Title

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Publication Date
2007-05-20

Chapter 4

Fisheries, Aquatic Ecosystems and Water Quality

Assessment of Freshwater Mussel Relocation as a Conservation Strategy

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Abstract: Over the last 30 years, relocation of freshwater mussels has been used as a conservation strategy for potential impacts from bridge construction and dredging operations. Improved methods have effectively increased relocated mussel survivorship rates of target species from ~ 50% to ~ 90% under ideal circumstances. Success to date is largely based upon survivorship rates without consideration of relocation activity effects upon fitness and behavioral traits of mussels.

In 2003, the Federal Highway Administration (FHWA) and Arkansas Highway and Transportation Department (AHTD) funded research to: 1) determine the success of mussel relocation efforts associated with highway construction projects by investigating survivorship, movements, mortality, fitness (as indicated by condition factor), and fecundity of relocated and non-relocated adults and sub-adults, 2) determine success of mussel propagation efforts by investigating survivorship of juveniles returned to identified habitats and used for population enhancement (recruitment), and 3) determine impacts at highway construction sites by comparing pre- and post-construction mussel assemblage abundance and composition, sediment deposition downstream of the construction, and individual mussel fitness.

This project seeks, in part, to use the data acquired in the formulation of a programmatic biological assessment/biological opinion streamlining initiative for P. capax that will be proposed to the U. S. Fish and Wildlife Service by the ATHD and FHWA. Biochemical composition (i.e. condition factor) and movement (i.e. displacement) were monitored for two species of freshwater mussels subjected to relocation activity, the federally endangered P. capax and a species with a different life history, *Quadrula quadrula* and compared with control (i.e. non relocated) populations. Trends were identified in condition factor, through repeated measures ANOVA, associated with short (glycogen), moderate (lipid), and long term energy stores (proteins, RNA:DNA ratios) sampled pre- and post-relocation. Behavioral trends (i.e. displacement) between native and relocated populations of the two species were measured in both short-term (weeks) and long-term (quarterly) intervals. Results pertaining to population enhancement strategies, specifically field methodologies used for *in-situ* rearing of juvenile *P. capax* along with growth and survival rates of field reared and lab reared individuals are presented.

Introduction

Society has placed a value, though economic value is often difficult to estimate, on appropriately functioning ecosystems and the species that inhibit those environs (Wilson and Carpenter 1999). This is evidenced by the multiple pieces of federal legislation enacted, such as the Clean Water Act (CWA), the Endangered Species Act (ESA) and the National Environmental Policy Act (NEPA). Because of these legislative actions, most activities, such as point source discharge, wetland filling, and non-point discharge are regulated and mitigation measures to buffer against unavoidable negative impacts are required. However, in many cases and especially those pertaining to endangered species, a widely accepted mitigation measure and implementation process may not have necessarily gone through a critical evaluation process. Rather, *ad hoc* responses to expected disturbances may have been established and implemented due to the nature and requirements of the authoritative legislation and the need for the given project.

One such example of this management scenario is mitigation prescribed for impacts to freshwater mussels (Mollusca: Unionoida). Freshwater mussels have gained increasing focus over the past three decades as researchers have been able to vastly expand the general knowledge base (Haag and Staton 2003; Strayer et al. 2004) and better understand the ecosystem function of these organisms (Howard and Cuffey 2006; Spooner and Vaughn 2006; Vaughn and Hakenkamp 2001; Welker and Walz 1998). Unfortunately, the impetus for this flurry of activity revolves around the fact that ~ 72% of North American freshwater mussel species have been accorded some protection designation such as endangered, threatened or special concern status (Bogan 1997; Williams et al. 1993). The causes of declines have historically been attributed to habitat degradation, commercial harvesting, and the introduction of exotic species (Anthony and Downing 2001; Bogan 1997; Williams et al. 1993). Other threats have also been identified, including landscape context (Arbuckle and Downing 2002; Poole and Downing 2004), diseases and parasites (Chittick et al. 2001), eutrophication (Patzner and Muller 2001), and host fish loss (Haag and Warren 2003). One major factor contributing to populations and species declines is the complex life history traits of these organisms, which allow freshwater mussels to be susceptible to a wide variety of environmental threats.

Anthropogenic activities linked with freshwater mussel declines include hydrologic alteration (Vaughn and Taylor 1999), sedimentation (Brim Box and Mossa 1999), incompatible land use (Arbuckle and Downing 2002; Poole and Downing 2004), eutrophication (Patzner and Muller 2001) and point source contamination (Gagne et al. 2001). The sources of these threats include conversion from forest to urban or agricultural land uses, transportation construction projects, dredging activities and mining. The unintended consequences of these activities can include in-stream habitat changes via sedimentation, increased sunlight exposure, nutrient enrichment, and hydrologic alteration.

