CONSERVATION AND MANAGEMENT OF FRESHWATER MUSSELS (BIVALVIA: UNIONIDAE): REPRODUCTION, NON-INVASIVE TECHNIQUES AND RELOCATION

A Dissertation

by

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ABSTRACT

Freshwater mussels (Bivalvia: Unionidae) represent one of the most endangered groups of aquatic organisms worldwide, yet efforts to mitigate the endangerment of this group are being outpaced by the rapid decline of species diversity. In this dissertation, I report on advancements to several methods used in conservation of freshwater mussels. First, I validated a non-lethal syringe technique used to quantify gamete production by extracting fluid from gonads. I specifically tested: (i) if gamete traits (sperm concentration, egg size and egg concentration) measured using the syringe technique were correlated with gamete traits measured using a histological technique; and (ii) if survival, growth and body condition (Fulton's K index) were affected by the syringe technique in a two-year mark-recapture field experiment. Gamete production measured over the first year of the study indicated that gamete estimates were positively correlated among techniques, and overall, the syringe technique had no discernible effect on survival probability, shell growth and Fulton's K index of mussels. Being both accurate and noninvasive, this technique can now be used to study the reproductive biology of threatened and endangered mussels quantitatively. Second, I reciprocally transplanted mussel populations within the same river and tested: (i) whether individual and population traits (i.e., survival probability, shell growth and reproduction) successfully acclimated to novel environments, and (i) which environmental conditions best explained seasonal variability in mussel performance? Mussels generally acclimated to the conditions of the sites such that performance was not greatly diminished, but the

minor effects that were observed, which originated from environmental and genotypic interactions, suggested some degree of local adaptation was apparent. Cumulative degree days, chlorophyll *a* and benthic organic matter were among the most import variables explaining trends in survival, growth and body condition; while, cumulative degree days, chlorophyll *a* and historical discharge were important in explaining gametogenic periodicity. Although mussels responded positively to relocation, my results suggest that resource managers should minimize geographic distances and ecological differences between sites to avoid relocating mussels to populations where variation in demographic phenotypes might hinder relocation success. Future research should investigate the roles of phenotypic variation and habitat quality in driving performance of relocated populations and, ultimately, success of mussel relocations.

To my parents,

Charles and Nancy K. Tsakiris,

And brothers,

Chuck and Jeff

To Farmingdale,

And all my friends present, past and beyond

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CHAPTER I

INTRODUCTION: THE STATE OF FRESHWATER MUSSEL CONSERVATION

Freshwater ecosystems are experiencing rapid species declines on a global scale (Dudgeon et al. 2006). One of the most endangered groups of freshwater organisms are mussels of the family Unionidae (Bivalvia). Species of freshwater mussels are in decline worldwide (Lydeard et al. 2004), and in North America, where mussels reach their highest diversity, more than two-thirds of the approximately 300 species are considered threatened, endangered or of special concern (Williams et al. 1993, Strayer et al. 2004). Threats to mussels have largely originated from anthropogenic factors, especially through habitat destruction, hydrologic change and extirpation of host fishes (Bogan 1993). Consequently, loss of mussels could further exacerbate ongoing changes to ecosystem function by eliminating the important roles they play in riverine environments. For example, through filter feeding and deposition of feces and pseudo-feces, mussels alter nutrient dynamics and enhance trophic interactions in aquatic and even semi-terrestrial communities (Spooner and Vaughn 2006, Vaughn 2010, Allen et al. 2012). Mussels also increase substrate stability and habitat heterogeneity where they occur in dense, species-rich assemblages (Vaughn and Hakenkamp 2001).

Freshwater mussels are long-lived, sedentary and have a unique life cycle. They are iteroparous and typically gonochoristic, though some species of mussel are known to exhibit sequential hermaphroditism (Coe 1943, Morton 1991, Henley 2002). Unlike in

other groups of bivalves, fertilization is internal in unionid mussels and takes place in the marsupial demibranches (modified gills) of females (Ortmann, 1911) that capture sperm shed directly into the water column by males (Matteson 1948). Fertilized eggs then develop into glochidia larvae that are obligate ectoparasites on the gills or skin of fishes (Matteson 1948). Glochidia may parasitize a host for days to months until they metamorphose into juveniles and drop from the host to settle onto the substrate (Neves and Widlak 1987). Perhaps the most important yet understudied stage within the mussel life cycle is the development and production of sperm and eggs (i.e., gametogenesis), which generally occurs year-round and peaks prior to spawning (Zale and Neves 1982, Haggerty et al. 1995, Smith et al. 2003).

In response to the catastrophic declines of populations throughout the United States, conservation efforts for freshwater mussels have expanded in recent decades (Haag and Williams 2014). In 1997, the National Strategy for the Conservation of Native Mussels was developed to improve conservation and restoration of endangered species of mussels (NNMCC 1997). Among the areas of research identified as deficient included knowledge of basic mussel biology, efficiency and guidance of management methods (e.g., relocation) and linking mussel responses to anthropogenic impacts. Despite these concerns outlined over 15 years ago and corresponding increase of published studies related to mussel ecology and conservation since the mid-twentieth century (Strayer et al. 2004, Lopes-Lima et al. 2014), population declines continue today and gaps in our fundamental knowledge of mussel biology persist (Strayer et al. 2004, Strayer and Dudgeon 2010, Haag and Williams 2014). Efforts to manage and protect freshwater

mussels will therefore be important in future decades, but there is a desperate need to address the disparity between conservation efforts and successful conservation of species and their populations. Advancements in conservation strategies are vital not only to the sound efforts aimed to monitor and maintain viable populations, but also for the assessment of impacts to mussels in changing environments (e.g., climate change).

Texas harbors approximately 52 species of freshwater mussels and, similar to other regions in the United States, populations of mussel have dwindled in recent decades as evidence from reduction in historical ranges (Howells et al. 1996, Howells et al. 1997). The imperilment of mussels in Texas has led to the listing of 15 species as state-threatened (Texas Register 35 2010), including six that are currently considered for federal protection under the Endangered Species Act (Federal Register 76 2011). Mussel conservation has now become a priority in Texas and represents one of the few regions of the United States in which the mussel faunas have been poorly studied. Although important efforts have laid the foundation for the current knowledge of mussels in Texas (Strecker 1931, Singley 1982, Howells et al. 1996, Howells 2006), relatively little is known about the biology of many endemic species, which renders conservation efforts challenging and difficult to implement. Furthermore, experience with mussel conservation in Texas is limited and few have experimented with how Texas mussels might respond to conservation techniques.

This dissertation reports advancements in freshwater mussel conservation, with particular emphasis on improving the methods needed to conserve threatened species. In the first of two studies, I explore the effectiveness of a non-lethal method used to

quantify gamete production (Chapter II). Studies on the reproductive biology of mussels have played an important role in the conservation of this group (Downing et al. 1989, Mcivor and Aldridge 2007, Haag 2013), but given the complexity of the unionid life cycle and diversity of mussels, studies on reproduction are relatively scarce, particularly studies on early reproductive stages (e.g., Haggerty and Garner 2000, Galbraith and Vaughn 2009). By validating this non-lethal technique, conservationists can use it to investigate the reproductive cycle of endangered species, and researchers could use it obtain large sample sizes often needed to explore the reproductive ecology of mussels, as I show in the subsequent chapter.

In Chapter III, I examined how relocation as a conservation strategy affects individual and population performance. Relocation as a strategy for mussel conservation was historically met with challenges because of the high mortality that occurs from transplanting mussels between different populations (Cope and Waller 1995). Despite some methodological improvements to relocation practices over the years (Dunn et al. 1999, Cope et al. 2003, Peck 2010), relatively few studies have adequately investigated whether relocation has negative effects on mussel performance nor has any study adequately investigated how individuals and populations respond to novel environments when relocated for conservation purposes. I use reciprocal transplant experimentation to examine how mussels respond to relocation and look to life history theories to ascertain how mussels perform when acclimating to novel environments. To accomplish this study, I used mark-recapture and regression techniques to model environmental characteristics that best explain variability in mussel performance in two common

(*Amblema plicata*, threeridge; and *Quadrula apiculata*, southern mapleleaf) and two threatened species of mussels (*Quadrula houstonensis*, smooth pimpleback; and *Quadrula petrina*, Texas pimpleback) endemic to Texas rivers. Finally, I outline the major findings of this study in the conclusions of this dissertation (Chapter IV), where I also discuss future research directions that might improve mussel relocations.

CHAPTER II

EFFECTIVENESS OF A NON-LETHAL METHOD TO QUANTIFY GAMETE PRODUCTION IN FRESHWATER MUSSELS

INTRODUCTION

In North America, freshwater mussel (Bivalvia: Unionidae) conservation has rapidly expanded over recent decades in an attempt to reduce population declines of threatened species (Williams et al. 1993, Haag and Williams 2014). The study of mussel reproductive biology has contributed greatly toward such efforts, particularly for analyzing population structure and understanding mussel life histories (Downing et al. 1989, Mcivor and Aldridge 2007, Haag 2013). However, methods used to study early stages of mussel reproduction have relied primarily on lethal preservation of specimens for laboratory dissection; e.g., studies have analyzed sex ratios (Morton 1991), gametogenic periodicity (Haggerty and Garner 2000) and hermaphroditism (Downing et al. 1989) by creating histological thin-sections of gonad tissue. Histological methods are commonly used and preferred because they illuminate reproductive development at the cellular level, and although lethal, histology has played an important role in our understanding of mussel reproduction for over a century (e.g., Lefevre and Curtis 1910). However, the level of detail gained from histological analysis of gonad tissues is not necessarily needed (e.g., quantifying non-gamete germline cells) to elucidate important aspects of mussel reproduction (Henley 2002), and sacrificing live mussels is not always a viable option, especially for threatened and endangered species.

A non-lethal method that involves the use of a hypodermic syringe needle to extract fluid from gonads (hereafter, "syringe technique") was used previously to evaluate various reproductive traits of mussels; e.g., Bauer (1987) identified the sex of mussels of non-sexually dimorphic species by confirming the presence of male (spermatozoa) or female (oocytes) gametes in gonadal fluid extracted using a syringe. Christian et al. (2000) used this technique to extract gametes from mussels (although post-mortem) to determine age of maturation, Shiver (2002) used this method to qualitatively assess reproductive timing of mussels, and Henley (2002) developed a protocol using this technique to determine sex, hermaphroditism and gametogenic stage. In a laboratory experiment, Saha and Layzer (2008) validated the lethality and accuracy of the syringe technique but only for determining sex in non-sexually dimorphic species and gametogenic stage qualitatively. Galbraith and Vaughn (2009) later used this method to quantitatively assess factors influencing timing and rate of gamete production, which represents the first and only attempt to use the syringe technique in a quantitative fashion. Finally, others have used this technique to examine the prevalence of hermaphroditism and parasitism of mussels in disturbed habitats (e.g., Moles and Layzer 2008, Galbraith and Vaughn 2011).

Use of the syringe technique by mussel researchers has increased because it has several important management and conservation implications. The method can be used to rapidly assess sex or reproductive condition (Henley 2002), allows for large sample sizes (Galbraith and Vaughn 2009) and limits impacts to mussels (Saha and Layzer 2008), while still providing the information necessary for conservation and management.

While studies have concluded this technique can provide accurate representation of sex and gametogenic stage without increasing mortality under laboratory conditions (Henley 2002, Saha and Layzer 2008), the syringe technique was not properly validated for its use to quantitatively assess gamete production in mussels (Galbraith and Vaughn 2009), nor was it tested for its lethal and sublethal affects (e.g., on growth) to mussels under natural conditions. The goal of this chapter is to evaluate the effectiveness of the non-lethal syringe technique used to quantitatively assess gamete production in freshwater mussels. I specifically investigate whether gamete production measured using the syringe technique is positively correlated with gamete production measured using the traditional histological technique, and if survival, growth and body condition of mussels is affected by the syringe technique in natural populations.

METHODS

Study sites

I established three sites in two western Gulf Coast Rivers in south-central USA to validate the syringe technique. Two sites were selected in the lower San Saba River, a tributary of the Colorado River, Texas and one site in the Navasota River, a tributary of the Brazos River, Texas (Figure 1). The San Saba River is located on the Edwards Plateau and surrounded by the Montane ecoregion with uplands of limestone bedrock, relatively little soil cover and semiarid to subtropical-subhumid climate (Blum et al. 1994). This river is relatively high gradient, resulting in long periods of low flow and short, high-magnitude flows during heavy rainfall (Blum et al. 1994). In contrast, the Navasota River occurs in the Southern Post Oak Savanna ecoregion, characterized by

alluvial sediments from sandy loams to clay and subtropical-subhumid climate (Clark, 1973). During periods of heavy rainfall, the Navasota River experiences high flows and extended flooding.

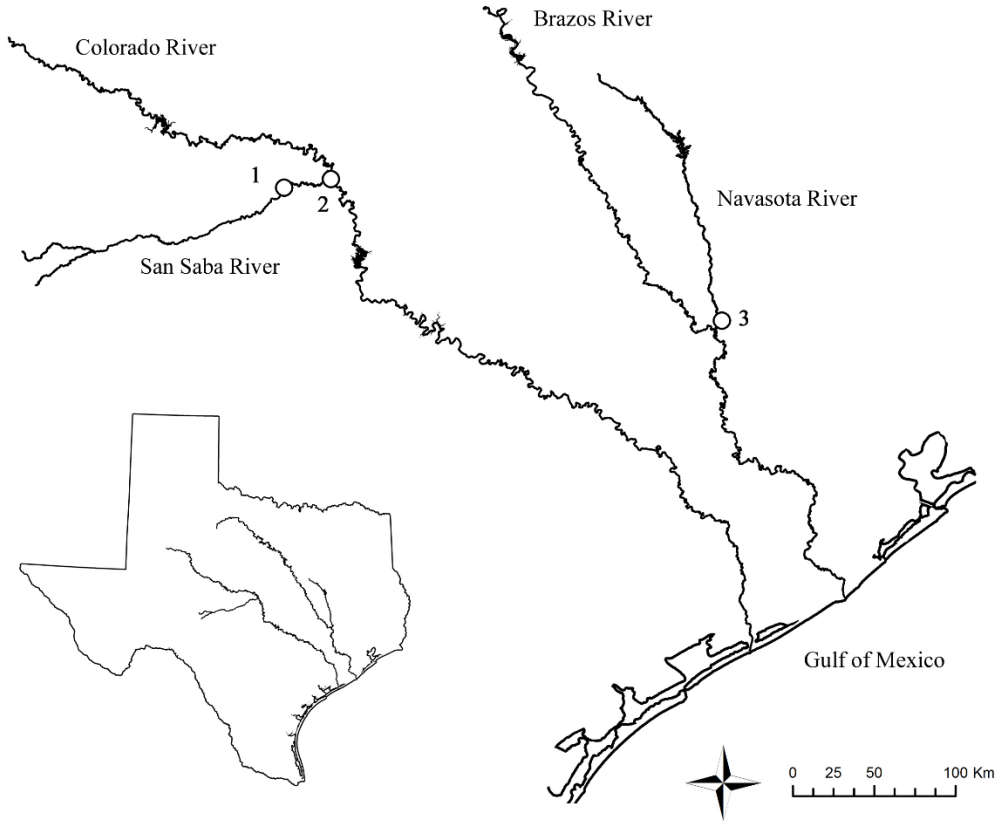

Figure 1. Experimental sites (circles) located on the San Saba River (Sites 1 and 2) and Navasota River, Texas (Site 3).

Study species

Between both San Saba and Navasota Rivers, four mussels of the genus *Quadrula* were targeted for this study: *Quadrula apiculata* (Say 1829) (southern

mapleleaf), *Quadrula houstonensis* (Lea 1859) (smooth pimpleback), *Quadrula petrina* (Gould 1855) (Texas pimpleback) and *Quadrula verrucosa* (Rafinesque 1820) (pistolgrip). *Quadrula apiculata* occurs widely among Gulf Coast drainages, ranging from the Rio Grande to Mississippi River (Williams et al. 2008), and *Q. verrucosa* is distributed throughout most of the eastern United States, including Gulf coastal and Atlantic slope drainages (Williams et al. 2008). In contrast, *Q. petrina* and *Q. houstonensis* are both endemic species, restricted to rivers of Central Texas (Howells et al. 1996) and are currently considered state-threatened (Texas Register 35 2010) and federal candidates (Federal Register 76 2011) for listing under the Endangered Species Act (ESA). The gametogenic cycle is known only from *Q. verrucosa* (Jirka and Neves 1992), which produces gametes throughout the year, typically peaking between early spring and summer. I make the assumption that *Q. petrina* and *Q. houstonensis* will have a similar gametogenic cycle to *Q. verrucosa* and other species of *Quadrula* (Williams et al. 2008). Due to differences in distribution and abundance of my focal species between study sites, *Q. petrina* and *Q. verrucosa* were studied only from the San Saba River (Sites 1 and 2), *Q. apiculata* was studied only from the Navasota River (Site 3), and *Q. houstonensis* was studied in both the San Saba and Navasota Rivers (Sites 2 and 3; Figure 1; Table 1).

Table 1. Treatments, their respective sample sizes (n) and mean (± SD mm) initial shell length (sl) used to study the effectiveness of the syringe technique, which includes an experimental treatment for quantifying gamete production using the syringe technique (Syringe), validation treatment for quantifying gamete production using the histological technique (Histology), and non-reproduction treatment to control for the effects of the syringe technique on survival and growth (Control).

