicates that some of our candidate restoration streams are suitable.

Candidate streams were assigned scores for their ability to support seasonal profiles of mussel condition index and proximate biochemical composition (see methods for scoring criteria). This weight-of-evidence scoring approach was arbitrary but was needed to integrate the various tissue-based metrics into a common index that can be used to prioritize streams for restoration. The weighting basis was grounded on the most current literature regarding the relative importance of the different metrics for reflecting mussel fitness. For example, more study of seasonal protein and lipid demands of freshwater mussels is needed to provide greater understanding of their utility in assessing fitness of mussels; hence they were assigned half the weighted score values compared to condition index and carbohydrate. The overall score should be interpreted with caution as a preliminarily guide with which to grade the candidate streams for their relative restoration promise.

The weighted scoring approach furnished a means to qualitatively discern among the

candidate streams, indicating that RCL, MWC, and EBC are suitable for mussels because they scored 8 points, identical to "best" source stream, Ridley Creek. CC and BC were judged to be slightly less suitable (6 and 5 points, respectively) but still capable of supporting mussels. In contrast, WB scored only 1 point and would require more study and possible improvement before mussel restoration should commence.

To summarize the seasonal patterns of condition and tissue biochemistry seen in relatively healthy mussels living in southeast Pennsylvania, Figure 5 depicts seasonal variation in the four key sublethal measures studied, averaged among mussels from the Ridley Creek and the three top ranked candidate streams. Although more studies are needed to assess seasonal patterns of tissue metrics in other areas and mussels species, our results suggest that the condition index and tissue carbohydrate content of freshwater mussels follows a similar seasonal pattern for many marine bivalves, being greatest during autumn. However, seasonal peaks of protein and lipid contents in freshwater mussels were earlier (winter) compared with marine mussels (spring), possibly reflecting earlier seasonal reproductive conditioning in the short-term brooder, *E. complanata*. . Further research of freshwater mussel biochemical physiology is warranted. Indeed, this study represents one of the few studies to monitor seasonal changes in the biochemical composition of freshwater mussels (e.g. Baker and Hornbach 2001).

The tissue-based ranking information showing which streams sustain similar seasonal biochemical composition compared to healthy source populations was found to be useful for discerning subtle differences in condition maintenance among streams in SE Pennsylvania, thereby helping to prioritize streams for mussel restoration and ensuring that

precious resources are invested strategically in locations where mussel populations are more likely to thrive and be self-sustaining.

Acknowledgments:
Many thanks to Angela Padeletti for her extensive contributions to the field work for this project as well as her editorial assistance with this manuscript. Additional help on this manuscript was provided by Drs. Susan Kilham and David Velinsky as well as two anonymous reviewers. MWG would like to thank the Partnership for the Delaware Estuary for hosting him as a graduate research fellow from 2007-2009 and partially funding this research. This research was funded by ConocoPhillips through a restoration and research grant to the Partnership for the Delaware Estuary.

References

Anthony JL, Kesler DH, Downing WL, Downing JA. 2001. Length-specific growth rates in freshwater mussels (Bivalvia: Unionidae): extreme longevity or generalized growth cessation? *Freshwater Biology* **46**: 1349–1359.

Baker SM, Hornbach DJ. 2001. Seasonal metabolism and biochemical composition of two unionid mussels, *Actinonaias Ligamentina* and *Amblema Plicata*. *Journal of Molluscan Studies* **67**: 407–416.

Bayne BL. 1976. Marine mussels, their ecology and physiology. Cambridge University Press. Cambridge.

Bayne BL, Moore MN, Widdows J, Livingstone DR, Salkeld P, Crisp DJ, Morris RJ, Gray JS, Holden AV, Newell RC. 1979. Measurement of the responses of individuals to environmental stress and pollution: studies with bivalve molluscs [and Discussion]. *Philosophical Transactions of the Royal Society of London*. B, Biological Sciences **286**: 563–581.

Beninger PG, Lucas A. 1984. Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussates* and *Tapes philippinarum*. *Journal of Experimental Marine Biology and Ecology* **79**: 19–37.

Boening DW. 1999. An Evaluation of Bivalves as Biomonitors of Heavy Metals Pollution in Marine Waters. *Environmental Monitoring and Assessment* 55(3): 459–70.

Bogan AE. 1993. Freshwater bivalve extinctions (Mollusca: Unionoida): a search for causes. *American Zoologist* **33**: 599–609.