Since 2002, the Federal Highway Administration (FHWA)-Arkansas Division, U.S. Fish and Wildlife Service Arkansas Ecological Services Field Office, U.S. Army Corps of Engineers Memphis District, Arkansas Game and Fish Commission, Arkansas Department of Environmental Quality, Arkansas Highway and Transportation Department (AHTD), and Arkansas State University have collaborated to construct and implement a local recovery strategy for the endangered fat pocketbook mussel, *Potamilus capax* (Green 1832), that is anticipated to lead to stabilizing and restoring habitat, increasing the number of reproducing and recruiting populations, and recovery of the species so that Federal Endangered Species protection is no longer required..

The fat pocketbook mussel, *Potamilus capax*, was listed as endangered throughout its native range in 1976 by the USFWS (USFWS 1989). The historic range of *P. capax* includes the upper and middle reaches of the Mississippi River and the middle and lower reaches of the Ohio River. Major tributaries to these systems where the fat pocketbook has been documented include the Wabash River in Indiana and Illinois and the lower White River of Arkansas (Cummings and Mayer 1993; USFWS 1989). Perhaps the best remaining population, however, is found in the St. Francis River Drainage of southeastern Missouri and eastern Arkansas (Ahlstedt and Jenkinson 1991). Habitat harboring populations of *P. capax* has been described as moderate to large rivers with slow moving water and mud, sand, and clay substrates (Ahlstedt and Jenkinson 1991; Cummings and Mayer 1993; USFWS 1989). More specifically in the St. Francis Drainage, this species has been found in habitats ranging from the downstream, inside vertical clay banks of meanders to mid-channel habitats with soft substrates consisting of a sand, silt and clay mixture. This species, however, appears to be more abundant in lower reaches of the main river channel and the larger drainage ditches.

Specific information needs were identified by the *Potamilus capax* work group in 2003, and in 2004, the FHWA and AHTD requested letters of interest from qualified governmental agencies, research institutions, and regional universities to conduct ecological research to address these information needs. Subsequently, a proposal was submitted by Arkansas State University (ASU) to address each of the information needs identified by the work group.

The ASU proposal included two major research components or tasks. Task I – Effects of Construction and Mitigation Efforts - included 1) determining the success of relocation efforts for *Potamilus capax* associated with highway construction projects (i.e. survivorship, movements and/or mortality, condition factor, fecundity of relocated and non-relocated adults and sub-adults, and genetic considerations for maintaining genetic diversity), 2) determining the success of propagation efforts for *P. capax* resulting from highway construction projects (i.e. survivorship of juveniles after return to field and population enhancement (recruitment)), and 3) determining impacts to *P. capax* and associated mussel community at highway construction sites (i.e. pre- and post-construction community composition, sediment deposition downstream of construction site, mussel condition factor pre- and post-construction).

Task II - Tyronza River Drainage Restoration - centered around restoring a degraded river system and included 1) determining the status of the freshwater mussel community of Tyronza River (i.e. survey for mussels, summarize existing physical and chemical data for Tyronza River, summarize existing land use patterns in Tyronza River drainage), 2) determining the suitability of Tyronza River for reestablishment of *P. capax* (i.e. toxicity to juveniles, sediment deposition rates vs. survivorship of juveniles and survivorship of trans-located adults, stream restoration and sediment reduction techniques to benefit mussels, availability of host fish for natural reproduction and recruitment, locate “preferred” sites for *P. capax* reestablishment), and 3) preparing an Ecosystem Recovery Plan for Tyronza River Drainage (i.e. target mussel community restoration, *P. capax* restoration, and fish community restoration).

A contract was issued by the AHTD in 2004 to implement Task I research. This paper focuses on results of research directed towards assessing the success of mussel relocation combined with mussel population augmentation as a mitigation technique.

Mitigation Practices

Transportation projects have produced a relatively large footprint on the North American landscape. For example, Forman and Alexander (1998) estimated 15 – 20% of the United States is influenced by road networks. These networks are necessary for social and economic reasons. However, they do cause habitat fragmentation and degradation of both terrestrial and aquatic systems (Forman and Alexander 1998; Jones et al. 2000; Trombulak and Frissell 2000; Wheeler, Angermeier, and Rosenberger 2005). Understanding the expected ecological consequences of transportation development can potentially provide critical information to aid in the conservation and restoration of threatened ecological systems and biota. Mitigation techniques, such as wetland mitigation banking and stream restoration (i.e. bio-engineering) to counteract the negative aspects of development, have recently begun to gain acceptance in both the political and science arenas (Bonnie 1999; Fox and Nino-Murcia 2005; Green and O’Connor 2001). Though these techniques may not completely negate the impacts of development and policies governing their implementation are still being developed, the application of techniques at the interface between ecosystems and development are quite promising (Fox and Nino-Murcia 2005; Mills 2004).

Some states require impact mitigation when specifically dealing with freshwater mussels and bridge construction. The U. S. Fish and Wildlife Service almost always requires mitigation when an endangered or threatened mussel is present. Typically, impact mitigation is achieved through relocation of mussel aggregations within the estimated construction footprint and a larger zone of potential impact. This entails the total removal of individuals within a pre-defined area at the construction site, and the subsequent relocation to suitable safe habitat within the same stream or a nearby stream within the same drainage. This practice has received the scrutiny of scientific research (Cope and Waller 1995; Dunn, Sietman, and Kelner 1999; Hamilton, Brim Box, and Dorazio 1997; Newton et al. 2001) though the efficiency and efficacy of the practice is still in question, due to observed mortality rates. In Arkansas, the success of relocations is unknown because, in many instances, relocated individuals have not been found in follow-up surveys, thus their fate (live or dead) is unknown (J. Harris, personal communication).