	Q. apiculata		*Q. houstonensis*		*Q. petrina*		*Q. verrucosa*	
Treatment	n	sl	n	sl	n	sl	n	sl
Site 1 (San Saba River)								
Syringe	---	---	---	---	63	55.6 ± 3.8	96	87.8 ± 6.8
Histology	---	---	---	---	---	---	---	---
Control	---	---	---	---	40	54.4 ± 4.7	40	84.7 ± 7.1
Site 2 (San Saba River)								
Syringe	---	---	105	43.9 ± 5.7	110	47.6 ± 6.5	96	79.4 ± 16.0
Histology	---	---	105	43.2 ± 7.3	100	45.5 ± 9.0	---	---
Control	---	---	40	45.2 ± 5.5	40	46.0 ± 6.1	40	77.4 ± 16.1
Site 3 (Navasota River)								
Syringe	74	54.6 ± 9.5	79	42.6 ± 5.0	---	---	---	---
Histology	78	55.4 ± 9.2	79	41.5 ± 5.7	---	---	---	---
Control	---	---	---	---	---	---	---	---

Experimental design

Three treatment groups of mussels were established to examine the effectiveness of the syringe technique, which included (1) syringe (experimental), mussels used to measure gamete production with the syringe technique; (2) histology (validation), mussels used to measure gamete production with the histological technique; and (3) non-gamete (control), mussels in which gamete production was not measured (Table 1). Syringe treatment groups served two important purposes in this experiment. The first was to validate the syringe technique by comparing gamete estimates between syringe and histology treatments, and the second was to determine the effect of the syringe technique on mussels by comparing survival, growth and body condition between

syringe and control treatment groups. Since mussel assemblages varied across rivers, I established either a syringe/non-gamete (Sites 1 and 2) and/or syringe/histology (Sites 2 and 3) treatment pairing to assess the accuracy of the syringe technique or examine the effects of the syringe technique on mussels, respectively (Table 1).

Starting in July 2012, I collected adult mussels of similar size at Sites 1 and 2, marked each with a 12.5 mm passive integrated transponder (PIT) tag (Biomark, Inc., Boise, Idaho) and randomly assigned them to treatments (Table 1). Each PIT tag has a unique identification number and was affixed to a mussel shell using non-toxic marine epoxy putty (Kurth et al. 2007). Once marked and assigned to a treatment, initial measurements of shell length (anterior to posterior, mm) and wetted weight (g) were recorded to estimate growth and body condition (see below). Mussels from each treatment were then placed into 5 × 5 m plots (1 treatment per plot) in which densities were kept at 8 mussels/m^2. At these sites (Sites 1 and 2), syringe and control treatments were monitored (see below) to assess mussel survival, growth and body condition. At Site 3, I only compared gamete production using the two techniques (i.e., syringe and histology; Table 1) and therefore did not mark mussels with PIT tags or use capture-recapture methods.

I examined reproduction in syringe and histology treatments roughly every 4 – 6 weeks for one year. Survival, growth and body condition were examined for two years, which allowed me to test for sublethal effects associated with the syringe technique. An antenna receiver was used to locate 8 – 10 mussels from syringe treatments and/or histology treatments from Sites 1 and 2. For histology treatments I preserved each

individual by cutting its adductor muscles and placing it directly into 10% neutral buffered formalin. I followed Galbraith and Vaughn (2009) for syringe treatments and extracted gonadal fluid from each individual by inserting a 20 gauge hypodermic needle through the foot, positioned approximately midline to the shell and half way into the visceral mass. The location of the gonads for these species were confirmed *a priori* by examining cross-and longitudinal-sections of reproductive tissues (see Henley 2002). I extracted 0.25 – 0.50 ml of gonadal fluid per individual, which was then fixed in 10% buffered formalin and placed on ice for transport to the laboratory. Mussels in syringe treatments were sampled for gonadal fluid only once and were placed back into their respective plots for the duration of the study. At Site 3, I randomly collected mussels, sampled gonadal fluid from 8 – 10 individuals, and preserved 8 – 10 individuals for histological analysis. Because mussels sampled with the syringe technique were not fitted with PIT tags at this site, I used a paint pen to mark their shells to ensure gonadal fluid was sampled only once from an individual.

Survival, growth and body condition were assessed in syringe and non-gamete treatments approximately quarterly for two years (7 encounter periods total). During each assessment, mussels were collected by locating PIT tags using an antenna receiver, combined with visual and tactile searches within and around the study plots. I searched for mussels until all individuals were recovered or PIT tags were no longer detected with the antenna receiver, which typically took 1 – 3 days per site. This consisted of searching the entire study area (50 m up- and downstream) to re-capture any individuals that might have migrated out of the plots. All mussels collected were placed into mesh bags, which

were kept submerged in areas with sufficient flow. Data collected on recapture occasions were organized into the following for each mussel: not encountered, live encounter or dead recovery. Shell length (mm) and wetted weight (g) were also measured and used to estimate yearly proportional shell growth (mm/yr) and Fulton's K body condition factor:

$$\text{shell growth rate} = \frac{\text{new shell length / initial shell length}}{\text{time (yrs) since the begining of the study}}$$

$$\text{Fulton's K} = \frac{\text{wetted weight}}{\text{shell length}^3} \times 10^6$$

Quantifying Gamete Production

Following Galbraith and Vaughn (2009), gamete production was quantified in syringe treatments from gonadal fluid by estimating mean sperm concentration (no./ml) for males and mean egg concentration (no./ml) and diameter (μm) for females. I first determined sex by identifying male or female gametes in a small drop of each sample. Methylene Blue was added to samples to help identify gametes (see Saha and Layzer 2008 for details and descriptions of gametes). Sperm concentration was quantified with a hemocytometer under a compound microscope (400×):

$$\text{sperm concentration} = \frac{\text{no. of cells counted} \times \text{dilution} \times 4000}{\text{number of small squares counted}}$$

where the number of cells count is equivalent to the total number of sperm counted in a sample, dilution is the ratio between volume of gonadal fluid extracted to total volume of solution (gonadal fluid + formalin), and number of small squares where counts on the grid where recorded. Hemocytometers have been traditionally used to determine blood

or reproductive cell density in humans, but has also been successfully applied to non-human subjects (e.g., Navarro et al. 1998). For females, I mixed the contents of each sample, placed 3 µl onto a glass slide using an automatic pipettor (GeneMate, ISC BioExpress, Kaysville, UT), and began by counting the number of eggs under a compound microscope (100×). Egg concentration was estimated by extrapolating the number of eggs to 1 ml volume of gonadal fluid. Mean egg diameter was estimated by measuring 50 randomly selected eggs with an ocular micrometer.

Mussels sacrificed for histological examination were fixed in 10% neutral buffered formalin for at least two weeks and subsequently transferred to 70% ethanol. Tissue and slide preparation were conducted following Kiernan (1999). Mussels were dissected in the laboratory by excising the visceral mass and cutting a 2 – 4 mm section located slightly anterior of the midline of the shell. This area was chosen to mirror the location where gonadal fluid was sampled in mussels using the syringe technique. Gonad tissue was then dehydrated to 100% ethanol, cleared in toluene and embedded in paraffin wax. Transverse sections of gonad tissue (7 µm) were cut using a Spencer 820 rotary microtome (American Optical Co., Buffalo, NY). Tissues sections were mounted on glass slides and stained with hemotoxylin and eosin. Gamete production was quantified by counting or measuring the number of gametes through the center of 10 randomly selected follicles (Haggerty et al. 1995, methods described in Haggerty and Garner 2000, and Jones et al. 1986). Using the eyepiece pointer on a compound microscope (1000×), the number of sperm per follicle were counted from transects by moving the microscope stage along an x- or y-axis, and the diameter of the first 50 eggs were measured along

transects positioned randomly through the entire tissue section. Only eggs that were sectioned through the nucleus were measured, and diameter was estimated for each egg by averaging length and width measurements.

Statistical analyses

To examine the accuracy of the syringe technique, I performed a Pearson's Product Moment Correlation analysis to determine if estimates of mean monthly gamete production using the syringe technique (sperm concentration, egg diameter and egg concentration) were correlated with gamete production using the histological technique (sperm density, egg diameter and egg density). This analysis allowed me to test whether timing of peak gamete production estimated from the two techniques were positively correlated, which would indicate the ability of the syringe technique to accurately estimate gametogenic periodicity in relation to the traditional histological technique. Because of differences in timing of peak gamete production among species and sites, each syringe-histology pairing (i.e., the same species at the same site) was analyzed separately. Prior to analysis, estimates of gamete production were scaled to a mean of 0 and standard deviation of 1. The strength of linear correlation among the treatments were compared using the correlation coefficient r, and the t-statistic was used to examine for a significant trend between gamete estimates. I performed these analyses using the R statistical package (R Development Core Team, http://www.R-project.org) and set $\alpha = 0.05$ for all statistical tests. Although I considered a significant correlation among gamete estimates to include $P < 0.05$, I considered a marginally significant trend when $P = 0.05 – 0.10$.

I used a joint live encounter and dead recovery mark-recapture analysis using the R package RMark (Laake 2013) to develop models in Program MARK (White and Burnham 1999), with the primary aim of modeling effects of the syringe technique on survival probability (Burnham 1993). This model accommodates data from encounters with both live and/or dead individuals to improve parameter estimation (Lebreton et al. 1992, Burnham 1993). Four parameters can be estimated using this model: (1) survival probability (S), probability of surviving the duration of an encounter interval; (2) recapture probability (p), probability of being observed, conditional on being alive and in the study area; (3) recovery probability (r), probability of being observed and reported dead; and (4) fidelity (F), the probability of remaining in the sampling area. Key assumptions necessary to implement this model include (1) all marked individuals have the same probability of surviving and being recaptured, (2) tags were not lost, (3) dead recovery rates are constant, (4) encounter intervals were relatively short in duration, and (5) dead recoveries occurred outside the sampling region (Lebreton et al. 1992, Burnham 1993). Since this model can differentiate between temporary and permanent emigration ($1 - F$), survival probability is considered true survival. However, because I only recovered dead individuals within the same sampling region as live recaptures (violating an important assumption for estimating fidelity), I fixed $F = 1$ for all models, making my estimates of S analogous to apparent survival.

To estimate S, p and r, I developed a candidate set of biologically relevant additive models based on my knowledge of freshwater mussel biology and stream ecology. Four predictor variables were considered as potential sources of variation:

sample date (time), site, species and treatment. For each of the three parameters, I included time and site effects, because variation in environmental conditions in streams over time and space may influence survival, recapture and recovery rates. Species effects were modeled with *S*. Because mortality schedules inherently vary among species as a result of trade-offs among life history traits (Stearns 1992), treatment effects were also modeled with *S* to test whether the syringe technique significantly effects survival. Since mussels belonging to the syringe treatment groups were not all sampled with the syringe technique at the same time, I accounted for this variability by including treatment effects as a time-varying, categorical covariate. Despite the fact that behavioral differences were found to influence recapture probability among species of mussels (Villella et al. 2004), I did not include species effects to estimate *p* and *r* because all mussels were marked with PIT tags and placed into study plots. PIT tags are known to significantly improve detection of mussels (Kurth et al. 2007), negating differences among species and influence on *p*. Treatment effects were not considered for *p* and *r*, because there is no discernible reason the syringe technique could significantly influence these parameters. Thus, my global model was $S_{\text{Treat + time + Sp + Site}}\, p_{\text{time + Site}}\, r_{\text{time + Site}}\, F_1$.

To test for adequate fit of the global model, I used a bootstrap goodness-of-fit test implemented in Program MARK. Level of fit was determined by ranking and counting the number of models from 1000 simulations with deviance ≥ observed deviance. Since my model lacked fit ($P = 0.001$), I corrected for overdispersion by estimating the variance inflation factor ($\hat{c} = 1.73$) by dividing observed $\hat{c}$ by mean estimated $\hat{c}$ from the bootstrap simulations. After correcting for overdispersion, an

information-theoretic approach was used to assess the candidate models (Burnham and Anderson 2002). I ranked the models based on lowest quasi-likelihood Akaike's Information Criterion ($QAIC_c$) corrected for small sample sizes to determine the most parsimonious model (Burnham and Anderson 2002). The top-ranked models within $\Delta QAIC_c < 2$ were considered to have substantial support, though models fitting this criterion with a difference of only one parameter and minimal difference in maximum log-likelihood are typically not considered competitive because of the inclusion of an uninformative parameter (Burnham and Anderson 2002, Arnold 2010). Thus, to make further inferences regarding the best-approximating model(s) and the importance of variables, I averaged parameter estimates for models within $\Delta QAIC_c < 2$ to account for model selection uncertainly. I also used $QAIC_c$ weights (w) to determine the relative importance of each model, based on the ratios among weights (i.e., evidence ratios), and I estimated relative variable importance $w_{+(j)}$ by summing w across all candidate models that contained each predictor variable x_j (Burnham and Anderson 2002). Higher $w_{+(j)}$ values (ranging from 0 – 1) indicate greater support for a particular variable. The advantages of investigating relative variable importance are that inferences can be drawn beyond variables occurring in the best-approximating model (Burnham and Anderson 2002, Wagenmakers and Farrell 2004), but can only be done when the variables occur in equal numbers throughout the candidate model set, as was the case in my analyses.

I use both linear mixed models (LMM) and generalized additive mixed models (GAMM) to examine variation in Fulton's K condition factor and yearly proportional growth rate, respectively. Mixed models are useful regression analyses for grouped data

(e.g., repeated measures on experimental units) because of their flexibility in handling covariance structures and unbalanced designs (Pinheiro and Bates 2006). Both response variables were modeled with time as a continuous fixed variable and allowing all possible combinations of species, site and treatment effects as categorical grouping variables. The lowest experimental unit (mussel) was modeled as a random effect, in which intercepts were allowed to vary to account for heterogeneity and non-independence of (repeated) measurements over time (Pinheiro and Bates 2006). Because of the nonlinear rate at which mussels grow over time (Zuur et al. 2009), I used the R package mgcv (Wood 2001) to model growth using GAMM. GAMM was implemented with a Gaussian identity link function and cubic smoothing splines to characterize the nonlinear relationship between time and growth (Zuur et al. 2009, Zuur et al. 2014). Exploratory analysis of normalized residuals indicated heterogeneity; therefore, I square-root transformed growth to meet model assumptions. The fit of the smoothing term was evaluated by the effective degrees of freedom (edf), where edf > 1 is defined by the degree of nonlinearity, and significance was determined by an *F*-ratio tests (Zuur et al. 2014). Because these data displayed a linear trend over time, LMM was used to model Fulton's K using the R package lme4 (Bates 2010). Since length-wet weight ratios vary widely among species (i.e., shell morphology varies in size and weight relative to tissue mass), I also included species as a random effect to account for this variation (Bates et al. 2015). For both GAMM and LMM, model selection was implemented using the lowest ranked AIC value to determine the most parsimonious model, and evidence ratios

and relative variable importance based on AIC *w* were used to measure relative support of the models and individual variables.

RESULTS

I studied a total 1,185 mussels from 4 species across two rivers to analyze reproduction and conduct a mark-recapture experiment that included 875 mussels marked with PIT tags. On average, I extracted 0.37 ± 0.1 ml (SE) of gonadal fluid from 528 individual mussels using the syringe technique, and I successfully quantified gametes (sperm or eggs) from approximately 77.8% (n = 411) of these samples. For the other gonadal fluid samples, 21.8% (n = 115) did not have gametes, largely due to digenetic trematodes parasitizing mussel gonads, and the remaining two samples contained neither trematodes nor gametes 0.4% (n = 2). I was able to assess gamete production from 290 individual mussels among the 339 mussels sacrificed for histological analysis (85%), which excludes 23 mussels initially marked and not sampled. The other 49 individuals (14.5%) lacked gametes because they were parasitized by trematodes.

Accuracy of the syringe technique

Mean scaled estimates of gamete production measured using the syringe technique (egg concentration, egg size and sperm concentration) and the histological technique (egg density, egg size and sperm density) were comparable among the 12 syringe-histology treatment comparisons (4 groups × 3 gamete estimates) when plotted over time. In general, estimates of egg size (Figure 2a – d) and sperm concentration/density (Figure 2e – h) produced estimates that were similar over time;

whereas, egg concentration/density (Figure 2i – l) notably varied over time. In most cases, the timing of highest and lowest points of gamete production aligned across treatments, especially for egg size (Figure 2a – d). However, some peak estimates between sperm concentration and sperm density (*Q. houstonensis* at Site 3; Figure 2h) and egg concentration and egg density (*Q. apiculata* at Site 3; Figure 2k) were not aligned. Based on Pearson's correlations, gamete estimates from syringe and histology treatments were positively correlated (Figure 3). Egg size was correlated among treatments for all four groups ($P < 0.05$, $r = 0.88 – 0.92$; Figure 3a – d). Sperm concentration and sperm density also generally correlated (Figure 3e – h), with one group (*Q. petrina* at Site 2) significant at $P < 0.05$ ($r = 0.94$) and three groups marginally significant at $P < 0.10$ ($r = 0.61 – 0.64$; Figure 3). In contrast, egg concentration and egg density were only correlated in some groups (Figure 3i – l), including one group (*Q. apiculata* at Site 3) significant at $P < 0.05$ ($r = 0.78$) and one group (*Q. houstonensis* at Site 2) marginally significant at $P < 0.10$ ($r = 0.64$). The other two groups (*Q. petrina* at Site 2 and *Q. houstonensis* at Site 3) were not significantly correlated ($P > 0.10$).