Burns, KA, Smith JL. 1981. Biological Monitoring of Ambient Water Quality: The Case for Using Bivalves as Sentinel Organisms for Monitoring Petroleum Pollution in Coastal Waters. *Estuarine, Coastal and Shelf Science* **13**: 433–43.

Cope WG, Hove MC, Waller DL, Hornbach DJ, Barsch MR, Cunningham LA, Dunn HL, Kapuscinski AR. 2003. Evaluation of relocation of unionid mussels to in situ refugia. *Journal of Molluscan Studies* **69**: 27–34.

Cope WG, Waller DL. 1995. Evaluation of freshwater mussel relocation as a conservation and management strategy. *Regulated Rivers: Research & Management* **11**: 147–155.

Cosgrove PJ, Hastie LC. 2001. Conservation of threatened freshwater pearl mussel populations: river management, mussel translocation and conflict resolution. *Biological Conservation* **99**: 183–190.

Crosby MP, Gale LD. 1990. A review and evaluation of bivalve condition index methodologies with a suggested standard method. *Journal of Shellfish Research* **9**: 233–237.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**: 350–356.

Dunn HL, Sietman BE, Kelner DE. 2000. Evaluation of recent unionid (Bivalvia) relocations and suggestions for future relocations and reintroductions. In Freshwater Mollusk Symposia Proceedings (R. A. Tankersley, D. I. Warmolts, G. T. Watters, B. J. Armitage, P. D. Johnson & R. S. Butler, eds), p. 169–183.Ohio Biological Survey, Columbus Ohio.

Folch JM, Lee M, Sloane-Stanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**: 497–509.

Gabbott PA. 1983. Developmental and seasonal metabolic activities in marine molluscs. *The mollusca* **2**: 165–217.

Garner JT, Haggerty TM, Modlin RF. 1999. Reproductive cycle of *Quadrula metanevra* (Bivalvia: Unionidae) in the Pickwick Dam Tailwater of the Tennessee River. *The American Midland Naturalist* **141**: 277–283.

Geist J. 2010. Strategies for the conservation of endangered freshwater pearl mussel (*Margaritifera margaritifera* L.): a synthesis of conservation genetics and ecology. *Hydrobiologia* **644**: 69-88.

Gum B, Lange M, Geist J. 2011. A critical reflection on the success of rearing and culturing juvenile freshwater mussels with a focus on the endangered freshwater pearl mussel (Margaritifera margaritifera L.). *Aquatic Conservation: Marine and Freshwater Ecosystems* **21**: 743–751.

Gunther AJ, Davis JA, Hardin DD, Gold J, Bell D, Crick JR, Scelfo GM, Sericano J, Stephenson M. 1999. Long-term Bioaccumulation Monitoring with Transplanted Bivalves in the San Francisco Estuary. *Marine Pollution Bulletin* **38**: 170–81.

Haag WR, Williams JD. 2013. Biodiversity on the Brink: An Assessment of Conservation Strategies for North American Freshwater Mussels. *Hydrobiologia*. http://dx.doi.org/10.1007/s10750-013-1524-7

Havlik ME. 1997. Are unionid mollusk translocations a viable mitigation technique? The Wolf River, Wisconsin, experience, 1992-1995. In Conservation and management of freshwater mussels II: initiatives for the future (K.S. Cummings, A.C. Buchanan, C.A. Mayer, and T.J. Naimo, eds.), p. 184–195. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Hopkins AE. 1949. Determination of condition of oysters. *Science* **110**: 567.

Hubbs CRJ, Edwards RJ, Garrett GP. 1991. An annotated checklist of the freshwater fishes of Texas, with keys to identification of species. Texas Academy of Science. Available from: http://www.texasacademyofscience.org

Jenkinson JJ. 1985. Freshwater mussel transplants evaluated. *American Malacological Union News* **16**, 3.

Kreeger DA. 1993. Seasonal patterns in utilization of dietary protein by the mussel *Mytilus trossulus*. *Marine Ecology Progress Series* **95**: 215–232.

Kreeger DA. 2005a. A holistic concept for the conservation and propagation of populations of freshwater, brackish and estuarine bivalves for ecosystem services. *Journal of Shellfish Research* **24**: 662.

Kreeger DA. 2005b. The mighty *Elliptio* and stormwater runnoff. *Estuary News* **16**: 10–11.

Kreeger DA, Langdon CJ. 1993. Effect of dietary protein content on growth of juvenile mussels, *Mytilus trossulus* (Gould 1850). *Biological Bulletin* **185**: 123–139.