Relocation Analysis

Relocating mussels from areas of perceived threats to suitable safe habitat has been occurring in the United States for more than 30 years (Cope and Waller 1995). Relocation is a logical response to policy and legislative authorities implemented at the federal level and resource management objectives applied at the state and local levels. However, evaluations of relocation as a viable technique did not begin to appear in the literature until the mid 1990s. In one of the first such efforts, Cope and Waller (1995), reviewed 33 papers and/or reports discussing relocation activities and found that nearly 90,000 mussels had been relocated during 37 projects. The impetus for 43% of these relocations was construction or dredging activities in proximity to endangered species, which invoked statutes of the Endangered Species Act of 1973 (ESA). The remaining projects were associated with management objectives such as population enhancement, protection from invasive species, and research efforts.

Their initial review estimated that the survival rate for relocated animals was ~50%, but more accurate estimates were not possible due to inadequate post-relocation monitoring efforts in 78% of the cases. The effectiveness of relocation was left in question due to inconsistent reporting, lack of long-term monitoring, and difficulty in recapturing relocated animals. More specifically, the authors pointed out that 30% of reports documented mortality rates >70% and a mean mortality rate of 49% (based on a 43% recovery rate). The authors also noted that half of the reported relocation efforts occurred in the southern and southeastern United States from July through September. Furthermore, the authors noted that the factors influencing survival rates were not well understood and recommended a research agenda that included improved habitat characterization of relocation sites, better defined test methods, increased duration of post-relocation monitoring, and greater publication of relocation projects.

Another technique often employed by resource managers is the captive propagation of juvenile mussels to augment impacted aggregates (Neves 1997). Propagation involves a multi-step process from collecting gravid target species females to placing the propagated juveniles at the impacted (Barnhart 2003, 2004, 2005; Barnhart and Roberts 1997, 1997). Briefly, gravid females are collected in the field and transported to a propagation facility. Larval mussels are non-lethally removed from the marsupia of the gravid female and exposed to suitable host fish for encystment via either direct or indirect techniques. Exposed host fish are held in tanks modified for the collection of excysted larvae for several weeks until glochidia have had time to develop on the host fish and are ready to excyst (Barnhart 2005). Excysted larval mussels are collected from the collection tanks periodically and held in a culturing tank until release into the field. Costs for such mitigation activities can swell into the many of thousands of dollars for relocation alone and can, in some cases, substantially delay the construction permit process, ultimately increasing construction costs and time to completion.

Methods

Two mussel species, *Potamilus capax* and *Quadrula quadrula*, exhibiting contrasting life history traits (Subfamily Lampsilinae versus Subfamily Ambleminae), were used for the relocation study that was conducted in the Mississippi Delta Ecoregion of eastern Arkansas. Much of this region is included in the St. Francis River Watershed, and land use is generally characterized by row crop agriculture, with the primary products being rice, soybeans, and cotton. Because of the negligible topographic relief in the region, many of the stream systems have been dredged and channelized to facilitate hydraulic conveyance. The resulting drainage ditches are maintained through dredging activities managed by local water management districts and the Army Corps of Engineers. However, several of these ditches also harbor the endangered mussel species *P. capax*, as well as representative large river species. Due to the presence of *P. capax*, management activities, such as dredging and highway bridge and culvert construction, require mitigation that has been historically achieved through relocation.

Ditch 10, west of Truman, Poinsett County, Arkansas, was scheduled for dredge maintenance during late Summer 2005 and harbored a known population of *P. capax* and several associated Mississippi Delta mussel species, including *Q. quadrula* (figure 1). A similar dredging operation in 2003 on Stateline Ditch north of Dell, Mississippi County, Arkansas revealed a similar species composition and harbored large numbers of *P. capax* (J. Harris, unpublished data). Both drainage systems facilitate agricultural land use, with the main crops consisting of cotton and soybeans, and they are managed for water evacuation. Based on the similarity of the two systems and the presence of large *P. capax* populations in Stateline Ditch, it was determined by the Army Corp of Engineers and the U.S. Fish and Wildlife Service that mussels found in the footprint of the Ditch 10 dredge operation would be relocated into a reach of Stateline Ditch (subsequently named Stateline Ditch Experimental Reach). One of the deciding factors in this decision was that this reach of Stateline Ditch had recently (2003) undergone maintenance dredging and represented a safe harbor against being disturbed in the foreseeable future. Relocation activities were completed by Ecological Specialists, Inc. in the Spring 2005, using previously described methods (Cope and Waller 1995; Dunn, Sietman, and Kelner 1999).