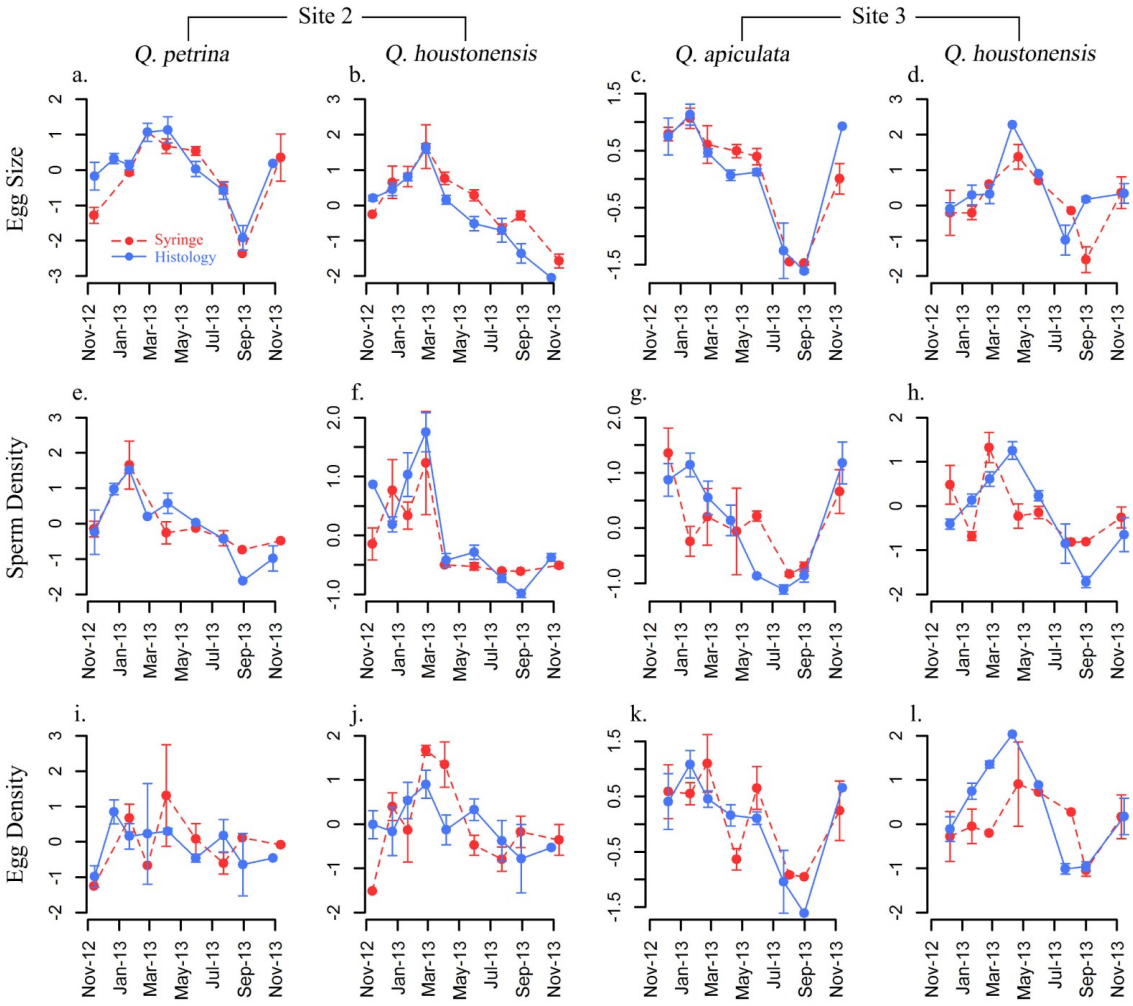

Figure 2. Scaled estimates of gamete production (sperm density, egg size and egg density) comparing the syringe treatment groups and histology treatment groups among sites (12 comparisons = 4 groups × 3 gamete estimates) and species (*Quadrula apiculata*, *Q. petrina*, *Q. houstonensis* and *Q. verrucosa*). Error bars indicate the standard error of the mean.

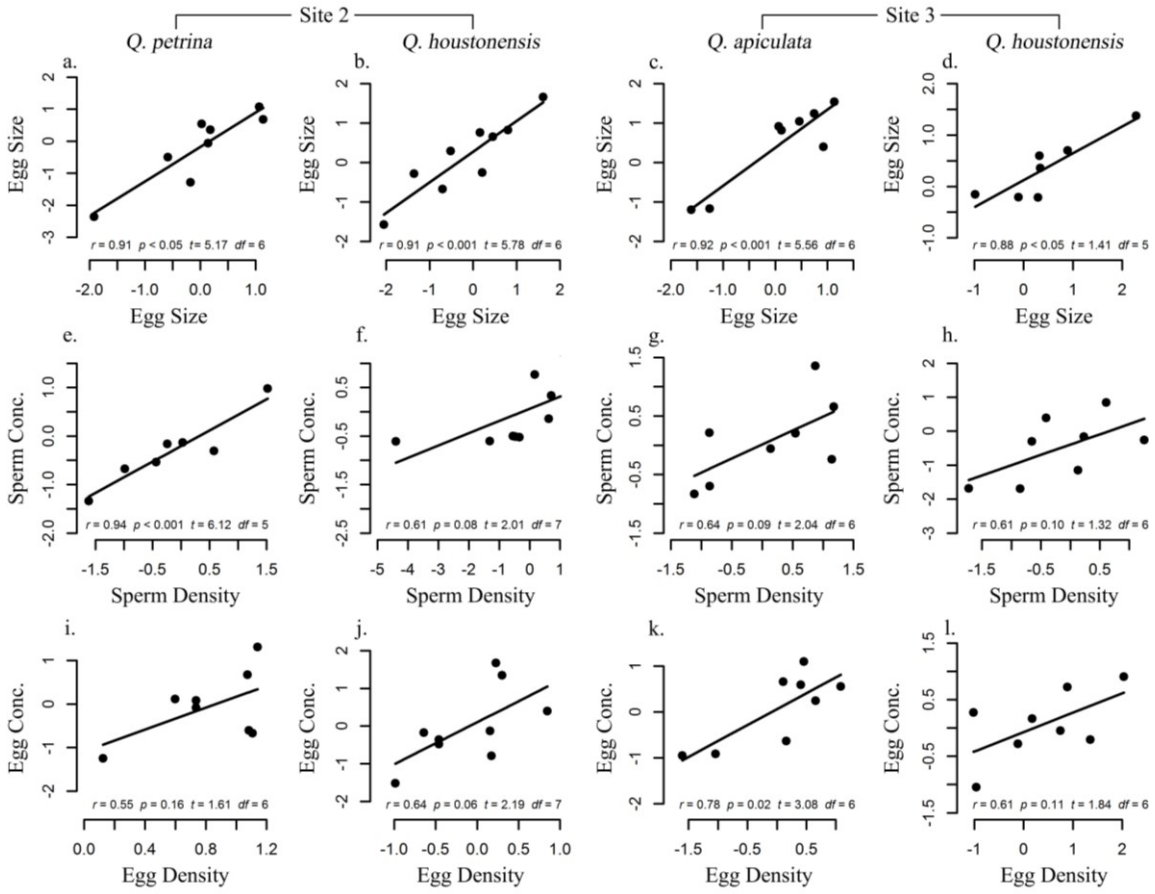

Figure 3. Pearson's correlations comparing gamete production measured from syringe treatment groups (sperm concentration, egg size and egg concentration) and histology treatment groups (sperm density, egg size and egg density) among sites (12 comparisons = 4 groups × 3 gamete estimates) and species (*Quadrula apiculata*, *Q. petrina*, *Q. houstonensis* and *Q. verrucosa*). Statistics include correlation coefficient (*r*), *p* value, test statistic (*t*), and degrees of freedom (*df*).

Survival, recapture and recovery probabilities

My candidate model set consisted of 128 models within which I estimated three parameters (*S*, *p*, and *r*) represented by several variables (time, species, site and treatment). The best-approximating model ($S_{\text{time + Sp + Site}}\, p_{\text{time + Site}}\, r_{\text{time}}$, QAIC_c = 1291.55) indicated *S* (survival probability) varied with time, species and site, *p*

(recapture probability) varied with time and site, and *r* (recovery probability) varied with time (Table 2). All three parameters were consistently time dependent (Table 2). Based on the $\Delta QAIC_c < 2$ criterion, the top three $QAIC_c$ ranked models were supported. The second best-approximating model ($S_{Treat + time + Sp + Site}\ p_{time + Site}\ r_{time}$, $QAIC_c = 1291.94$) was similar to the first but included treatment as an additional factor explaining *S*, whereas the third best-fit model ($S_{time + Sp + Site}\ p_{time + Site}\ r_{time + Site}$, $QAIC_c = 1293.42$) was similar to the first except site was an additional factor explaining *r* (Table 2). However, only the first and second top-ranked AIC models were supported, because the third model had a similar maximum log-likelihood value as the first model and differed by only one parameter, which indicates that it is less parsimonious compared to first and second top-ranked models (Table 2). Additionally, *w* ($QAIC_c$ weight) of the first model indicated it was 2.5 times more supported than the third model, while the first model was only 1.2 times more supported than the second model. The variables species, site and time all significantly explained variability in *S* ($w_{+(j)} = 0.999$ for all three variables; Table 3), whereas treatment was 2.2 times less important than the other variables considered ($w_{+(Treat)} = 0.452$; Table 3). Time and site were relatively important variables in explaining *p* ($w_{+(Time)} = 1.0000$, $w_{+(site)} = 0.9167$; Table 3). Time also was important in explaining *r* ($w_{+(Time)} = 1.0000$), whereas site had considerably less support (3.5 times) explaining *r* ($w_{+(site)} = 0.2817$; Table 3).

Table 2. Fifteen best-approximating models ranked by lowest quasi-likelihood Akaike Information Criterion ($QAIC_c$) from my joint live encounter and dead recovery analysis of three freshwater mussel species (*Quadrula petrina*, *Q. houstonensis* and *Q. verrucosa*) at two sites (Sites 1 and 2) in the San Saba River, Texas. Model parameters included survival probability (*S*), recapture probability (*p*), dead recovery probability (*r*) and fidelity (*F*), and were tested for variation among time (time), treatment (Treat), site (Site) and species (Sp), though only *S* was tested for treatment and species effects and *F* was fixed to 1 for all models. I also estimated $\Delta QAIC_c$, Akaike weights (w_i), and negative two log-likelihood (-2ln(*L*)) for each candidate model.

Competing Models	***k***	**$QAIC_c$**	**$\Delta QAIC_c$**	***w_i***	**-2ln(*L*)**
$S_{(time + Sp + Site)}\, p_{(time + Site)}\, r_{(time)}\, F_{(1)}$*	21	1291.55	0.000	0.358	2161.33
$S_{(Treat + time + Sp + Site)}\, p_{(time + Site)}\, r_{(time)}\, F_{(1)}$*	22	1291.94	0.384	0.296	2158.49
$S_{(time + Sp + Site)}\, p_{(time + Site)}\, r_{(time + Site)}\, F_{(1)}$	22	1293.42	1.871	0.141	2161.07
$S_{(Treat + time + Sp + Site)}\, p_{(time + Site)}\, r_{(time + Site)}\, F_{(1)}$	23	1293.81	2.256	0.116	2158.23
$S_{(time + Sp + Site)}\, p_{(time)}\, r_{(time)}\, F_{(1)}$	20	1296.35	4.798	0.033	2173.12
$S_{(Treat + time + Sp + Site)}\, p_{(time)}\, r_{(time)}\, F_{(1)}$	21	1296.74	5.187	0.027	2170.30
$S_{(time + Sp + Site)}\, p_{(time)}\, r_{(time + Site)}\, F_{(1)}$	21	1298.21	6.656	0.013	2172.84
$S_{(Treat + time + Sp + Site)}\, p_{(time)}\, r_{(time + Site)}\, F_{(1)}$	22	1298.60	7.046	0.011	2170.02
$S_{(time + Sp + Site)}\, p_{(time + Site)}\, r_{(1)}\, F_{(1)}$	16	1301.73	10.180	0.002	2196.40
$S_{(Treat + time + Sp + Site)}\, p_{(time + Site)}\, r_{(1)}\, F_{(1)}$	17	1302.11	10.560	0.002	2193.57
$S_{(time + Sp + Site)}\, p_{(time + Site)}\, r_{(Site)}\, F_{(1)}$	17	1303.73	12.181	0.001	2196.37
$S_{(Treat + time + Sp + Site)}\, p_{(time + Site)}\, r_{(Site)}\, F_{(1)}$	18	1304.11	12.562	0.001	2193.54
$S_{(time + Sp + Site)}\, p_{(time)}\, r_{(1)}\, F_{(1)}$	15	1306.42	14.865	0.000	2208.00
$S_{(Treat + time + Sp + Site)}\, p_{(time)}\, r_{(1)}\, F_{(1)}$	16	1306.80	15.230	0.000	2205.17
$S_{(time + Site)}\, p_{(time + Site)}\, r_{(time)}\, F_{(1)}$	19	1308.03	16.477	0.000	2196.82

*Estimates averaged due to model selection uncertainty

Table 3. Relative variable importance ($w_{+(j)}$) of grouping variables, including time (time), treatment (Treat), site (Site) and species (Sp), for the parameters survival probability (*S*), recapture probability (*p*), dead recovery probability (*r*), growth and Fulton's K body condition index. Number of models includes the number of times a variable occurred within the candidate model set, and relative variable importance was estimated by summing their Akaike weights (w_i).

Parameter	Grouping Variable	No. of models	Importance $w_{+(j)}$
S	Sp	64	0.9999
	Site	64	0.9999
	Time	64	0.9999
	Treat	64	0.4521
p	Site	64	0.9167
	Time	64	1.0000
r	Site	64	0.2817
	Time	64	0.9940
Growth	Sp	4	0.9662
	Site	4	0.8485
	Treat	4	0.2741
Fulton's K	Sp	4	1.0000
	Site	4	0.3208
	Treat	4	0.3997

Parameter estimates were averaged for the two top $QAIC_c$ ranking models because of model selection uncertainly (Table 2). Despite some evidence indicating treatment is an important predictor explaining variability in *S*, the small differences in mean model estimates between syringe and control treatments suggest treatment effects have little biological significance (Figure 4). Most differences in mean estimates between treatments were within 0.01 – 0.03 probability, and the largest difference was for *Q. petrina* at Site 1, which varied in as little as 0.03 – 0.05 probability (Figure 4), supporting earlier conclusions that treatment has little influence in explaining *S*. Regardless of treatment, *S* was generally high for most species and sites but declined

slightly over time (Figure 4). Over the two year period of this study *Q. petrina* had the lowest *S* and ranged from 0.75 – 0.93 (Site 1) and 0.53 – 0.84 (Site 2), whereas *Q. verrucosa* ranged from 0.80 – 0.95 (Site 1) and 0.91 – 0.98 (Site 2) and *Q. houstonensis* ranged from 0.83 – 0.96 (Site 2) across sites (Figure 4). Estimates of *p* averaged over the two best-approximating models were also high and varied little over time, which ranged from 0.77 – 1.00 and 0.87 – 1.00 across Sites 1 and 2, respectively (Table 4). For most recapture periods, $p > 0.97$ except for March 2014 in which estimates dropped significantly at both sites and represented the lowest recapture rates. In contrast, mean model estimates for *r* varied widely over time, which ranged from 0.34 – 0.93 probability (Table 4). Estimates of *r* were lower earlier in the study, increased by more than 100% by April 2013 and steadily dropped towards the end of the study (Table 4).

Table 4. Parameter estimates and 95% confidence intervals for recapture probability (*p*) and dead recovery (*r*) averaged over the two best-approximating models (see Table 2).

	Site 1		Site 2		Sites 1 and 2	
Date	**Recapture (*p*)**	**CL**	**Recapture (*p*)**	**CL**	**Recovery (*r*)**	**CL**
15-Jul-2012	---	---	---	---	0.44	0.20 – 0.69
6-Nov-2012	0.98	0.97 – 1.00	0.99	0.99 – 1.00	0.44	0.19 – 0.68
1-Apr-2013	0.98	0.97 – 0.99	0.99	0.98 – 1.00	0.93	0.84 – 1.01
23-Jul 2013	0.97	0.96 – 0.99	0.99	0.98 – 1.00	0.69	0.52 – 0.86
28-Oct-2013	0.97	0.96 – 0.99	0.99	0.98 – 1.00	0.45	0.19 – 0.70
10-Mar-2014	0.77	0.71 – 0.83	0.87	0.84 – 0.90	0.34	0.16 – 0.52
9-Jun-2014	1.00	1.00 – 1.00	1.000	1.00 – 1.00	---	---

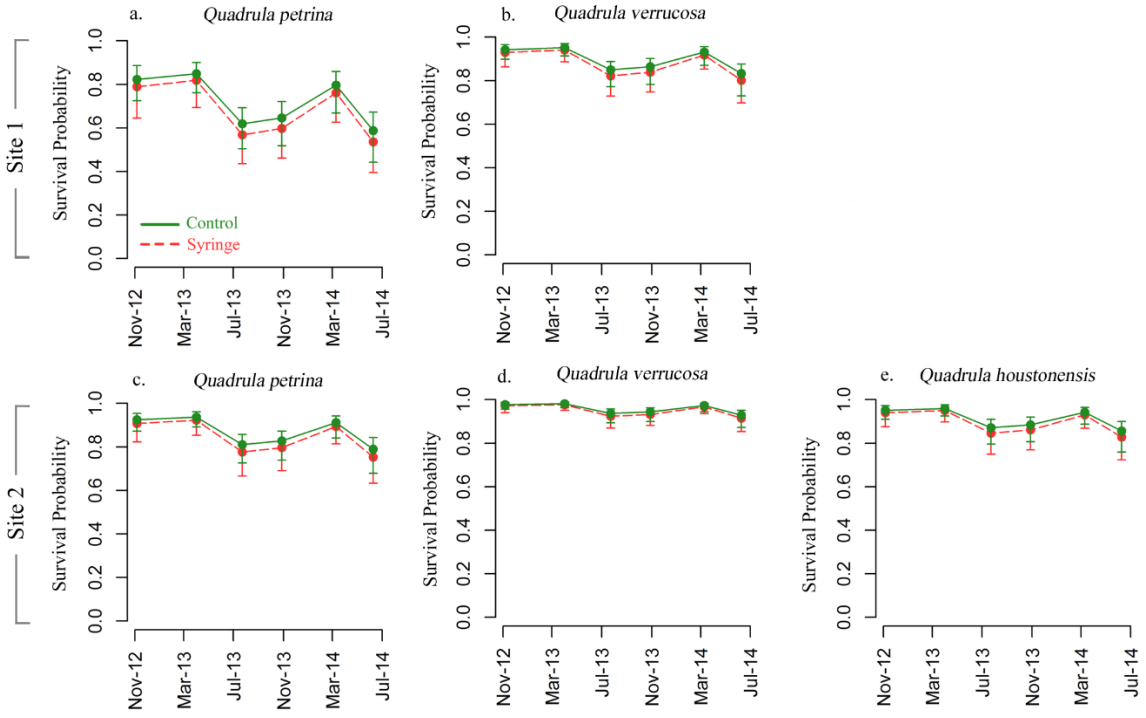

Figure 4. Survival probability (*S*) of three freshwater mussel species (*Quadrula petrina*, *Q. houstonensis* and *Q. verrucosa*) at two sites (Sites 1 and 2) in San Saba River, Texas. Estimates from the two best-approximating QAICc models were average due to model selection uncertainty (Table 2).