Layzer JB, Gordon ME. 1993. Reintroduction of mussels into the upper Duck River, Tennessee. Conservation and management of freshwater mussels. Upper Mississippi River Conservation Committee, Rock Island, Illinois: 89–92.

Livingstone DR. 1993. Biotechnology and pollution monitoring: use of molecular biomarkers in the aquatic environment. *Journal of Chemical Technology & Biotechnology* **57**: 195–211.

Lydeard CR, Cowie RH, Ponder WF, Bogan AE, Bouchet P, Clark SA, Cummings KS, Frest TJ, Gargominy O, Herbert DG, *et al*. 2004. The global decline of non-marine mollusks. *BioScienc*e **54**: 321–330.

Master L. 1990. The imperiled status of North American aquatic animals. *Biodiversity Network News* **3**: 1–2.

Monserrat JM, Martinez PE, Geracitano LA, Amado LL, Martins CMG, Pinho GLL, Chaves IS, Ferreira-Cravo M, Ventura-Lima J, Bianchini A. 2007. Pollution biomarkers in estuarine animals: critical review and new perspectives. Comparative Biochemistry and Physiology Part C: *Toxicology & Pharmacology* **146**: 221–234.

Murphy TA, Kapustka L. 1989. Capabilities and limitations of approaches to in situ ecological evaluation. In in situ Evaluation of Biological Hazards of Environmental Pollutants. Environmental Protection Agency, Washington D.C.

Neves RJ, Bogan AE, Williams JD, Ahlstedt SA, Hatfield PW. 1997. Status of aquatic mollusks in the southeastern United States: a downward spiral of diversity. In Aquatic Fauna in Peril: The Southeastern Perspective (Benz, G.W., and D.E. Collins, eds). Special Publication **1**, Southeast Aquatic Research Institute, Lenz Design and Communications, Decatur, GA; 43–

86.

Okumus I, Stirling HP. 1998. Seasonal variations in the meat weight, condition index and biochemical composition of mussels (*Mytilus edulis*) in suspended culture in two Scottish sea lochs. *Aquaculture* **159**:249-261.

Ortmann AE. 1919. A monograph of the naiades of Pennsylvania. Part III. Systematic account of the genera and species. Memoirs of the Carnegie Museum 8:21.

Strayer DL, Downing JA, Haag WR, King TL, Layzer JB, Newton TJ, Nichols SJ. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience* **54**: 429–439.

Thomas RD, Kreeger D, Klein S, Padeletti A, Ruge Z, Gray M, Gatenby C. 2011. Occurrence of freshwater mussels (Unionids) in surveyed streams of southeastern Pennsylvania, 2000-2010. In: P. Cole and D. Kreeger (eds.), Proceedings of the Fourth Delaware Estuaries Science & Environmental Summit. Partnership for the Delaware Estuary Report #11-01. 154 pp. Willmington, DE.

USGS. 2012. Chester County Water-Quality and Bacteria Monitoring Network - USGS Water Resources of Pennsylvania. *Biology data for West Branch Brandywine Creek at Modena*. http://pa.water.usgs.gov/projects/assessments/chesco/biochemsite.php?site_id=01480617&site_name=West%20Branch%20Brandywine%20Creek%20at%20Modena. [26 July 2012]

Vaughn CC, Taylor CM. 1999. Impoundments and the decline of freshwater mussels: a case study of an extinction gradient. *Conservation Biology* **13**: 912–920.

Widdows J, Burns KA, Menon NR, Page DS, Soria S. 1990. Measurement of physiological energetics (scope for growth) and chemical contaminants in mussels (*Arca zebra*) transplanted along a contamination gradient in Bermuda. *Journal of Experimental Marine Biology and Ecology* **138**: 99–117.

Zale AV, Neves RJ. 1982. Reproductive biology of four freshwater mussel species (Mollusca: Unionidae) in Virginia. *Freshwater Invertebrate Biology* **1**: 17–28.

Zandee DI, Kluytmans JH, Zurburg W. 1980. Seasonal variations in biochemical composition of *Mytilus edulis* with reference to energy metabolism and gametogenesis. Netherlands Journal of Sea Research. *Netherlands Journal of Sea Research* **14**: 1–19.

Table 1. Number of cages of mussels deployed from two source streams into seven recipient streams (including candidate restoration streams and reciprocal controls)

Source	***Recipient Streams***						
	Brandywine	Middle Branch White Clay	East Branch White Clay	Red Clay Creek	West Branch Brandywine	Chester Creek	Ridley Creek
Brandywine	4	4	4	4	4	4	4
Ridley	4						4

Note that each cage had ≥ 15 mussels