Upon collection of *P. capax* and *Q. quadrula* individuals at the impact site (Ditch 10), standard measurements including length, width, depth, and wet mass were recorded in the field. A unique identification number was etched into each mussel shell using a handheld rotary drill, and a passive integrated transmitter (PIT) tag (Germano and Williams 1993; MacGregor and Reinert 2001) was attached to the dorsal posterior margin near the hinge and umbo using a waterproof epoxy. The location of PIT tag placement was selected based on the size of the tag and the desire to not impede mobility, feeding, or respiration. Relocation activities for Ditch 10 animals began in April 2005 and continued through May 2005, and individuals were corralled until their transfer to Stateline Ditch Experimental Reach.

Following transit to the relocation site (Stateline Ditch Experimental Reach), the mussels were placed into one of two 5 m x 5 m grid systems constructed in the stream. These grids were divided into 1-m^2 cells with a rebar pin placed in the center and the relocated mussels were placed against the pin. Each cell was given a unique number and the individual mussels placed within each cell were recorded. GPS coordinates were collected for each pin, and the grid system allowed for free movement into and out of the system. Due to low numbers of P. capax collected at Ditch 10 (n = 18), other sites were searched for individuals and treated similarly. One additional specimen was relocated from the St. Francis River at Parkin, Cross County, Arkansas, and 11 specimens were relocated from a reach of Stateline Ditch substantially downstream (> 5.0 mi) of the relocation study site in October 2005.

Figure 1. *Potamilus capax* distribution within Arkansas and identification of study reaches. Solid black circles represent distribution records prior to 1986, and solid black squares represent distribution records acquired 1986-1996. Black dashed ovals indicate relocation study areas with the southern oval representing Ditch 10 (evacuation reach) and the northern most red oval representing Stateline Ditch (receiving reach).

Condition Factor Sampling and Analysis

Monitoring events were conducted in August 2005, October 2005, and June 2006. The first step was to visit the relocation grids and search for relocated mussels by hand. When a mussel was located, a buoy containing the individual ID number written in wax was used to mark the location until the mussel could be processed, thus minimizing emersion time of individuals (Greseth et al. 2003). Mussels were removed from the substrate, processed, and returned to their capture location that was recorded using a Trimble GPS unit.

Following the grid search, the entire relocation reach (~300 m) was searched using hand techniques. The search began at the downstream extent of the reach and proceeded by systematically moving bank-to-bank and upstream. Native, naïve (i.e. never being collected before) *P. capax* and *Q. quadrula* individuals encountered through this effort were measured, etched, and affixed with a PIT tag. Capture location of the individual was obtained via GPS and recorded. Upon initial capture and each successive recapture, a 50 mg mantle tissue snip was taken from each individual, placed on dry ice in the field, and stored in a -80o C freezer until analysis.

In the laboratory, tissues were processed for condition factor analysis focused on two macro-molecules assays: glycogen and lipids. Each 50 mg mantle tissue sample was portioned into 10 mg (± 2 mg) sub-samples and collection data, animal identification, and sub-sample mass for each portion was recorded. Tissue portions were stored in a -80o C freezer. Glycogen concentration (mg glycogen per g tissue mass) analysis was conducted according to Naimo *et al.* (1998) with aliquots reserved for future analysis, if necessary. Lipid concentration (mg lipids per mg tissue) analysis followed van Handel (1985), except for the addition of a 1 hr sonication step to facilitate lipid extraction from whole tissue sub-samples. A portion of the lipid extraction liquid was used for analysis.

Statistical analysis for condition factor consists of repeated measures ANOVA, as the goal is to sample multiple individuals from each treatment group on multiple occasions. Species, treatment group, and season serve as effects and the appropriate condition factor indicators serve as analytical response variables.

Movement Study

Because recapture rates using PIT tags were not efficient due to the unexpected movement distances of *P. capax*, radio telemetry techniques were implemented to monitor movement patterns of *P. capax* following relocation. Transmitters, specifically designed to match the water quality conditions (primarily conductivity) at Stateline Ditch and the shell sculpturing of *P. capax*, were used to track a sub-set of both native and relocated *P. capax*. Transmitter battery life was limited to 3 months due to size and signal strength requirements. Transmitters were placed on the dorsal posterior shell margin so transmitters would not interfere with feeding or vertical and/or horizontal movement. Two sampling periods have been completed and include October 2005 - January 2006 and July - October 2006. Transmitters were affixed to equal numbers of resident and relocated animals and positions were recorded with GPS. Locations of mussels carrying transmitters were monitored bi-weekly to monthly. Displacement was measured using GIS (ESRI 2002). Upon confirmation of identification, mussels were immediately placed back to the point of capture; neither size measurements nor tissue snips were collected upon recapture during these trials.

Statistical analysis of displacement was conducted in two forms. First, for individuals fitted with transmitters, repeated measures ANOVA served to analyze short-term movement patterns both between and within transmitter monitoring periods. A second repeated measures ANOVA compared long-term movement patterns of those individuals not fitted with a transmitter with the effects of species, treatment group, and season associated with monitoring events. In both cases, total displacement was used as the response.