Growth and body condition

Of the eight generalized additive mixed models (GAMM) within the candidate set, the best-approximating model explaining variability in growth included time, site and species ($Growth_{s(time) + Site + Sp}$, AIC = -7003.40; Table 5). The second top-ranked AIC model was also supported, which was a more complex version of the first and included treatment as an additional predictor variable ($Growth_{s(time) + Treat + Site + Sp}$, AIC = -7001.46; Table 5). However, based on AIC *w*, the first model was 2.6 times more supported than the second, and relative variable importance (i.e., summed AIC *w*) indicated that site

($w_{+(site)}$ = 0.8485) and species ($w_{+(species)}$ = 0.9662) were highly supported, while treatment ($w_{+(Treat)}$ = 0.2741) was weakly supported (Table 5). Overall, the coefficients derived from the best-approximating model indicated growth decreased over time (Table 6), and that this decreasing trend was significantly nonlinear (edf = 4.937, F = 910.8, P < 0.001; Table 6).

Of the eight linear mixed models (LMM) explaining variability in Fulton's K condition index, the best-approximating model varied with time and species (*Fulton's* $K_{time + Sp}$, AIC = 26523.18; Table 5). The second (*Fulton's* $K_{time + Treat + Sp}$, AIC = 26523.98; Table 5) and third best-fit models (*Fulton's* $K_{time + Sp + Site}$; AIC = 26524.66; Table 5), which included treatment and site effects, respectively, were also well supported. Based on AIC w, the first best-approximating model was 1.5 times more supported than the second and 2.1 times more supported than the third. Relative variable importance indicated that treatment ($w_{+(Treat)}$ = 0.3997) and site ($w_{+(site)}$ = 0.3208) were weakly supported within the candidate model set, indicating the added variables within the second (treatment) and third (site) best-fit models were not important in explaining heterogeneity in Fulton's K index. Coefficients from the first top-ranked model indicated a linear increase in Fulton's K over time, and Fulton's K was highly dependent on species from both fixed (time and species) and random effects (species and mussel; Table 6).

Table 5. Candidate model set for generalized additive mixed models (GAMM) used to analyze growth over time and linear mixed models (LMEM) used to analyze Fulton's K condition index over time. Models are ranked according to their lowest Akaike Information Criterion (AIC). I estimated change in AIC value (ΔAIC) and AIC weight (w_i) and log-likelihood for each model. Parameters included time, site (Site), species (Sp) and Treat (Treatment). For GAMM models, s(time) indicates smoothing term was applied for time.

Parameter	Competing Models	AIC	ΔAIC	w_i	ln(*L*)
Growth	s(time) + Site + Sp	-7003.40	0.0000	0.6057	3509.70
	s(time) + Treat + Site + Sp	-7001.46	1.9437	0.2292	3509.73
	s(time) + Sp	-6999.71	3.6920	0.0956	3506.86
	s(time) + Treat + Sp	-6997.74	5.6628	0.0357	3506.87
	s(time)	-6995.97	7.4325	0.0147	3502.99
	s(time) + Site	-6995.18	8.2251	0.0099	3503.59
	s(time) + Treat	-6993.99	9.4108	0.0055	3503.00
	s(time) + Treat + Site	-6993.21	10.1942	0.0037	3503.60
Fulton's K					
	time + Sp	26523.18	0.0000	0.4061	-13254.59
	time + Treat + Sp	26523.98	0.7933	0.2731	-13253.99
	time + Sp + Site	26524.66	1.4752	0.1942	-13254.33
	time + Treat + Sp + Site	26525.51	2.3306	0.1266	-13253.76
	time	26545.20	22.0145	0.0000	-13267.60
	time + Treat	26546.00	22.8167	0.0000	-13267.00
	time + Site	26546.65	23.4710	0.0000	-13267.33
	time + Treat + Site	26547.52	24.3362	0.0000	-13266.76

Table 6. Coefficients for the top generalized additive mixed model for growth and linear mixed model for Fulton's K Condition Index, including fixed and random effect coefficient for each model. Approximated estimates for smooth terms consist of effective degrees of freedom (edf), *F* statistic and significance level (*P*).

Model	**Fixed Effects**	**Estimate**	**SE**	***t***
Growth:	Intercept	0.271	0.010	26.97
s(time) + Site + Sp	s(time)	-0.114	0.002	-54.8
	Site (Site 1)	-0.032	0.013	-2.38
	Species (*Q. petrina*)	0.027	0.014	1.92
	Species (*Q. verrucosa*)	0.051	0.014	3.50
	Random Effects	**Variance**	**SD**	---
	Mussel	0.115	0.041	---
	Smooth Terms	**edf**	***F***	***P***
	s(time)	4.937	910.8	< 0.001
Fulton's K Index:	**Fixed Effects**	**Estimate**	**SE**	***t***
time + Sp	Intercept	403.7	56.0	7.21
	Time	283.4	0.0	25.80
	Species (*Q. petrina*)	-63.2	79.2	-0.80
	Species (*Q. verrucosa*)	-257.3	79.1	-3.25
	Random Effects	**Variance**	**SD**	---
	Mussel	894.9	29.9	---
	Species	3129.4	55.9	---
	Residuals	190.0	13.8	---

DISCUSSION

I used the syringe technique to extract gonadal fluid from freshwater mussels, and gametes were observed in the majority of the samples (77.8%) but absent from samples that contained digenetic trematode parasites, which are known to castrate mussels (Laruelle et al. 2002). Histological investigation of mussels parasitized with trematodes showed that gonads were, in fact, devoid of gametogenic tissues, suggesting that the absence of gametes in fluid extractions was not because I had failed to locate the gonads. I attribute my high extraction rate success to an *a priori* histologically

examination of the viscera in individuals belonging to my target species, as recommended by Henley (2002), which enabled me to select and target a suitable location from which to accurately and consistently sample gonadal fluid with a syringe needle. Saha and Layzer (2008) also had high success when using the syringe technique to extract gametes from *Eliptio dilatata* (spike) for sex determination, but had slightly lower success with *Actiononaias ligamentina* (mucket), which is a species known to pause gameteogensis (Jirka and Neves 1992). The success rate of exacting gametes may therefore vary over the course of a year for species reported to have reduced or inactive periods of gametogenesis (e.g., Quadrula cylindrica, Yeager and Neves 1986, Cyclonaias turberulata, Haggerty et al. 1995). In contrast, species known to produce gametes year-round, including the species examined in my study, might result in higher extraction rate success (e.g., Villosa nebulosa, Zale and Neves 1982, Ellipto dilatata, Jirka and Neves 1992, Anodonta anatina, Hinzmann et al. 2013).

Gamete production was estimated in mussels using the syringe technique with relatively high accuracy. Mean egg diameter had the highest correlated estimates among treatment groups, which was not unexpected because direct measurements of egg diameter were made using both techniques. The only difference observed was that mean egg diameter tended to be smaller when measured with the histology technique (130.4 ± 19.3 µm SE) than the syringe technique (157.3 ± 19.3 µm SE); an artifact likely attributed to tissue shrinkage from embedding and thin-sectioning during slide preparation (Kiernan 1999). Measurements obtained from eggs collected via the syringe technique may be closer to the actual size of the eggs, which is not relevant to

quantifying gametogenic periodicity. Sperm concentration estimated from the syringe technique was generally correlated with sperm density estimated from the histological technique but with some variability among treatments. This variability was small in most cases, but high enough that peak estimates did not align on the same sample period for a few treatment comparisons. This could be attributed to limited sample sizes in some treatments, particularly in treatment groups where parasitism by trematodes was high (e.g., *Q. petrina* and *Q. houstonensis* at Site 2). Increasing sample size and the frequency of sampling in future studies may reduce this variability. In contrast, correlations among egg concentration and egg density were significant in only some cases (e.g., *Q. houstonensis* at Site 2 and *Q. apiculata* at Site 3) and not in others (e.g., *Q. petrina* at Site 2 and *Q. houstonensis* at Site 3), suggesting that quantifying egg concentration using syringe technique could lead to inaccurate results. Quantification of gamete production with the syringe technique can therefore be accomplished with reasonable accuracy, particularly when estimating sperm concentration and egg diameter, but not egg concentration.

My mark-recapture analyses indicated that I had high recapture probability, which varied by time and site, whereas (dead) recovery probability varied by time. Since mussels were marked with PIT tags, variability in environmental conditions (e.g., temperature, turbidity and flow) that could affect mussel behavior and my ability to find mussels (e.g., Villella et al. 2004, Wisniewski et al. 2013) likely had minimal influence in recapture probability. In fact, most estimates were high except for a slight drop in March 2014, and I attribute this to a technical malfunction with the antenna receiver.

Despite the presumed invasiveness of inserting a syringe needle into the viscera of mussels, I failed to find negative effects of the syringe technique to survival. Support for treatment effects was apparent in some models, but differences in survival probability among control and syringe treatments were not biologically meaningful. Saha and Layzer (2008) conducted a one-year laboratory experiment and also found no indication of increased mortality due to the syringe technique. Moreover, I failed to detect sublethal effects to mussels based on my mixed model analyses of growth and Fulton's K index, despite the fact growth did vary significantly with time. Although mark-recapture methods could bias growth estimates due to impacts associated with PIT tags or increased handling (Waller et al. 1999, Haag 2009, Wilson et al. 2011), these biases were likely not an issue since both control and syringe treatments were marked with PIT tags, and although I did not test for the effects of handling associated with the syringe technique (i.e., since syringe treatment mussels were handled more than control treatment mussels), the lack of finding support for treatment effects in my models indicated the added handling in the syringe treatment group was not important.

My results suggest that mussels are not impacted by the syringe technique on both lethal and sub-lethal levels. However, this does not necessarily preclude the possibility of inflicting stress to mussels on other measurable levels or causing permanent, long-term effects. The reproductive anatomy of bivalves is arranged in a relatively complex manor. The gonads, along with the intestinal track, digestive gland and kidney, are housed within the visceral mass, and are generally fused throughout the anteroventral to posteroventral region, depending on the species (Cummings and Graf

2009). Consequently, it could be relatively easy to damage these organs by inserting a syringe needle into the viscera of a mussel. I did notice a slight darkish yellow discoloring in several samples of gonadal fluid, suggesting I inserted the needle through the intestinal track of the mussels, though the fate of these individuals was unknown. This was confirmed by the presence of undigested food particles (e.g., phytoplankton) in these samples when examined under the microscope. Galbraith and Vaughn (2009) similarly noted that inserting the needle into the visceral mass evidentially led to extraction of intestinal fluids. The effects of the syringe technique on reproduction itself are not completely understood, though Saha and Layzer (2008) did examine gonad tissues histologically in mussels subsequent to extracting gametes twice with the syringe technique. Although they concluded that there were no significant effects on reproduction, future studies should only extract gonadal fluid from a mussel once to avoid permanent damage to reproductive tissues, and mussels should be properly marked or tagged subsequent to using the syringe technique to avoid this issue. As such, implementation of the syringe technique should be done cautiously, and resource managers should consult the small but growing body of literature on the use of the syringe technique prior to implementation (e.g., Bauer 1987, Henley 2002, Shiver 2002, Moles and Layzer 2008, Saha and Layzer 2008, Galbraith and Vaughn 2009).

Histological techniques have historically been the preferred method to examine reproductive traits of freshwater mussels, such as the timing and duration of spawning periods (Zale and Neves 1982, Smith et al. 2003), gametogenic periodicity (Haggerty et al. 1995, Haggerty and Garner 2000), and sex ratios (Morton 1991). In response to the

growing imperilment of freshwater mussels, researchers have adopted the syringe technique as a method to qualify reproductive traits (e.g., Shiver 2002, Moles and Layzer 2008). The results of my study indicate that the syringe technique can now be extended to studies attempting to quantify mussel reproduction. Caveats notwithstanding, benefits of the syringe technique are that it could be used to examine the reproductive biology of threatened and endangered species in a non-lethal manor and could be used to help resolve the status of mussel populations in future conservation efforts (Saha and Layzer 2008). For example, physiochemical changes in aquatic systems, such as through hypolimnetic impoundment releases or increased pollution, have been known to suppress gamete production and spawning in freshwater mussels (Heinricher and Layzer 1999, Bringolf et al. 2010). The syringe technique could be used to investigate the reproductive viability of populations exposed to such abnormal conditions, or it could be used in relic populations experiencing low recruitment rates due to unknown causes. Furthermore, implementing this technique would be less costly, relatively easy to learn and more time efficient (Saha and Layzer 2008). Beyond conservation implications, the syringe technique opens the door to a new avenue of broader ecological research on mussels as larger numbers of individuals (sampled in a non-lethal manor) can be used to explore aspects of reproductive ecology (e.g., Galbraith and Vaughn 2009). Finally, despite the advantages of the syringe technique, there is still a need for continuing histological research on mussels, given that the latter provides a more complete and accurate analysis of reproduction that is not possible with the syringe technique. I predict that the syringe

technique will be most useful in conservation studies of threatened and endangered species or ecological studies that require large sample-sizes.

CHAPTER III

EFFECTS OF RELOCATION ON PERFORMANCE OF FRESHWATER MUSSELS: IMPLICATIONS OF PHENOTYPIC VARIATION AMONG POPULATIONS

INTRODUCTION

Species relocation is a common strategy used in conservation (Griffith et al. 1989). Relocation is broadly considered as the intentional movement of populations, including: (1) moving populations outside the historic range of a species (introductions); (2) re-establishing populations to areas from which a species has been previously extirpated (reintroductions); (3) moving populations from one part of a species current range to another (translocations); and (4) adding individuals to an existing population (augmentation or supplementation) (IUCN 1998, Fischer and Lindenmayer 2000, Chauvenet et al. 2013). The usefulness of species relocation as an effective conservation strategy remains subject to debate largely due to low rates of success (Fischer and Lindenmayer 2000, Massei et al. 2010), confusion over acceptable criteria for success (Chauvenet et al. 2013), and genetic and evolutionary consequences for target species (Weeks et al. 2011). Despite these concerns, relocation is still being used as a conservation tool, particularly in cases where endangered species recovery conflicts with economic development (Fischer and Lindenmayer 2000). Efforts to improve and validate the efficacy of species relocation, however, has been limited by the lack of experimental evidence coupled with the absence of rigorous protocols for some taxonomic groups.

Historically, relocation has been heavily biased towards terrestrial vertebrates, especially birds and mammals (Fischer and Lindenmayer 2000, Massei et al. 2010). Given the increasing awareness of threats to freshwater ecosystems and global decline of species inhabiting freshwaters (Dudgeon et al. 2006), relocation of aquatic organisms (vertebrates and invertebrates) are becoming commonplace (Olden et al. 2011). For example, freshwater mussels of the family Unionidae are experiencing precipitous population declines (Strayer et al. 2004), and relocation as a strategy for mussel conservation has rapidly increased in recent decades even though success has been limited in past attempts (Cope and Waller 1995, Haag and Williams 2014). Relocation has been justified and implemented for freshwater mussels for a variety of reasons, including: (1) preventing or minimizing the impacts from construction activities or invasive species; (2) augmenting existing populations; (3) reintroducing species into historic ranges; and (4) temporarily housing populations in artificial refugia (Cope and Waller 1995, Haag and Williams 2014). In a review of past relocation studies, Cope and Waller (1995) inferred that only 43% of mussels relocated for conservation purposes were recovered and less than half (49%) of those individuals survived. Later research has indicated that higher post-relocation survival rates can be achieved for mussels by limiting handling and emersion and improving habitat selection for relocated populations (Havlik 1997, Dunn et al. 1999, Cope et al. 2003, Peck et al. 2014). However, success in mussel relocations is often judged on crude end-points such as survival (Cope and Waller 1995), in part, because mussels are long-lived and estimating demographic rates (e.g., population growth, recruitment) would require years of monitoring (e.g., Jones et

al. 2012). Other reasons for limited investigations stem from the high cost and reportedly lack of guidance on how to implement a successful relocation (Cope and Waller 1995, Haag and Williams 2014). Although some studies have explored detailed short-term criteria as measures of success (e.g., glycogen reserves as stress responses; Newton et al. 2001, Kesler et al. 2007, Peck 2010), few studies have specifically addressed how relocation can affect individual and population-level performance and sought to discern the underlying causes affecting relocation success.

Performance, such as survival, somatic growth and reproduction, represent important traits that directly affect demographics and might be useful for understanding success of mussel relocation in the short-term (e.g., < 5 years). Life history theory indicates that because energy available to an organism is finite trade-offs exist between survival, growth and reproduction to maximize fitness (R. Levins and R.H. MacArthur's principle of allocation; Cody 1966). Variation among life history traits are driven by local environmental conditions that can have different fitness consequences for populations across variable environments (Williams 1966, Stearns 1992, Martone and Micheli 2012). For example, conditions leading to increased reproductive investment and decreased survival or growth may lead to lower life-time reproductive success, whereas increased growth and decreased reproductive investment may result in fewer offspring at larger body sizes (Schaffer 1974, Stearns 1992, Jokela 1997, Martone and Micheli 2012). Spatial variability in performance traits has not been considered with respect to mussel relocations, and it could impact establishment of populations

transplanted across riverscapes into novel environments where genotypic and phenotypic variation may be high (Weeks et al. 2011).

Differentiating factors affecting relocated mussels has been given little precedence. Ignoring such details has led to the practice of relocating mussels to sites with existing populations where habitat conditions are presumably ideal (Cope and Waller 1995, Dunn et al. 1999), but this does not take into consideration variability in performance among populations and assumes mussels can sufficiently acclimate to environmental conditions of transplanted sites. The ability of organisms to successfully acclimate to environments depends upon plasticity of phenotypes set through adaptations to local environments, and responses may therefore vary by genetic and environmental factors, or interaction among the two (genotype × environment) (Schaffer 1974, Crowe and Underwood 1999, Kawecki and Ebert 2004). Performance of relocated populations that conform to novel environments should be equally fit and allow for a synchrony of life history events with respect to resident populations at the site of relocation. In contrast, performance of populations relocated to novel environments might be constrained genetically (Crowe and Underwood 1999, Weeks et al. 2011), which can lead to a state of stress if exposed to conditions that exceed their capacity for adaptive responses (Petes et al. 2008) and, in turn, mismatch in life history events (e.g., dyssynchrony in spawning periods or lifetime reproductive success) between transplanted and resident populations. Establishment of populations with insufficient variation in performance traits may be of limited evolutionary potential that could lead to poor performance under future changing environments (Weeks et al. 2011).

Freshwater mussels are sedentary and relatively immobile whose physiological processes (e.g., metabolic, filtration and respiration rates) are dependent upon the environmental conditions within their immediate surroundings (i.e., ectothermic) (Cummings and Graf 2009). Performance and demographic responses of mussel populations, therefore, have evolved to maximize fitness within local or regional environments (Jokela 1997, Haag 2012). There are several variables known to influence physiological processes that might affect performance of relocated mussels. Water temperature and food availability have been found to directly effect survival, growth and reproductive rates (Heinricher and Layzer 1999, Kesler et al. 2007, Galbraith and Vaughn 2009, Galbraith et al. 2012) and other environmental variables, such as hydraulic and substrate stability (Strayer 1999, Hardison and Layzer 2001, Gangloff and Feminella 2007) and variability in stream flow (Inoue et al. 2014), have been linked to population structure and survival rates, respectively. Despite evidence indicating the environment has the propensity to effect performance of mussels, habitat is typically not monitored in relocation studies (Cope and Waller 1995, but see Bolden and Brown 2002). As such, relocating mussels from the conditions in which they have adapted to maximize fitness could lead to unpredictable performance and ultimately changes to demographic rates (Jokela and Mutikainen 1995, Bolden and Brown 2002, Kesler et al. 2007). Therefore, identifying variation in mussel performance and testing their responses to relocation could be important starting point for predicting success in future relocations.

The objective of this study was to examine the effects of relocation on individual and population performance of freshwater mussels. Using reciprocal transplant experimentation, I relocated freshwater mussel populations for conservation purposes (in this case translocation) within the same river and tested: (1) whether the source of variation in mussel performance (i.e., survival probability, shell growth, body condition and reproduction) came from environmental effects, genetic effects or the interaction among both when transplanted to novel sites; and (2) what effects do seasonal variations in environmental conditions have on the performance of local (resident) mussel populations? I predicted that performance traits of relocated mussels was explained more by environmental variation, in contrast to being constrained genetically that would otherwise lead to stressed-induced responses in mussel performance. I also predicted that physiochemical conditions of the sites (e.g., temperature, food availability and flow) will be important determinants of mussel performance.

METHODS

Site Selection

I studied effects of relocation on mussel performance in the San Saba River. Because of their relatively high abundance within the San Saba River, two state-threatened, endemic species (*Quadrula houstonensis*, smooth pimpleback; and *Quadrula petrina,* Texas pimpleback) and two common species (*Quadrula verrucosa*, pistolgrip; and *Amblema plicata*, threeridge) were targeted for this research. Two sites were selected for relocation among six candidate sites within the lower San Saba River based on the following criteria: the sites had (1) similar population and assemblage

characteristics (i.e., abundance, richness, and evidence of recent recruitment) and (2) similar physical habitat characteristics. Population characteristics were determined from unpublished survey data and habitat characteristics were quantified at the six candidate sites in the lower San Saba River (Figure 5). Freshwater mussels generally persist in areas that are stable and protected from scour during higher flows (i.e., hydraulic refugia; Strayer 1999, Hardison and Layzer 2001), and research suggests that relocation is more successful when transplanted to these refugia (Cope et al. 2003). Therefore, I quantified substrate stability at these sites by estimating mean bankfull depth and median substrate particle size from six cross-section profiles and surface water slope from a longitudinal profile at each site. These estimates were then used to empirically derive reach-scale bankfull shear stress (i.e., the force of water on the stream bottom; Statzner et al. 1988):

$$\tau = \rho \times d \times g \times S$$

where τ is shear stress (N/m^2) or force of water in Newton's per unit area, ρ is water density at 25ºC, d is mean depth (m) at bankfull, g is the gravitational constant, and S is the slope of the energy line (i.e., water surface slope, m). Substrate instability index was then quantified by dividing shear stress by median particle size (Gangloff and Feminella 2007). Low and high index values correspond to high and low bed stability, respectively (Cobb and Flannagan 1990).

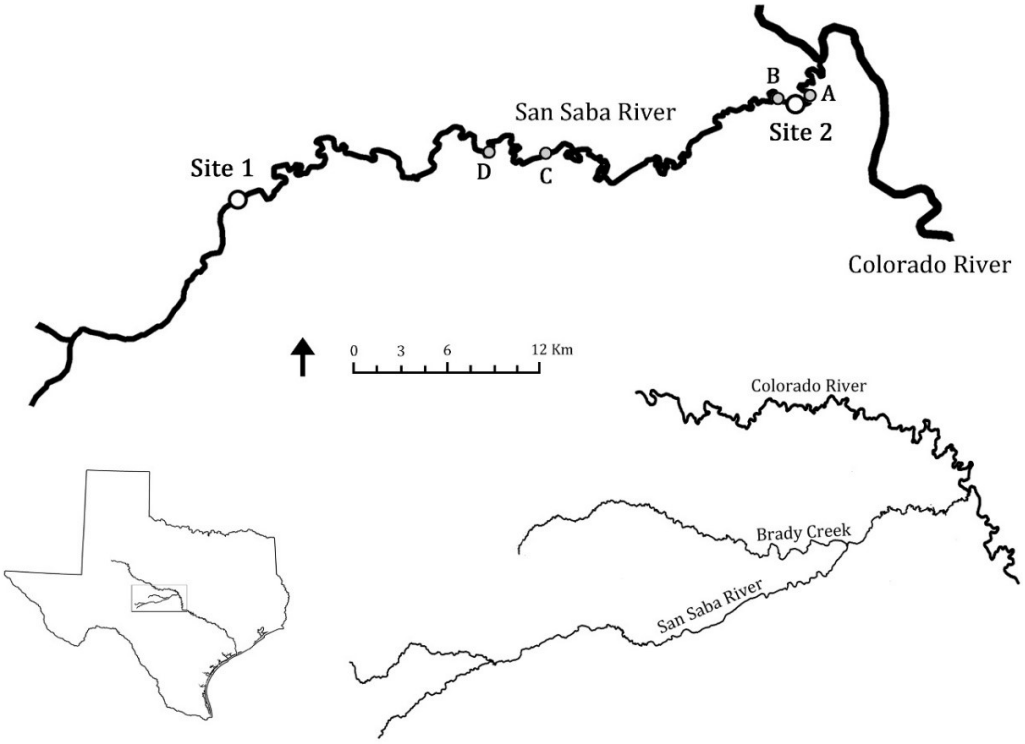

Figure 5. Map of the two relocation sites (Sites 1 and 2, white circles) and four potential relocation sites initially examined for their suitability (Sites A – D, gray circles).

Experimental design

I used reciprocal transplant experimentation by transplanting populations within and between sites to examine performance responses to relocation. Reciprocal transplant studies were traditionally used to investigate local adaptation or variation in phenotypes between populations across heterogeneous environments (Stearns 1992, Jokela and Mutikainen 1995, Crowe and Underwood 1999, Kawecki and Ebert 2004). Because I am interested in exploring transplantation from a conservation perspective (i.e., translocation), my goal was not to determine the extent with which performance can acclimate to specific environments but rather to simulate the procedures used by

conservationists to relocate mussels to existing populations with a lack of in depth knowledge regarding the environmental characteristics of the sites (e.g., temperature and food availability). Conservationists often relocated mussels to sites with existing populations because identifying suitable habitat in the absences of mussels is difficult, so their presence is presumed to be an indication of suitable habitat (Dunn et al. 1999, Cope et al. 2003). Reciprocal transplant experimentation will allow me to identify any habitat effects on mussels that might not be readily observable or detectable between sites and, therefore, if performance traits of transplanted mussels acclimate to the conditions of novel sites. Performance of transplanted mussels may be influenced by and respond similar to the (1) site of origin, indicating a stronger genetic basis in the responses of performance traits (Figure 6a); (2) site of destination, indicating a stronger environmental basis (Figure 6b); or (3) influenced by the interaction between the two (Figure 6c, d).

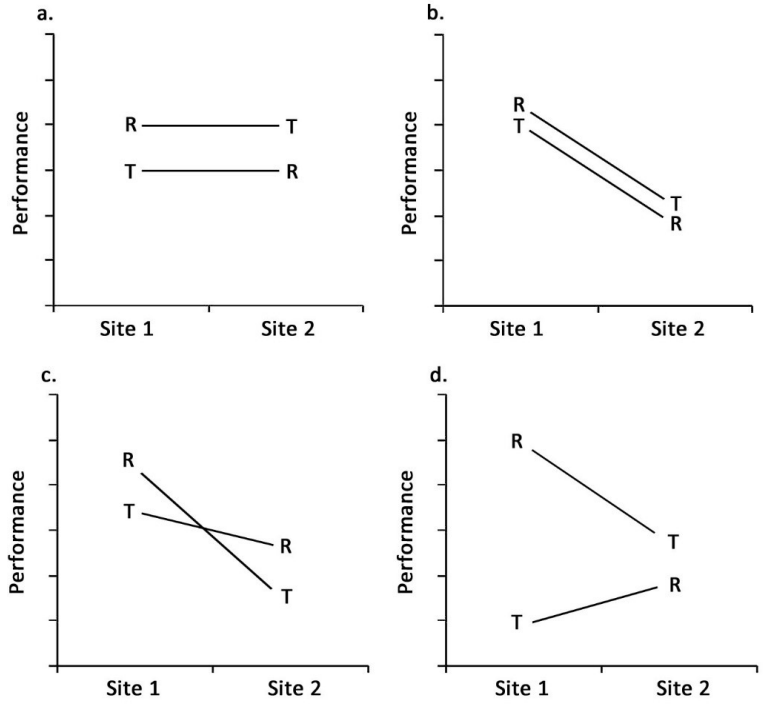

Figure 6. Time-invariant hypothetical outcomes of performance between two reciprocally transplanted populations (R = resident, T = transplant). Performance between resident and transplant populations which (a) is constrained more by genetic factors, (b) influenced by local environmental factors, or (c and d) the interaction among the two. Adapted from Reed and Martiny (2007) and Kawecki and Ebert (2004).

Adult mussels of similar sizes from the target species were collected at the sites and assigned into 1 of 5 treatment groups randomly: (1) resident, non-gametes; (2) transplant, non-gametes; (3) resident, gametes; (4) transplant, gametes and (5) resident, undisturbed (Table 7). Resident treatment groups were used as controls to compare survival, growth and reproduction (i.e., gamete production) of mussels with transplant treatment groups. Non-gamete treatment groups were used to assess survival, growth and body condition, whereas gamete treatment groups were only used to assess reproduction, because the method used to assess reproduction (i.e., the syringe technique) could have

potential additive negative effects on survival and growth of transplanted mussels. The "undisturbed" treatment was established at Site 1 to control for the effects of handling on survival. Each treatment (except for the undisturbed treatment) was studied at the sites using randomized, nested block design. Non-gamete treatment groups consisted of 5 replicates of 8 individuals per species (40 mussels total), except one treatment (transplant from Site 1 → Site 2) that received 30 individuals (Table 7). Gamete treatment groups consisted of one replicate with roughly twice the number of mussels as non-gamete treatment groups (Table 7).

Table 7. Treatments (resident, transplant, undisturbed), their respective sample sizes (n), and mean (± SD mm) initial shell length (sl) used to study the effects of relocation on mussel performance in the San Saba River, Texas. Sample sizes either consisted of replicates with the number of individuals per replicate in parentheses (non-gamete treatment groups) or total number of individuals (gamete treatment groups).

	A. plicata		***Q. houstonensis***		***Q. petrina***		***Q. verrucosa***	
Treatment	**n**	**sl**	**n**	**sl**	**n**	**sl**	**n**	**sl**
Site 1								
Non-gamete								
Resident	5 (8)	79.7 ± 13.2	5 (8)	59.5 ± 4.4	5 (8)	61.8 ± 6.5	5 (8)	92.1 ± 13.9
Transplant	--	--	5 (8)	49.5 ± 6.6	5 (8)	51.5 ± 6.8	5 (8)	81.1 ± 16.5
Undisturbed	--	--	--	--	--	--	5 (8)	--
Gamete								
Resident	--	--	--	--	64	61.9 ± 5.0	96	98.5 ± 14.3
Transplant	--	--	--	--	--	--	96	78.5 ± 16.1
Site 2								
Non-gamete								
Resident	--	--	5 (8)	46.8 ± 5.1	5 (8)	48.8 ± 5.4	5 (8)	84.3 ± 18.9
Transplant	5 (8)	89.4 ± 5.7	--	--	5 (6)	57.3 ± 7.9	5 (8)	96.6 ± 14.7
Gamete								
Resident	--	--	--	--	105	49.0 ± 7.0	96	85.3 ± 18.7
Transplant	--	--	--	--	64	58.6 ± 7.0	96	89.5 ± 18.5

In July 2012, mussels were collected at each site, marked with 12.5-mm passive integrated transponder (PIT) tags (Biomark, Inc., Boise, Idaho) using marine epoxy putty and randomly assigned to treatment groups (Table 7). PIT tags were used to increase the detection of mussels for a more accurate estimation of survival (Kurth et al. 2007). Once marked and assigned to the treatments, initial measurements of shell length (mm) and wetted weight (g) were recorded. Mussels from resident treatments were then placed into 16 or 25 m^2 plots with each replicate placed into $1m^2$ subplots (blocks) in which densities were kept at 8 mussels/m^2. Unpublished survey data indicated density within the study plots was twice that of natural densities in lower San Saba River (~4.5 mussels/m^2). Mussels belonging to the undisturbed treatment were not placed into the plots and were studied at the location where they were found. These mussels (n = 40) were located visually during initial surveys and marked with a flag so they could be processed *in situ*. PIT tags were affixed to these individuals by removing them from the substrate for ≤ 1 min and placing them back into their exact location and orientation. These mussels were not disturbed for the remainder of the study. This treatment was used to compare survival with the resident treatment at the same site and will allow me to test the effects of (1) handling during processing and (2) transplanting resident mussels within the same site (i.e., to the study plots). Only individuals of *Q. verrucosa* were assigned to the undisturbed treatment and this species was used as a representative organism for the other species (Table 7).

Once the mussels assigned to transplant treatment groups were initially processed, they were prepared for transport by wrapping them in a paper napkin,

submerging them in the river and placing them inside an unsealed plastic bag. They were then placed into an ice chest with the bottom of the chest lined with ice, and multiple layers of cardboard were inserted above the ice so that the mussels did not come into direct contact with the ice or ice water. This procedure was used to minimize stress during handling and transport by keeping mussels relatively cool and completely moist (Chen et al. 2001, Yusufzai et al. 2010). Mussels were transported by vehicle to and from each site for a trip time of 45 min one way. Upon arriving at a site, mussels were unwrapped and placed into the study plots so that they could be monitored along with resident treatment groups.

Mussel Performance

Survival, growth and body condition were assessed in syringe and non-gamete treatments approximately every 3 – 4 mo for two years. During each post-relocation assessment, mussels were collected by locating PIT tags using an antenna receiver, combined with visual and tactile searches within and around the study plots. The entire site was searched for mussels, and searching ended once all individuals were recovered or PIT tags were no longer detected with the antenna receiver, which typically took 1 – 3 d per site. Mussels were placed into mesh bags submerged in areas with sufficient flow. To minimize handling during processing, mussels were brought to shore in a small group (< 20 individuals) at any one time and removed from the water for ≤ 7 min. During emergence, I recorded the encounter history for each mussel (not encountered, live encounter or dead recovery). Shell length (mm) and wetted weight (g) were measured and used to estimate yearly proportional shell growth (mm/yr) and Fulton's K body

condition factor (see Chapter II). Gamete production was assessed using the syringe technique by extracting gonadal fluid (~0.5ml) from 8 – 10 mussels every 4 – 6 weeks for the first year of the study. Gonadal fluid was only sampled once for a given mussel. Gamete production was quantified for males using a hemocytometer to estimate sperm concentration (no./ml) and quantified for females by estimating the proportion of eggs within the 80th percentile, based on mean diameter of oocytes (µm) measured with an ocular micrometer (Galbraith and Vaughn 2009).

Environmental Variables

To examine the effects of seasonal environments on mussel performance, I quantified characteristics of food availability, water temperature and stream flow. Food availability was quantified by estimating chlorophyll *a* (µg/L) and fine particulate organic matter (mg/L; POM; < 250 µm) concentration from the water column and percentage of total benthic organic matter (BOM) from sediment samples collected from each site (Wallace et al., 1996; Galbraith and Vaughn, 1999). Three 50- and 100-ml water samples were collected approximately once a month from each site, passed through a 250-µm sieve and filtered through glass-fiber-filters (0.7 µm porosity) *in situ* to estimate chlorophyll *a* and POM in the laboratory, respectively. Three sediment samples were collected near the study plots on a bi-monthly bases to estimate BOM. I attempted to use cores to collect sediment sample; however, the complexity of the substrate (i.e., sand, small and large gravel, and cobble) was such that cores could not be inserted into the substrate. Therefore, a shovel was used to extract sediment samples that were stored frozen in plastic bags until processing. In the laboratory, Chlorophyll *a* was

extracted from filters (50-ml samples) using the acetone method and measured using standard fluorometric procedures (APHA 1998). Both filters (100-ml samples) and sediment samples (three ~50 ml subsets per sample) were weighed (to the nearest 0.0001 g), dried for at least 3 days, and ashed for 1 h in a muffle furnace (550ºC) to obtain ash-free-dry mass.