In-situ Juvenile Rearing

In order to assess the effectiveness of propagation efforts, four gravid *P. capax* females were collected from Ditch 10, transported to the lab facility, and following the propagation procedure in April 2005, they were relocated to the Stateline Ditch Experimental Reach in July 2005. Juvenile *P. capax* propagated in the lab facility were reared using a bucket grow out method (Barnhart 2005) for several months prior to placement into either constructed cages or *in-situ*.

From this propagation effort, three groups of juveniles were exposed to 2 different rearing treatments: in laboratory bucket rearing (1 group) and *in-situ* cage rearing (2 groups). In September 2005, the first treatment of cage-reared juveniles (n = 400) was placed in Stateline Ditch Experimental Reach in a cage constructed of 2 in. x 6 in. untreated lumber with a screen top enclosure and then filled with surrounding sediments. An additional 1600 juveniles from the propagation effort were placed in Stateline Ditch Experimental Reach *in-situ* with locations referenced with GPS coordinates. In December 2005, another treatment of caged reared juveniles (n = 500) was placed into a second cage with a solid bottom and welded frame covered with screen, and this cage was also filled with surrounding sediment. At this time, an additional 200 juveniles were placed *in-situ* of the State Line Ditch Experimental Reach. In June 2006, the third group of juveniles (n = 129), entirely bucket reared, were measured and released in-situ at the State Line Ditch Experimental Reach. Also in June 2006, the sediments from both grow out cages were sieved and individuals were counted and measured to determine survival and growth of cage-grow out juveniles. Individuals from each group were analyzed via ANOVA using mean length and survival as the factors and the release date as the treatment groups.

Results

Condition Factor

Initial results of the condition factor, movement, and *in-situ* rearing are providing insight to effects of relocation on freshwater mussels. The repeated measures ANOVA for glycogen concentration (mg/g) showed no significant differences between or within any of the factors (F = 0.31; df = 3, 12; p = 0.33). The native, naïve treatment groups for both *P. capax* and *Q. quadrula* show a decreasing trend in glycogen stores through time, but the remaining treatment groups of native, recapture, and relocated are consistent through time (figure 2). Glycogen stores at any one time ranged between 0.08 mg/g to 9.80 mg/l in *P. capax* samples and 0.30 mg/g and 18.07 mg/g in *Q. quadrula*.

Lipid concentrations (mg/g) were analyzed using repeated measures ANOVA and provide greater insight to energy storage. The overall model for lipid concentration was significant (F = 3.02; df = 3, 10, p = 0.002) with a significant species difference (F = 1.01; df = 1, 10; p = 0.01). The temporal trend between the study species show lipid stores at Time 1 (August 2005) are similar between the species but as time progressed *Q. quadrula* increased at a greater rate and to higher concentrations than *P. capax* (figure 3). The temporal trend of lipid concentrations for native individuals of both species showed an increasing trend from Time 1 to Time 2 (October 2005), and a stable energy reserve from Time 2 to Time 3 (June 2006). The trend for relocated individuals indicated a stable lipid concentration between Time 1 and Time 2 followed by an increase from Time 2 to Time 3. The overall trend for all specimens was relative stability between Time 1 and Time 2 with an increasing lipid concentration between Time 2 and Time 3.

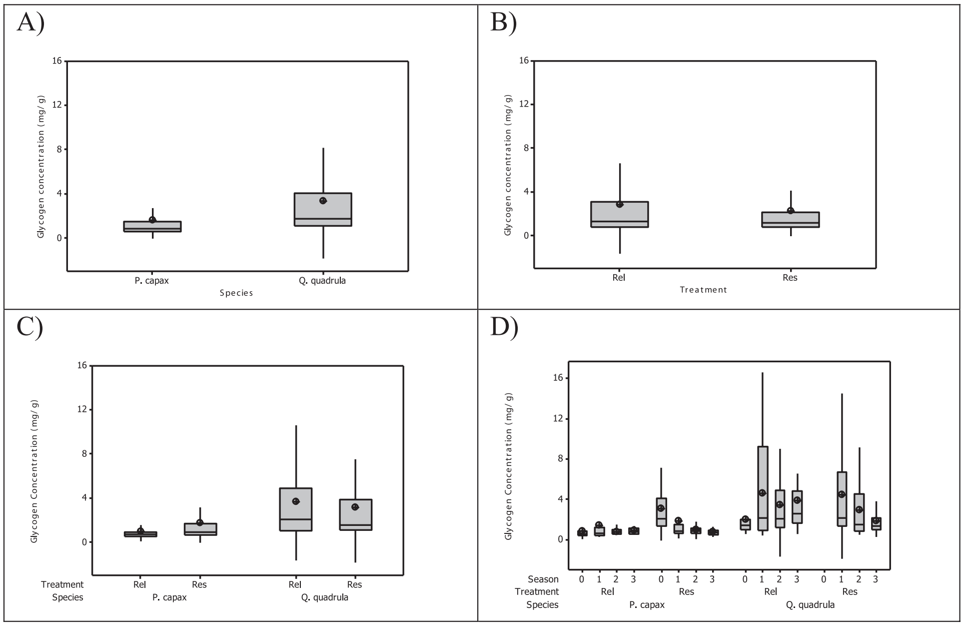

Figure 2. Box and whisker plots of glycogen concentration data with shaded areas representing interquartile range (25% - 75%), lower whiskers represent lowest values (Quartile 1 – 1.5), upper whiskers represent highest values (Quartile 1 + 1.5), horizontal bars within the shaded box represents the median value, stars represent the mean value. Panels represent glycogen concentration by A) species, B) treatment, C) species and treatment, D) species, treatment, and sample period/season after relocation (1 = August 2005 2 = October 2005 3 = June 2006).

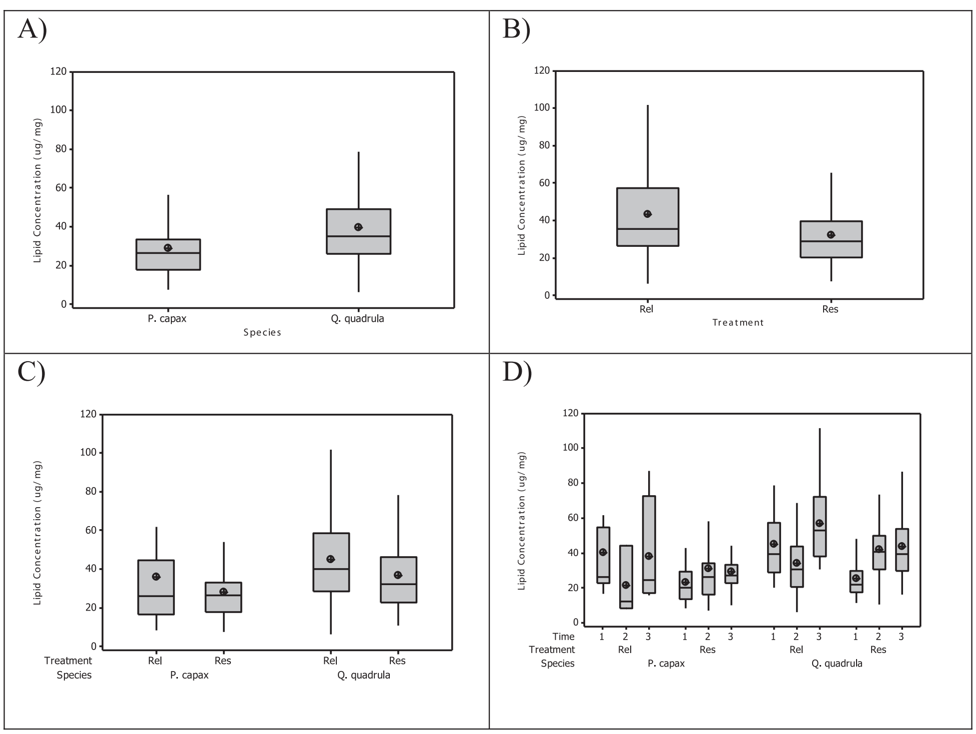

Figure 3. Box and whisker plots of lipid concentration with shaded areas representing interquartile range (25% - 75%), lower whiskers represent lowest values (Quartile 1 – 1.5), upper whiskers represent highest values (Quartile 1 + 1.5), horizontal bars within the shaded box represents the median, stars represent the mean value. Panels represent lipid concentration by A) species, B) treatment, C) species and treatment, D) species, treatment, and sample period after relocation (1 = August 2005 2 = October 2005 3 = June 2006).

Movement

Total displacement during two short-term monitoring periods, utilizing only *P. capax* individuals, showed no significant treatment group differences. Overall, individuals from both treatment groups tended to move greater distances immediately following initial handling (figure 4). However, as the study progressed, the displacement distances decreased dramatically toward the end of both trials. Displacement distances ranged from 0 m to 27 m over the course of both three month trials.

Long term displacement measured from location of initial capture to location of last recapture over the course of all four intensive sampling periods provide greater insight to movement patterns. This data set was analyzed with a 3-way ANOVA, with the overall model being significant (F = 8.68; df = 3, 107; p = < 0.0001). Species differences were significant with P. capax having an average displacement of 19.5 m (± SD 25.4 m) and Q. quadrula having an average displacement of 3.8 m (± SD 5.4 m; p = 0.016). The range of P. capax displacement was 0.8 m to 151.9 m. while the range of Q. quadrula displacement was 0.1 m to 30.8 m. Interestingly, resident individuals had significantly (p = 0.043) greater displacement distances than relocated individuals (figure 5).