Data loggers were deployed within plots to record water temperature (ºC) and level (m). Following Galbraith and Vaughn (2009), minimum and maximum daily water temperatures were used to calculate the number of accumulated degree days (Baskerville and Emin 1969) based on growth limits (10 and 30˚C) of a closely related species (Spooner and Vaughn 2008). Water level was used, along with stream channel characteristics derived from cross-section profiles, to estimate discharge following the slope-area method (Gordon et al. 2004):

$$Q = \frac{1}{\mathrm{n}} A R^{2/3} S^{1/2}$$

where Q is stream discharge (m^3/s), n is Manning's n, A is the cross-section area of flow based on water level, R is hydraulic radius and S is the slope of energy line (i.e., water surface slope) measured from a longitudinal profile. Cowan's method was used to determine Manning's n, which is based on visual qualification of stream characteristics at each site (Gordon et al. 2004). I also estimated median historical discharge from a USGS gauging station located in San Saba, Texas and roughly halfway between the two study sites. Historical discharge was used because it may be an important predictor of mussel performance, particularly through adaptations to seasonal reproductive cycles.

Statistical Analyses

Mark-recapture and regression statistics were used to model mussel performance (i.e., survival, growth, Fulton's K and gamete production) for each of the four study species. To differentiate the effects of mussel performance between sites and treatments based on the reciprocal transplant design of this study, I analyzed each response variable in two ways: (1) analysis by destination, where transplant treatments were contrasted with resident treatments at the destination site, and (2) analysis by origin, where transplant treatments were contrasted with resident treatments at the site of origin (Figure 7). Since all species did not have the treatments to satisfy a true reciprocal transplant, I derived most inferences regarding the source of variation in mussel performance (i.e., genetic basis, environmental basis, or their interaction) from *Q. petrina* and *Q. verrucosa*. Model section was used to test for differences in response variables among treatment and sites, and an information-theoretic approach was used to determine the most parsimonious model within the candidate set based on the lowest ranked Akaike's Information Criterion (AIC) (Burnham and Anderson 2002). Since a hierarchical approach was used in some analyses (see below), only the most parsimonious models based on lowest AIC value were considered (Burnham and Anderson 2002, Arnold 2010).

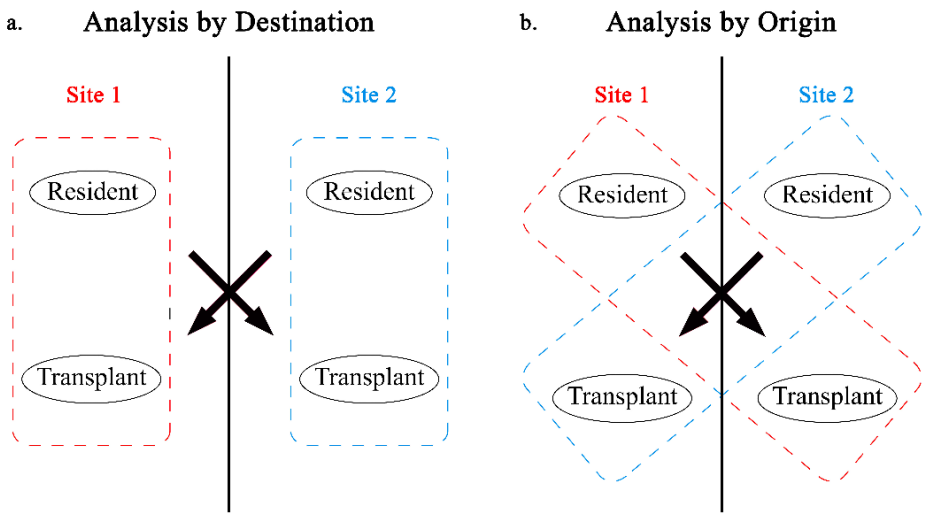

Figure 7. Reciprocal transplant study design comparing treatments (resident and transplant) between the sites in the San Saba River, Texas. (a) Analysis by destination depicts the transplant treatments grouped according to the site of destination, and (b) analysis by origin depicts the transplant treatment according to the site of origin.

Survival probability was analyzed using a live encounter and dead recovery mark-recapture analysis using the R package RMark (Laake 2013) and Program MARK (White and Burnham 1999) (see Chapter II). Since the primary goal of this study was to estimate survival probability (*S*), and because mussels were marked with PIT tags, thereby increasing detection during recapture attempts (Kurth et al. 2007), I considered recapture probability (*p*) and recovery probability (*r*) to be nuisance parameters. Therefore, to limit the number and complexity of models for estimating *S*, I used a hierarchical approach and started by identifying the most parsimonious model for *p* and *r*, with each model having the most complex model of *S* within the candidate set. A total of 15 models were developed to determine trends in recapture and recovery probabilities over time, which included all possible combinations of time and site (interaction

included). Survival probability was parameterized with the variables treatment, time, site and initial shell length (see Chapter II). Preliminary analysis indicated survival was not significantly different among replicates, and therefore, samples were pooled across replicates for each treatment. Shell length was included as an individual covariate to account for differences in length-specific survival rates. Thus, the global model for all parameters was considered: $S_{\text{Treat} \times \text{time} \times \text{Site} + \text{Length}}\, p_{\text{time} \times \text{Site}}\, r_{\text{time} \times \text{Site}}\, F_1$. Once the most parsimonious model for p and r was found, I then modeled S for each species with all possible combinations of the variables. A bootstrap goodness-of-fit test was used to assess model fit, and $\hat{c}$ was approximated to correct for over dispersion (see Chapter II).

Yearly proportional growth and Fulton's K body condition index were analyzed using generalized additive mixed models (GAMM, Zuur et al. 2009, Zuur et al. 2014) and linear mixed models (LMM, Pinheiro and Bates 2006), respectively. Gamete production (sperm concentration and egg proportion) were modeled with generalized additive models (fixed effects only) because each sample represents an individual mussel. All possible combinations of site and treatment were modeled as categorical grouping variables and shell length as a continuous covariate for each parameter. Time was included as a continuous variable for all candidate models. The lowest experimental unit (replicate) was modeled as a random effect to allow slopes to vary in mixed model analyses, accounting for non-independence due to repeated measures over time and randomization of the study plots (i.e., blocks). Response variables were either square root or log transformed when necessary to meet assumptions of equal variances. GAMM were implemented with a Gaussian identity link function and cubic smoothing splines to

characterize the nonlinear relationship between time and growth (Zuur et al. 2009, Zuur et al. 2014). Effective degrees of freedom (edf) and *F*-ratio tests were used to validate nonlinearity between the dependent and independent variables (Zuur et al. 2014).

Analysis of variance (ANOVA) was used to compare mean instability index among the six potential relocation sites (two study sites, plus four initial candidate relocation sites). Tukey's honestly significant difference *post hoc* test was used to contrast mean differences in the instability index. Two-way ANOVA was used to compare mean differences in the environmental variables (temperature, chlorophyll *a*, discharge, POM, and BOM) among sites and season, including an interaction term between site and season. To examine which environmental variables best explained variation in mussel performance, a set of candidate models were constructed for each response variable (survival probability, shell growth, Fulton's K and gamete production). Generalized additive mixed models were used to model environmental variables for shell growth and Fulton's K index, while generalized additive models (no random effects) were used to model gamete production. Each parameter was modeled separately for each site, was scaled and pooled across species, and allowed to vary among a subset of the following variables: mean monthly temperature, cumulative degree days, chlorophyll *a*, POM, BOM, mean monthly discharge, and median monthly historical discharge. If two or more variables were significantly correlated at $| r | > 0.6$ only one of the predictors were included in the analysis. My primary interest here is to determine which of these variables are most important and not necessarily which model or set of variables were more supported relative to models that include or exclude particular variables (Burnham

and Anderson 2002). Therefore, I used relative variable importance $w_{+(j)}$ based on the sum of AIC w for all candidate models that contained a predictor variable x_j (Burnham and Anderson 2002). The higher importance value $0 < w_{+(j)} < 1$ indicates higher support for a variable. The top three variables for each analysis were then plotted with the response variable to measure the strength and direction of the relationship between the independent and dependent variables. The advantages of investigating relative variable importance are that inferences can be drawn beyond variables occurring in the best-approximating model (Burnham and Anderson 2002, Wagenmakers and Farrell 2004), but can only be done when the variables occur in equal numbers throughout the candidate model set. To meet this criterion, each parameter was modeled with all possible combinations of the variables.

RESULTS

A total of 1,167 freshwater mussels were studied through mark-recapture experimentation from two common (*Amblema plicata* and *Quadrula verrucosa*) and two threatened species (*Quadrula houstonensis* and *Quadrula petrina*) in the San Saba River, Texas. Of those mussels, 486 were transplanted between the sites and subsequently monitored to estimate survival probability, yearly proportional growth rate, Fulton's K body condition index and gamete production along with resident treatment groups at the sites. Post-relocation monitoring was conducted for approximately two years and mussel performance was estimated from 7 recapture encounters following the initial transplantation.

Prior to relocation, ANOVA indicated there was a statistically significant difference in mean substrate instability index among sites ($F_{5, 30} = 3.56$, $p = 0.012$). Sites 1 and 2 had the lowest mean values (0.24 and 0.49, respectively) of the 6 sites initially examined in the San Saba River (Figure 5), and were not statistically different, based on a Tukey HSD *post hoc* test. This validates the suitability of the two relocation sites, based on the fact that substrate stability was similar and relatively lower than all of the other sites examined (Figure 8).

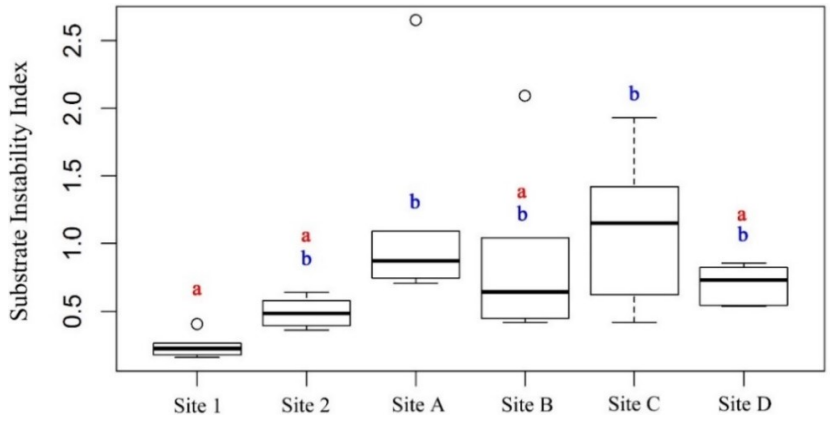

Figure 8. Mean substrate instability values among the two relocation sites (Sites 1 and 2) and four additional candidate sites (Sites A – D). Letters atop each boxplot indicates mean contrasts derived from Tukey's HSD tests.

Survival probability

Of the candidate models used to estimate recapture and recovery probabilities, the best-approximating model indicated that recapture probability varied by time and site ($p_{\text{Time + Site}}$) and recovery probability was constant (r) through time (Appendix A). Recapture probability for both treatments declined slightly over time, but Site 2 was

consistently higher throughout the study (Figure 9a). Survival probability for *Amblema plicata* and *Q. houstonensis* did not vary with time nor shell length and was best explained by variability among treatments, irrespective of analysis by destination or origin (Table 8, Appendix B). For both species, survival probability was significantly higher in resident treatment groups than transplant treatment groups. However, *A. plicata* only differed by 0.04 (Figure 9b), indicating marginal treatment effects, while *Q. houstonensis* differed by 0.12 (Figure 9c). The best-approximating model for *Quadrula petrina* varied by time and site in analysis by destination and varied by time in analysis by origin (Table 8), indicating that *S* was more effected by the environmental conditions of the relocation site. Survival probability was consistently higher at Site 2, which ranged from 0.95 – 0.81 from the beginning to the end of the study, while Site 1 ranged from 0.86 – 0.57 (Figure 9d). When analyzed by origin, *S* ranged from 0.91 – 0.69 for treatments for this species (Figure 9e).

The best-approximating models in the candidate model sets for *Quadrula verrucosa* indicated that *S* varied by site and shell length when analyzed by destination and varied by treatment when analyzed by origin (Table 8). Survival probability at Site 2 increased slightly during the study and ranged from 0.95 – 0.97, whereas *S* decreased with time at Site 1 and ranged from 0.98 – 0.70. Conversely, there was a significant site by treatment interaction in analysis of origin (Table 8), driven in part by the significantly lower *S* for the transplant treatment from Site 2 → Site 1 (Figure 9h), which ranged from 0.99 – 0.57. Survival probability for both resident and transplant treatments at Site 2 were relatively similar (Figure 9h). Overall, these results suggest a strong environmental

component to survival probability with only some evidence of interactive responses between genetic and environmental factors. These models also suggest that *S* varied as a function of shell length for *Quadrula verrucosa.* Survival probability was generally lower for mid-size individuals and higher for larger individuals (Figure 9f). Furthermore, the candidate model set used to compare survival between the resident and undisturbed treatments at Site 1 failed to detect any treatment effect, suggesting handling did not influence survival (Table 9).

Table 8. Summary of the top-ranked models (based on lowest AIC) for mark-recapture and regression analyses of mussel performance (survival probability, shell growth, Fulton's K, and gamete production) for the four study species (*Amblema plicata*, *Quadrula houstonensis*, *Quadrula petrina* and *Quadrula verrucosa*) in the San Saba River, Texas. Candidate model sets were parameterized with the variables time, treatment, site, and initial shell length. Time was included in all models for parameters growth, Fulton's K, sperm concentration (Sperm Conc.) and proportion of eggs within the 80th percentile (Egg Prop.). Complete candidate model sets for each for the parameters can be found in the appendices: Appendix B (survival), Appendix C (growth), Appendix D (Fulton's K) and Appendix E (reproduction).

	A. plicata	***Q. houstonensis***	***Q. petrina***	***Q. verrucosa***
Survival				
Destination	Treat	Treat	Time + Site	Site × Time + Length
Origin	---	Treat	Time	Site × Time × Treat + Length
Growth				
Destination	Time × Treat + Length	Time + Site	Time × Site × Treat	Time + Site × Treat
Origin	---	Time	Time + Site + Treat	Time × Site × Treat
Fulton's K				
Destination	Time × Treat + Length	Time × Site × Treat + Length	Time + Site + Treat + Length	Time × Site × Treat
Origin	---	Time × Site × Treat + Length	Time × Site × Treat + Length	Time × Site × Treat
Reproduction				
Sperm Conc.				
Destination	---	---	Time × Treat + Length	Time + Treat + Length
Origin	---	---	Time × Treat + Length	Time × Site + Length
Egg Prop.				
Destination	---	---	Time × Site	Time + Length
Origin	---	---	Time + Site	Time + Length

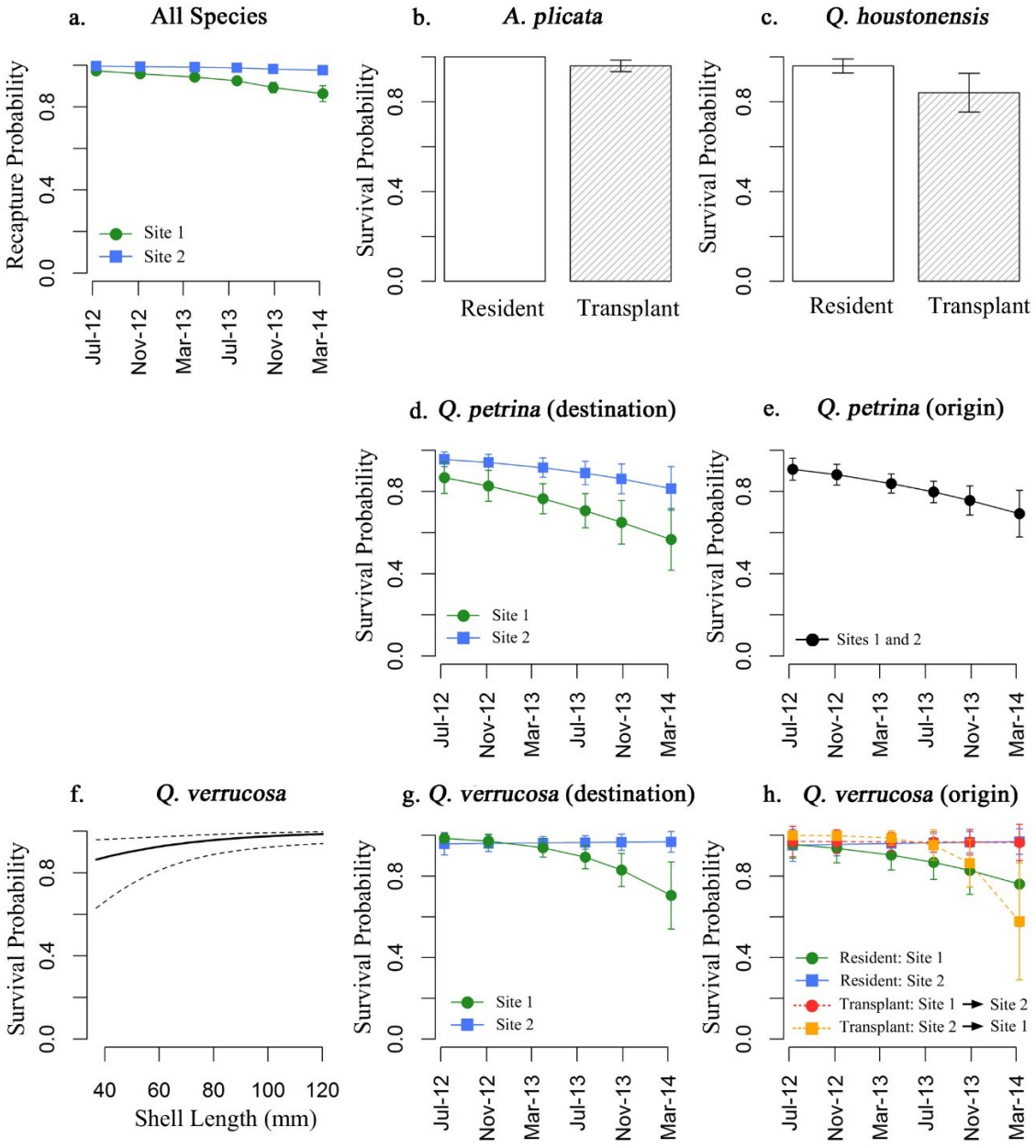

Figure 9. Parameter estimates for recapture probability (a) and survival probability (b – h). Boxplots represent time invariant survival probability for (b) *Amblema plicata* and (c) *Quadrula houstonensis* (S_{Treat}). (d) Analysis by destination for *Quadrula petrina* depicts differences among sites over time, while (e) analysis by origin depicts survival probability over time (S_{Time}). (f) Survival probability for *Quadrula verrucosa* varied by shell length for both analyses, and (g) was best explained by time and site when analyzed by destination ($S_{\text{Time + Site}}$) and (h) the interaction among time, site and treatment when analyzed by origin ($S_{\text{Time} \times \text{Site} \times \text{Treat} + \text{Length}}$).

Table 9. Eight candidate models used to examine the effects of handling on survival probability of mussels in the San Saba River, Texas. Treatment effects included both resident and undisturbed treatments for *Quadrula verrucosa* at Site 1.

Model	k	QAICc	ΔQAICc	w_i
$S_{\cdot}\ p_{(\mathrm{Time})}\ r_{\cdot}\ F_1$	4	153.92	0.00	0.55
$S_{\cdot}\ p_{(\mathrm{Time})}\ r_{(\mathrm{Time})}\ F_1$	5	155.89	1.97	0.21
$S_{(\mathrm{Time}\times\mathrm{Treat})}\ p_{(\mathrm{Time})}\ r_{\cdot}\ F_1$	7	156.66	2.74	0.14
$S_{(\mathrm{Time}\times\mathrm{Treat})}\ p_{(\mathrm{Time})}\ r_{(\mathrm{Time})}\ F_1$	8	158.65	4.74	0.05
$S_{\cdot}\ p_{\cdot}\ r_{\cdot}\ F_1$	3	159.88	5.96	0.03
$S_{\cdot}\ p_{\cdot}\ r_{(\mathrm{Time})}\ F_1$	4	161.84	7.93	0.01
$S_{(\mathrm{Time}\times\mathrm{Treat})}\ p_{\cdot}\ r_{\cdot}\ F_1$	6	162.58	8.67	0.01
$S_{(\mathrm{Time}\times\mathrm{Treat})}\ p_{\cdot}\ r_{(\mathrm{Time})}\ F_1$	7	164.56	10.65	0.00

Shell growth

Yearly proportional shell growth generally declined at a decreasing rate over time for all treatment groups. Within the candidate model set for *A. plicata*, the best-approximating model indicated shell growth was best explained by the interaction between time and treatment with the additive effects of length (Table 8, Appendix C). Mean values plotted for this species suggests the magnitude of shell growth rate was significantly greater in the resident treatment at Site 1 than the transplant treatment from Site 1 → Site 2 (Figure 10a). Mean shell growth rate also indicated that the transplant treatment (Site 1 → Site 2) was initially lower during the first few months following transplanting, then both treatments followed similar changes in growth over time, which initially decreased followed by a slight increase during spring and fall (Figure 10a). Of the candidate models used to model shell growth for *Q. houstonensis*, the best-approximating model suggested shell growth varied by time and site in analysis by destination and time in analysis by origin (Table 8, Appendix C), indicating stronger influence of environmental factors on growth. In fact, growth for the transplant treatment

from Site 2 → Site 1 actually increased and was more similar to the site of destination (Figure 10b). Despite this change in growth, all three treatments generally had similar growth rates (Figure 10b). Of the candidate models used to model shell growth for *Q. petrina*, the best-approximating model in analysis by destination varied as a function of the interaction between time, site and treatment (Table 8), while the best-fit model in analysis by origin was similar but lacked the interaction terms (Table 8, Appendix C). The site and treatment terms supported in these models indicate there is a potential interaction between genetic and environmental factors. However, growth between resident treatment at Site 1 and transplant treatment from Site 2 → Site 1 were similar, which would indicate a stronger influence of environmental factors. Support for treatment effects in these models appears to come from the significantly lower shell growth for the transplant treatment from Site 1 → Site 2 (Figure 10c). Of the candidate models used to model shell growth for *Q. verrucosa*, the best-approximating model varied as a function of either additive or interaction between time, treatment and site for both analyses (Table 8, Appendix C). Both transplant treatments were significantly lower than the resident treatments indicating growth for this species responded poorly to novel conditions, potentially due to genetic factors or genetic by environment factors (Figure 10d).

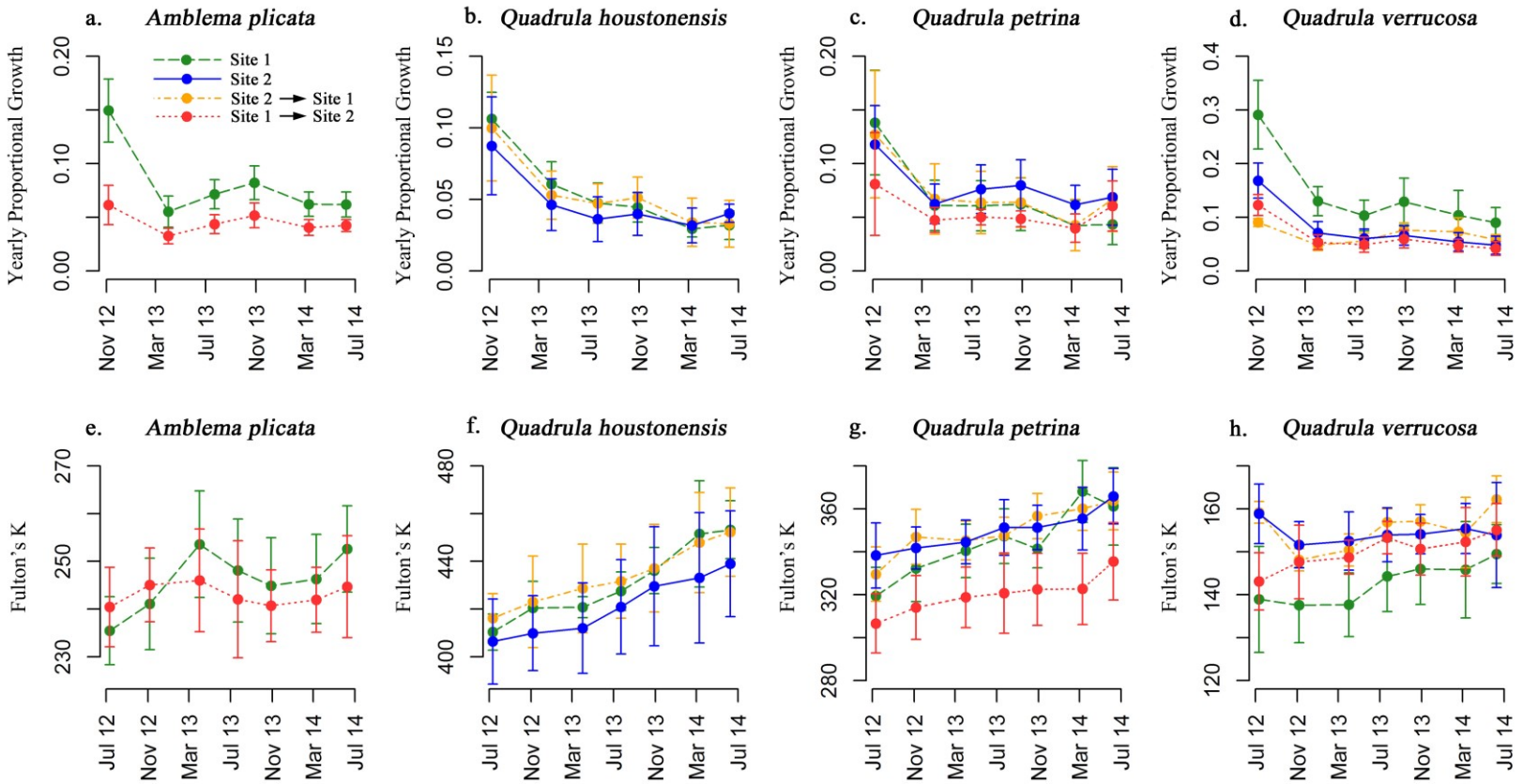

Figure 10. Mean yearly proportional growth rate (a – d) and mean Fulton's K body condition index of freshwater mussels in the San Saba River, Texas.

Fulton's K body condition index

Fulton's K body condition index increased linearly over time, and mixed model analysis supported several differences among treatments and sites. Of the candidate models used to estimate Fulton's K for *A. plicata*, the best-approximating model in analysis by destination was explained by the interaction between site and time and the addition of shell length as a covariate (Table 8). Mean Fulton's K values plotted over time suggested the one transplant treatment modeled for this species (Site 1 → Site 2) was initially higher in the first several months of the study, followed by a transition in which the resident treatment at Site 1 was higher for the remainder of the study (Figure 10e). The best-approximating models for *Q. houstonensis* varied as a function of the interaction between time, site and treatment and shell length as an additive covariate for both analyses by destination and origin. Mean Fulton's K values between the non-transplanted treatments suggest that the resident treatment at Site 1 was higher. The transplant treatment from Site 2 → Site 1 was also higher than the resident treatment at Site 2, the site with which it originated, but increased at a slightly lower rate than the resident treatment at Site 1 (Figure 10f). These results suggest the transplant treatment responded to the conditions at the destination site based on the increase in magnitude of Fulton's K index, but support for treatment effects from both analyses of destination and origin suggests a difference in the rate of increase among the treatments (Figure 10f).

Of the candidate set used to model Fulton's K for *Q. petrina*, the best-approximating model in analysis by destination varied as a function of time, site, treatment and shell length. The best-approximating model in the analysis by origin was

similar but included interaction terms between time, site and treatments. These results suggest that the condition of transplanted mussels might not have responded to environmental conditions at the transplanted sites, therefore differing from the resident at the site of origin and the site of destination. Mean Fulton's K values suggest the transplant treatment from Site 2 → Site 1was significantly lower and increased at a slower rate than the other transplant (Site 2 → Site 1) and resident treatments, which appeared to follow a similar trend over time (Figure 10g). Thus, treatment effects likely driven solely by the slower rate of increase for this one transplant treatment (i.e., Site 2 → Site 1). The full interactive model between time, site, and treatment and length as an additive factor were well supported in the confidence sets for *Q. verrucosa* in both analysis by destination and analysis by origin (Table 8). Mean Fulton's K for the resident treatment at Site 2 and the transplant treatment from Site 2 → Site 1 followed a similar pattern, which initially decreased. In contrast, the resident at Site 1 was significantly lower than the other treatments and increased at a similar rate to the transplant treatment from Site 1 → Site 2 (Figure 10h), suggesting that variation in mussel condition might come from sources other than environmental factors.

Gamete production

A total of 617 mussels was initially assigned to gamete treatment groups. Due either to incomplete recaptures or mortality, gonadal fluid was sampled from 513 of these individuals. However, gamete production was only quantified from a subset of gonadal fluid samples because the prevalence of castrating trematode parasites was relatively high. For *Q. petrina,* 63% of gonadal samples per treatment group contained

trematodes. Gamete production could not be estimated from these samples because neither sperm nor eggs were detected, leaving only the remaining 37% of samples on which to draw inferences. Gamete estimates for *Q. petrina* were therefore limited to only several periods throughout the first year of the study but the peak timing of gamete production was captured within my estimates. Prevalence of trematode infection for *Q. verrucosa* was less severe, occurring on average in 17.1% of samples per treatment groups, leaving the remaining 82.9% of samples to quantify gamete production.

Several differences in sperm concentration and proportion of eggs within the 80th percentile (egg proportion) were observed between the sites and treatments for both *Q. petrina* and *Q. verrucosa* (Appendix E). The best-approximating model in the candidate set for *Q. petrina* sperm concentration varied as a function of the interaction between time and treatment, including length as a covariate, in both analyses by destination and origin (Table 8). In contrast, egg proportion for *Q. petrina* varied only as a function of time and site in the best-approximating model for both analyses (Table 8). Inferences regarding the ability of gamete production to acclimate to novel environments were impossible to make for this species because of small sample sizes and absence of a second transplant treatment (Site 2 → Site 1). However, gamete production of the one transplant treatment appeared not to be affected by relocation (Figure 11a, b). In general, sperm concentration peaked between January and February in all three treatments examined, and it was highest in the one transplant treatment (Site 1 → Site 2) for this species and lowest in the resident treatment at Site 2 (Figure 11a). Sperm concentration peaked as high as 600,000 sperm/mL. Once sperm concentration peaked, it steadily

declined until reaching its lowest point in late spring and early fall and then began increasing throughout the fall and winter (Figure 11a). Egg proportion generally peaked between March and May, several months later than sperm concentration, and sharply declined where it reached its lowest point in early Fall (Figure 11b). Each treatment generally followed this pattern but peak sperm concentration was highest in the resident treatment at Site 2, whereas egg proportion in the resident treatment at Site 1 and transplant treatment (Site 1 → Site 2) followed similar patterns and peaked at a lower rate than the resident treatment at Site 1 (Figure 11a, b). Similar trends in sperm concentration between the resident treatment at Site 1 and transplant treatment (Site 1 → Site 2) reflect the lack of support for treatment effects in the best-approximating model. Sperm concentration also varied as a function of shell length, but the relationship between sperm concentration and shell length was weakly correlated ($r^2 = 0.15$)

The best-approximating model in the candidate set for *Q. verrucosa* sperm concentration varied as a function of time, treatment and length in analysis by destination and time, site and length in analysis by origin (Table 8). Mean sperm concentration estimates for *Q. verrucosa* generally peaked between November and January, reaching sperm concentrations as high as 4 million sperm/mL, followed by a relatively sharp decline to April (Figure 11c). Sperm production experienced a period of relatively low activity during summer months and subsequently increased in early fall (Figure 11c). Sperm concentration was substantially higher in both transplant treatments than the resident treatments and even peaked slightly earlier in one transplant treatment (Site 1 → Site 2) (Figure 11c). In contrast, *Q. verrucosa* egg proportion varied only by

time and shell length in analyses by destination and origin, indicating no differences among the treatments and sites (Table 8). Mean egg proportion peaked between December and February, declined at the onset of summer and increased early fall (Figure 14d). Support for initial shell length indicated gamete production varied by mussel size; however, the strength of these relationship for both sperm concentration ($r^2 < 0.00$) and egg proportion ($r^2 < 0.00$) were weak.

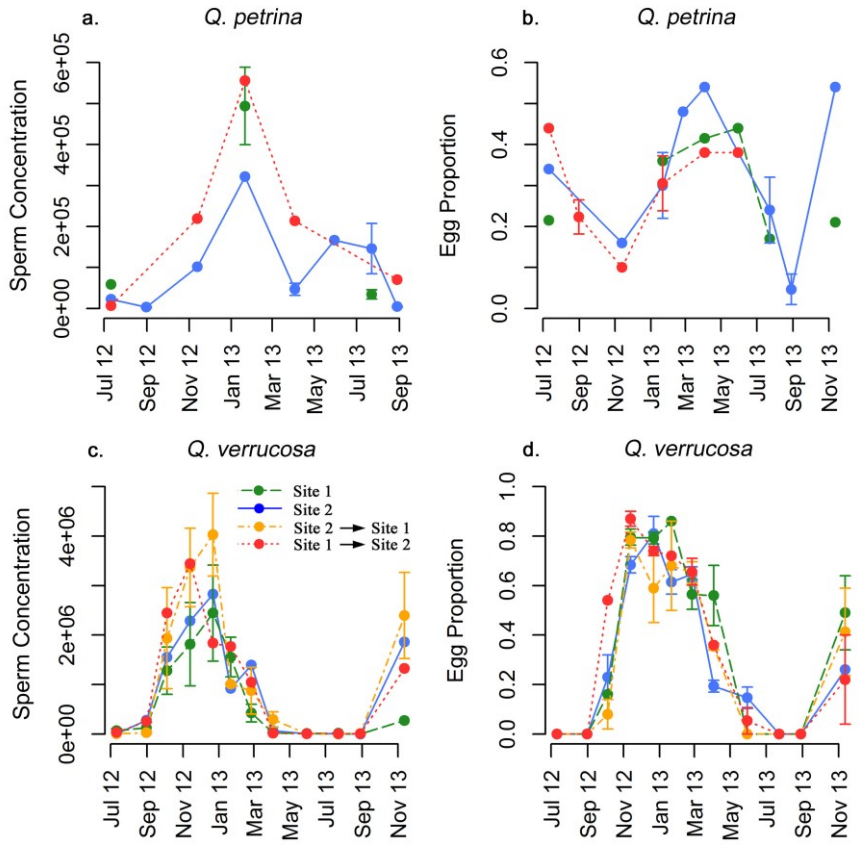

Figure 11. Variation in gamete production of mussels among species and treatments in the San Saba River, Texas: (a) *Quadrula petrina* sperm concentration (no./mL), (b) *Q. petrina* proportion of eggs within the 80th percentile (egg proportion), (c) *Quadrula verrucosa* sperm concentration, (d) and *Q. verrucosa* egg proportion.

Environmental factors influencing mussel performance

Two-way ANOVA indicated there were few differences in environmental variables examined between sites. Chlorophyll *a* was the only variable that was significantly different between sites and seasons (Table 10). Site 2 had a greater mean chlorophyll *a* concentration, reaching nearly twice the amount at Site 1 at some points, and was more variable than Site 2 (Figure 12a). None of the other environmental variables (temperature, discharge, POM and BOM) were statistically significant between the two sites (Table 10; Figure 12b, c, d, and f). Several of the environmental variables, however, were significantly different across seasons, including temperature and discharge (Table 10; Figure 12d, f). No significant interaction was supported in any of the variables tested.