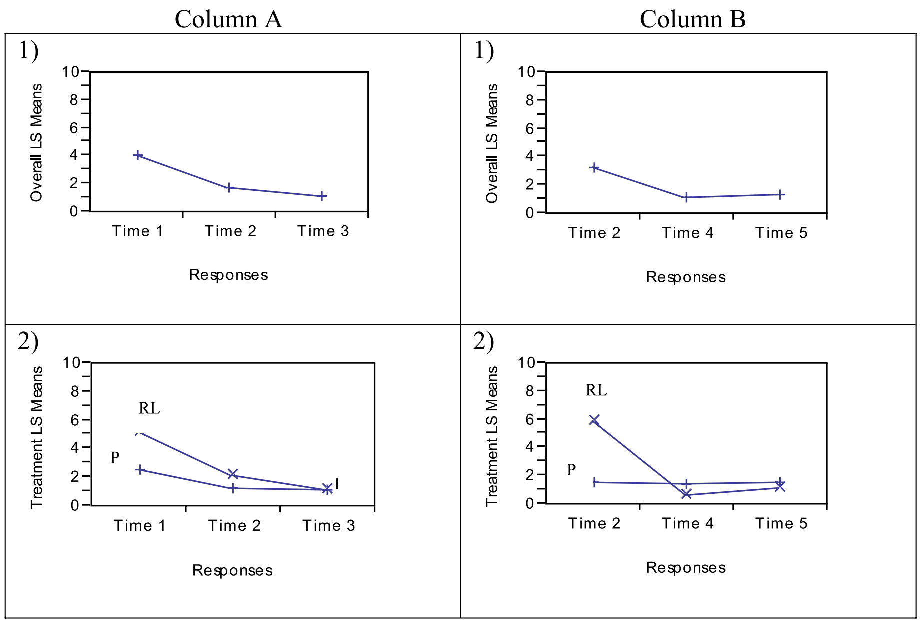

Figure 4. MANOVA repeated measures results of short-term (3 month) monitoring associated with total displacement (m) of *P. capax* by treatment (P = native animals, RL = relocated animals). Column A shows overall model results (1), treatment effects (2), and statistical analysis (3) of the first trial (October 2005 to January 2006). Column B shows identical information associated with the second monitoring period (July to September 2006); total displacement from Time 1 and Time 3 were omitted due to insufficient sample sizes. The Y-axis represents the response variable Treatment Least Squares (LS) Mean in m.

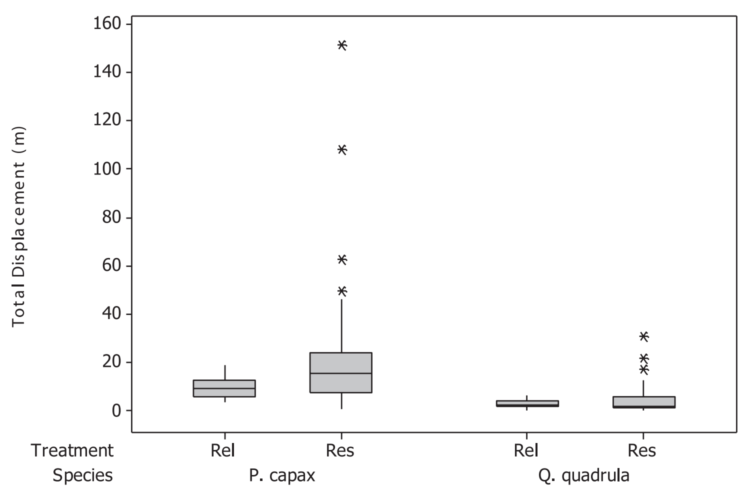

Figure 5. Boxplot of mean total displacement (August 2005 to June 2006) between species and treatment groups monitored for long-term movement study; boxplots as previously stated and asterisks representing statistical outliers.

Juvenile In-situ Rearing

The overall 1-way ANOVA model of P. capax juvenile growth in relation to rearing treatment was significant (F= 65.49; df = 2, 517; p = <0.0001). Even though all individuals were propagated in April 2005, and all measurements were obtained in June 2006, the Tukey's Honest Significant Difference (HSD) post-test showed that the September 2005 and January 2006 cage-reared cohorts were not significantly different from each other, both were significantly larger than the lab reared cohort. The average length of the group placed in the cage in September 2005 was 6.55 mm (± SD 3.82 mm, n = 18) with a 4.5% survival rate. The average length of the December 2006 caged reared group was 7.14 mm (± SD 2.05 mm, n = 371) with a 74.2% survival rate. The bucket-reared group had an average length of 4.83 mm (± 1.23 mm, n = 129; figure 6), survival rates of this group are unknown.

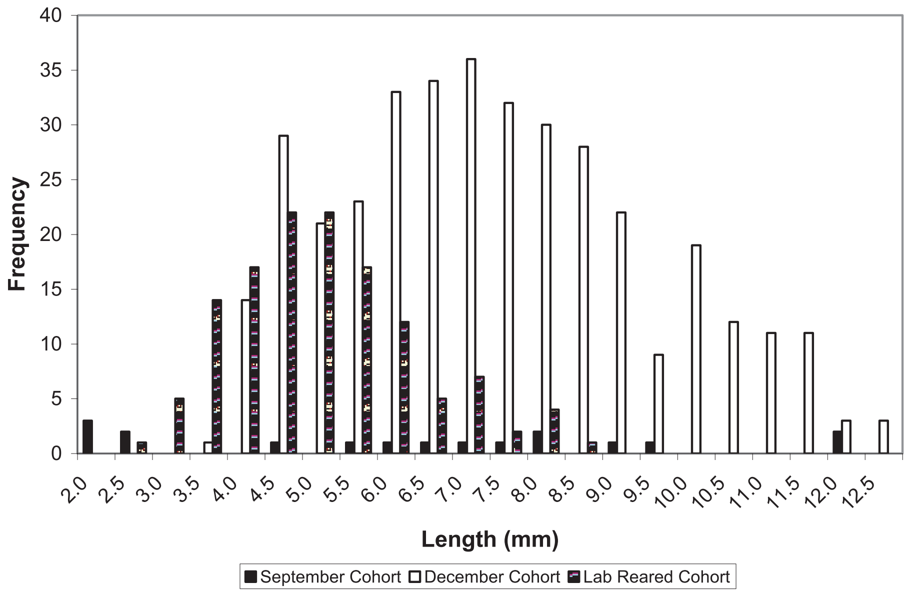

Figure 6. A bar graph depicting the frequency and distribution of measured shell lengths (mm) of the juveniles propagated in April 2005. Solid black bars represent the cohort placed in-situ September 2005 (n = 18); solid, unfilled bars represent the cohort released in December 2006 (n = 371); and the cross-hatched bars represent the laboratory reared cohort (n = 129).

Discussion

The results to date for the examination of freshwater mussel relocation as a viable mitigation method are promising on many levels. First, condition factor testing shows that repeated monitoring via non-lethal tissue samples is possible as no mortality has been detected as direct result of the relocation or tissue sampling. Most fatalities have been attributed to predation based on small mammal teeth marks on shells or midden (dead shell) piles surrounded by tracks. However, shell deformities have been observed, with greater deformities in *Q. quadrula* shell shape than in *P. capax (A. Peck, personal observation)*.

Lipid analysis appears to show the most promise for molecular testing on mussels, though other macro-molecules and methods are currently being tested and developed as part of this study. Glycogen concentration of the mantle tissue has not shown significant differences among treatments. This is interesting, as the original hypothesis was that glycogen concentrations would be a good predictor of intermediate effects of stress and that lipids would show the longer-term effects of stress.

Because the test species exhibit very different life history characteristics (e.g. brooding period and duration, movement behavior), significant species level differences were expected. Both the condition factor and movement data support this hypothesis. The causation of differences in molecular concentration will be difficult to pinpoint until better information regarding mussel diets, storage locations, and energy use are produced. One viable explanation, however, may be attributable to the difference in food types between the evacuation site and the relocation site. For example, Silverman et al. (1995; 1997)showed different species of mussels filtered different size particles. On the other hand, stable isotope studies have suggested that different species of freshwater mussels are feeding on the same food resource within a given system (Christian et al. 2004; Nichols and Darling 2000). Food selection was not tested in the present study, therefore there is no data to tease out potential differences in food and feeding (Christian et al. 2004; Nichols and Darling 2000).

The results of movement analysis indicate that relocation of more mobile species, like *P. capax*, may result in significant displacement patterns. This suggests that monitoring of the relocation reach areas should be adjusted to include a larger area than the initial relocation area to ensure recapture of highly mobile individuals.

Finally, *in-situ* rearing appears to not only be possible, but a viable and potentially a more biologically beneficial alternative (or supplement) to lab rearing based on the greater growth in the cage-reared group. Furthermore, appropriately designed cages increase the overall survival and growth rates of field reared individuals.

Data collection for this project continues, but the results analyzed to date related to survival, growth, fitness, and movement suggest that revised recommendations will be forthcoming regarding best management practices for mussel mitigation. The intensive relocation study will be continued in Summer 2007 and will include a modified short-term displacement study using radio telemetry. The bridge construction impact study, not addressed in this paper, where mussels were left in place during the construction period will continue. One pre-construction sample and one during construction sample have been collected, and two more sampling efforts will be conducted to address potential post-construction impacts and recovery. Finally, the policy evaluation will use all of the research results to develop an initial decision framework that will better incorporate site conditions, mussel community attributes, and construction methods into mitigation method selection. A final product reporting the findings associated with this research is expected by 2009.

Acknowledgements: Funding for the project was provided by two grants: a Federal Highway Administration and Arkansas Highway and Transportation Department grant (AHTD Job 001983) to ADC and JLF and a U.S. Fish and Wildlife Service grant (1448-40181-06-XXX) to AJP, ADC, and JLF. Assistance for the project was provided by personnel of the Arkansas Highway and Transportation Department, Arkansas Game and Fish Commission, Ecological Specialist, Inc, U.S. Army Corps of Engineers – Memphis District, and U.S. Fish and Wildlife Service – Arkansas Field Office. We thank R. Reed for assistance with the P. capax distributional map and M. Barnhart at Missouri State University for assistance in propagating juveniles. We also thank Josh Seagraves (AHTD), Leighann Gipson (US Army Corps of Engineers), members of the ASU Aquatic Ecology Lab, Bill Posey (AR Game and Fish Commission), Chris Davidson (US Fish and Wildlife Service), and Dr. Jeanette Loutsch (ASU Biology Department)for field and laboratory assistance.

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