Table 10. Two-way ANOVA table of the environmental variables compared among sites and seasons, including degrees of freedom (df), *F* statistic and p-value (*p*).

Habitat Variables		df	***F***	***p***
Temperature	Site	1	0.71	0.40
	Season	3	62.48	0.00
	Site × Season	3	0.04	0.99
Chlorophyll *a*	Site	1	13.70	0.00
	Season	3	4.59	0.01
	Site × Season	3	1.42	0.27
Discharge	Site	1	2.52	0.11
	Season	3	52.39	0.00
	Site × Season	3	1.97	0.12
Particulate Organic Matter	Site	1	0.25	0.63
	Season	3	1.37	0.28
	Site × Season	3	0.26	0.85
Benthic Organic Matter	Site	1	0.08	0.79
	Season	3	0.17	0.92
	Site × Season	3	0.21	0.89

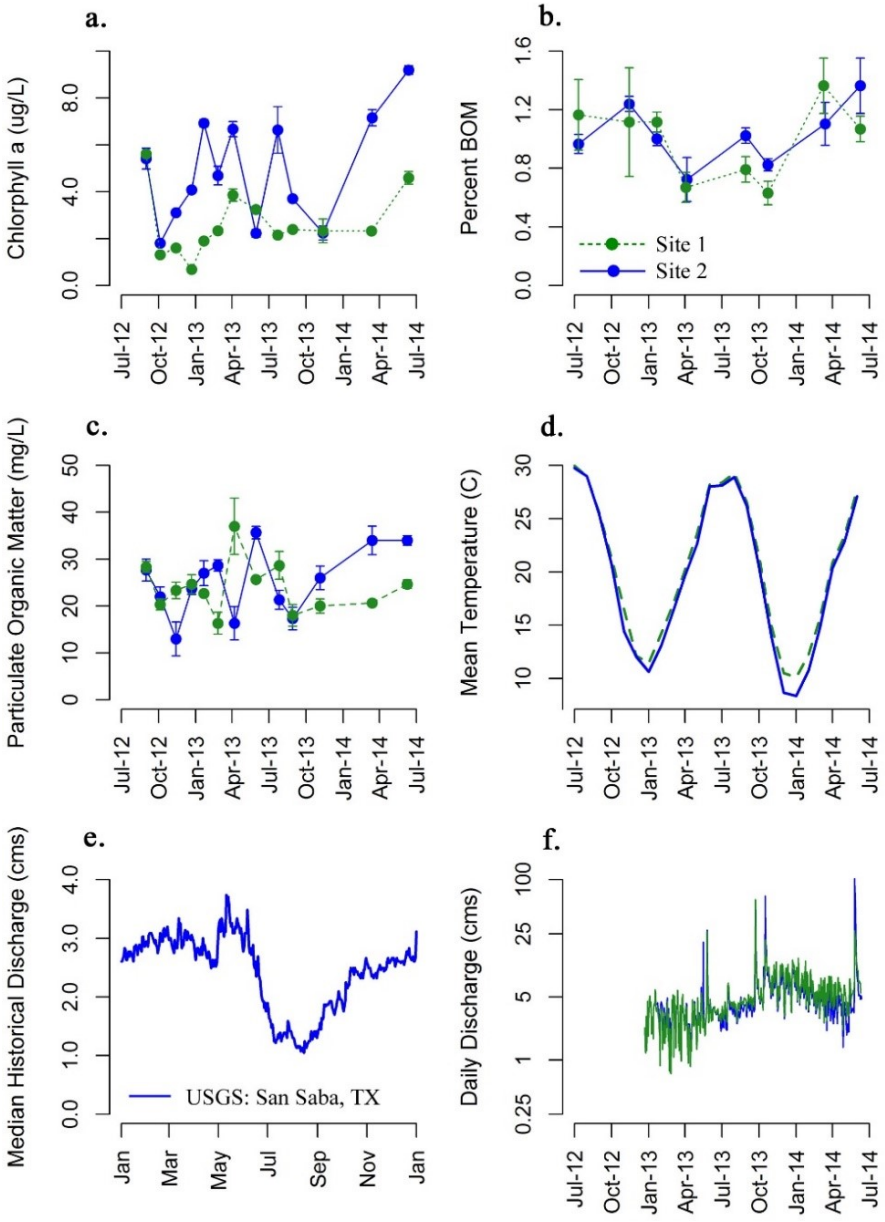

Figure 12. Environmental variables estimated for each relocation site in the San Saba River, Texas: (**a**) chlorophyll *a* concentration, (**b**) percentage of total benthic organic matter (BOM), (**c**) fine particulate organic matter concentration (POM; 7 – 250 µm), (**d**), mean monthly temperature, (**e**) median historical discharge (cms = m^3/s) estimated from 1915 – present at the USGS gauging station at San Saba River, Texas (08146000), and (**f**) mean daily discharge estimated from data loggers.

Survival probability, yearly proportional shell growth and Fulton's K body condition index were modeled with 6 environmental variables representing several biotic and abiotic habitat characteristics: water temperature, cumulative degree days, chlorophyll *a*, BOM, POM and discharge. Relative variable importance $w_{+(j)}$ indicated that no one variable substantially out supported the others in the candidate model sets for survival probability (Table 11). The most supported variable at Site 1, temperature ($w_{+(j)}$ = 0.66), was 1.4 times more supported than chlorophyll *a* ($w_{+(j)}$ = 0.48) and 1.6 times more supported than cumulative degree days ($w_{+(j)}$ = 0.42) (Table 11). Each of these variables were negatively related to survival probability, though the relationships were not strong (Figure 13a – c). BOM ($w_{+(j)}$ = 0.78) was the most supported variable at Site 2 and was 1.4 times more supported than POM ($w_{+(j)}$ = 0.54) and nearly twice as supported than chlorophyll *a* ($w_{+(j)}$ = 0.40) (Table 11). Similar to Site 1, these variables were negatively related to survival probability, with BOM having the strongest relationship among them (Figure 13d – f). For shell growth, cumulative degree days ($w_{+(j)}$ = 0.80) was most supported at Site 1 and was approximately 1.3 more supported than both BOM ($w_{+(j)}$ = 0.62) and discharge ($w_{+(j)}$ = 0.60) (Table 11). The strongest relationship among these variables with shell growth was cumulative degree days (r^2 = 0.36), which declined non-linearly with increasing heat accumulation (Figure 13j). Discharge also declined non-linearly with shell growth (r^2 = 0.23), whereas BOM was highest at intermediate values but was weakly related to shell growth (r^2 = 0.02) (Figure 13h). At Site 2, BOM ($w_{+(j)}$ = 0.97) was the most supported variable and was 1.6 times more supported than cumulative degree days ($w_{+(j)}$ = 0.60) and chlorophyll a ($w_{+(j)}$ = 0.60) (Table 11).

However, the relationship between BOM and shell growth (r^2 = 14) was weaker than both chlorophyll *a* (r^2 = 0.48) and cumulative degree days (r^2 = 0.36) (Figure 13j – l). Chlorophyll *a* and cumulative degree days declined non-linearly with shell growth (Figure 13k,l). The most supported variable explaining variability in Fulton's K index at Site 1 was cumulative degree days ($w_{+(j)}$ = 0.96) and was 1.3 times more supported than discharge ($w_{+(j)}$ = 0.72) and 2.9 times more supported than BOM ($w_{+(j)}$ = 0.33) (Table 11). The strong support for cumulative degree days was indicated by a linear increase (r^2 = 0.36) with heat accumulation (Figure 13m). Discharge (r^2 = 0.35) and BOM (r^2 = 0.10) also increased, though nonlinearly, with increasing values of Fulton's K index (Figure 13n, o). Similarly, cumulative degree days ($w_{+(j)}$ = 0.53) was the most supported variable at Site 2, but had nearly as much support as chlorophyll *a* ($w_{+(j)}$ = 0.49) (Table 11). These variables had nearly 1.5 times more support than POM ($w_{+(j)}$ = 0.36) (Table 11). Each variable increased linearly with Fulton's K and was strongly related to chlorophyll *a* (r^2 = 0.48) and weakly related to both cumulative degree days (r^2 = 0.10) and POM (r^2 = 0.10) (Figure 13p – r).

Table 11. Relative variable importance $w_{+(j)}$ of environmental covariates modeled with survival, shell growth and Fulton's K body condition index. Importance values highlighted in bold indicate the top three supported variables.

	Survival		Shell Growth		Fulton's K	
Habitat Variables	**n**	$w_{+(j)}$	**n**	$w_{+(j)}$	**n**	$w_{+(j)}$
Site 1						
Temperature	32	**0.66**	26	0.36	26	0.26
Cumulative Degree Days	32	**0.42**	26	**0.80**	26	**0.96**
Chlorophyll *a*	32	**0.48**	26	0.55	26	0.16
Benthic Organic Matter	32	0.40	26	**0.62**	26	**0.33**
Particulate Organic Matter	32	0.35	26	0.57	26	0.23
Discharge	32	0.38	26	**0.60**	26	**0.72**
Site 2						
Temperature	32	0.34	26	0.43	26	0.35
Cumulative Degree Days	32	0.36	26	**0.60**	26	**0.53**
Chlorophyll *a*	32	**0.40**	26	**0.60**	26	**0.49**
Benthic Organic Matter	32	**0.78**	26	**0.97**	26	0.16
Particulate Organic Matter	32	**0.54**	26	0.43	26	**0.36**
Discharge	32	0.34	26	0.52	26	0.35

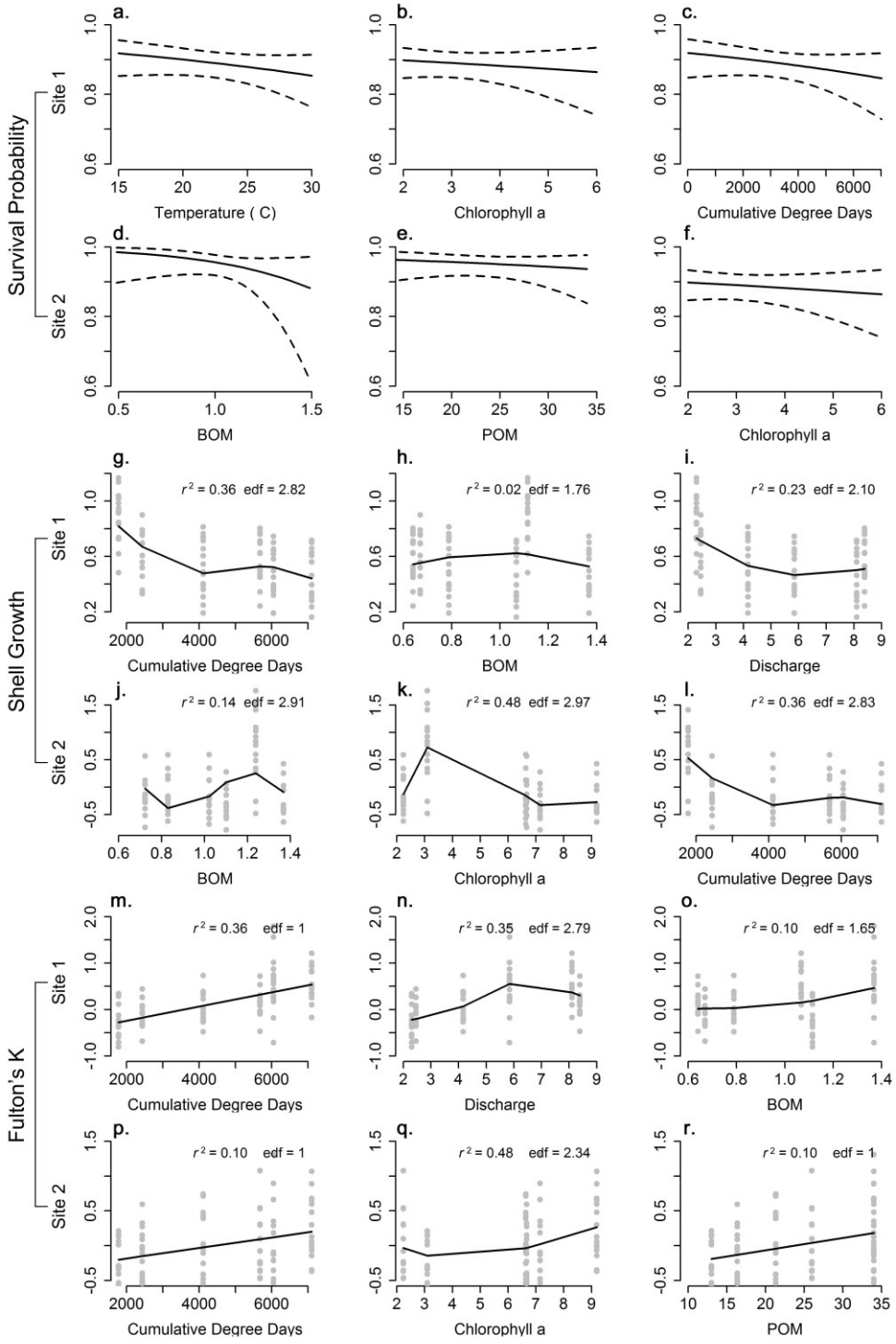

Figure 13. Relationship between environmental variables (temperature, ºC; chlorophyll *a*, µg/L; cumulative degree days, ºC; percent benthic organic matter; particulate organic matter, POM, mg/L; and discharge, m^3/s) and survival probability (a – f), shell growth (g – l), and Fulton's K (m – r). edf = effective degrees of freedom.

Gamete production was modeled with 6 environmental variables: temperature, cumulative degree days, chlorophyll *a*, median historical discharge, particulate organic matter and mean monthly discharge. Support for any one particular variable within the candidate model sets for both sperm concentration and egg proportion (and at either site) was not apparent giving the somewhat small variation in relative importance values (Table 12). Relative variable importance for sperm concentration at Site 1 indicated chlorophyll *a* ($w_{+(j)} = 0.72$) was the most supported variable but was only 1.2 times more supported than POM ($w_{+(j)} = 0.60$) and cumulative degree days ($w_{+(j)} = 0.60$) (Table 12). The strength in the relationship between sperm concentration and these variables were relatively strong. Sperm concentration declined non-linearly with chlorophyll *a* ($r^2 = 0.41$), was generally highest at intermediate values of POM ($r^2 = 0.32$) and varied cyclically with cumulative degree days ($r^2 = 0.44$) (Figure 14a – c). The relationship between sperm concentration and cumulative degree days was such that peak sperm concentration occurred when the rate of heat accumulation was highest. The same three variables were most supported with sperm concentration at Site 2 but were ranked differently. Cumulative degree days ($w_{+(j)} = 0.67$) was most supported followed by POM ($w_{+(j)} = 0.63$) and chlorophyll *a* ($w_{+(j)} = 0.61$) (Table 12). The relationship between sperm concentration and cumulative degree days was relatively strong ($r^2 = 0.39$) and was similar to Site 1 (Figure 14d). In contrast, sperm concentration and POM ($r^2 = 0.41$) were inversely related, whereby sperm concentration peaked (between November and February for both species) during periods of low POM (Figure 14e). The relationship between sperm concentration and chlorophyll at this site was not clear ($r^2 = 0.05$)

(Figure 14f). Of the variables modeled for egg proportion at Site 1, the most supported variables were median historical discharge ($w_{+(j)} = 0.77$) and chlorophyll a ($w_{+(j)} = 0.76$) and were 1.1 times more supported than POM ($w_{+(j)} = 0.69$). The relationships between egg proportion and historical discharge ($r^2 = 0.47$) and chlorophyll *a* ($r^2 = 0.52$) were relatively strong, while egg proportion was weakly related with POM ($r^2 = 0.18$) (Figure 14g – i). The relationship between egg proportion and historical discharge was negative (Figure 14g), whereby egg proportion was highest during lower median values of discharge that occurred over the past century. Chlorophyll *a* also was negatively related to egg proportion (Figure 14h), which is the similar trend observed for sperm concentration. Chlorophyll *a* ($w_{+(j)} = 0.79$) was the most supported variable explaining variability in egg proportion at Site 2 and was only 1.2 times more supported than temperature ($w_{+(j)} = 0.66$) and mean discharge ($w_{+(j)} = 0.65$) (Table 12). Egg proportion appeared to be highest at intermediate values of chlorophyll *a* ($r^2 = 0.30$), while a negative relationship was observed between egg proportion and temperature ($r^2 = 0.41$) (Figure 14j,k). Similarly, egg proportion was highest at intermediate values of mean discharge but this relationship was not strong ($r^2 = 0.17$) (Figure 14l).

Table 12. Relative variable importance $w_{+(j)}$ of environmental covariates modeled with sperm concentration (Sperm Conc.) and proportion of eggs within the 80th percentile (Egg Prop.) for *Quadrula petrina* and *Quadrula verrucosa* in the San Saba River, Texas. Importance values highlighted in bold are considered to have high support.

	Sperm Concentration		**Egg Proportion**	
Habitat Variables	**n**	$w_{+(j)}$	**n**	$w_{+(j)}$
Site 1				
Temperature	32	0.49	32	0.40
Cumulative Degree Days	32	**0.60**	32	0.53
Chlorophyll *a*	32	**0.72**	32	**0.76**
Particulate Organic Matter	32	**0.62**	32	**0.69**
Discharge	32	0.60	32	0.54
Historical Discharge	32	0.55	32	**0.77**
Site 2				
Temperature	32	0.56	32	**0.66**
Cumulative Degree Days	32	**0.67**	32	0.60
Chlorophyll *a*	32	**0.61**	32	**0.79**
Particulate Organic Matter	32	**0.63**	32	0.47
Discharge	32	0.46	32	**0.65**
Historical Discharge	32	0.49	32	0.58

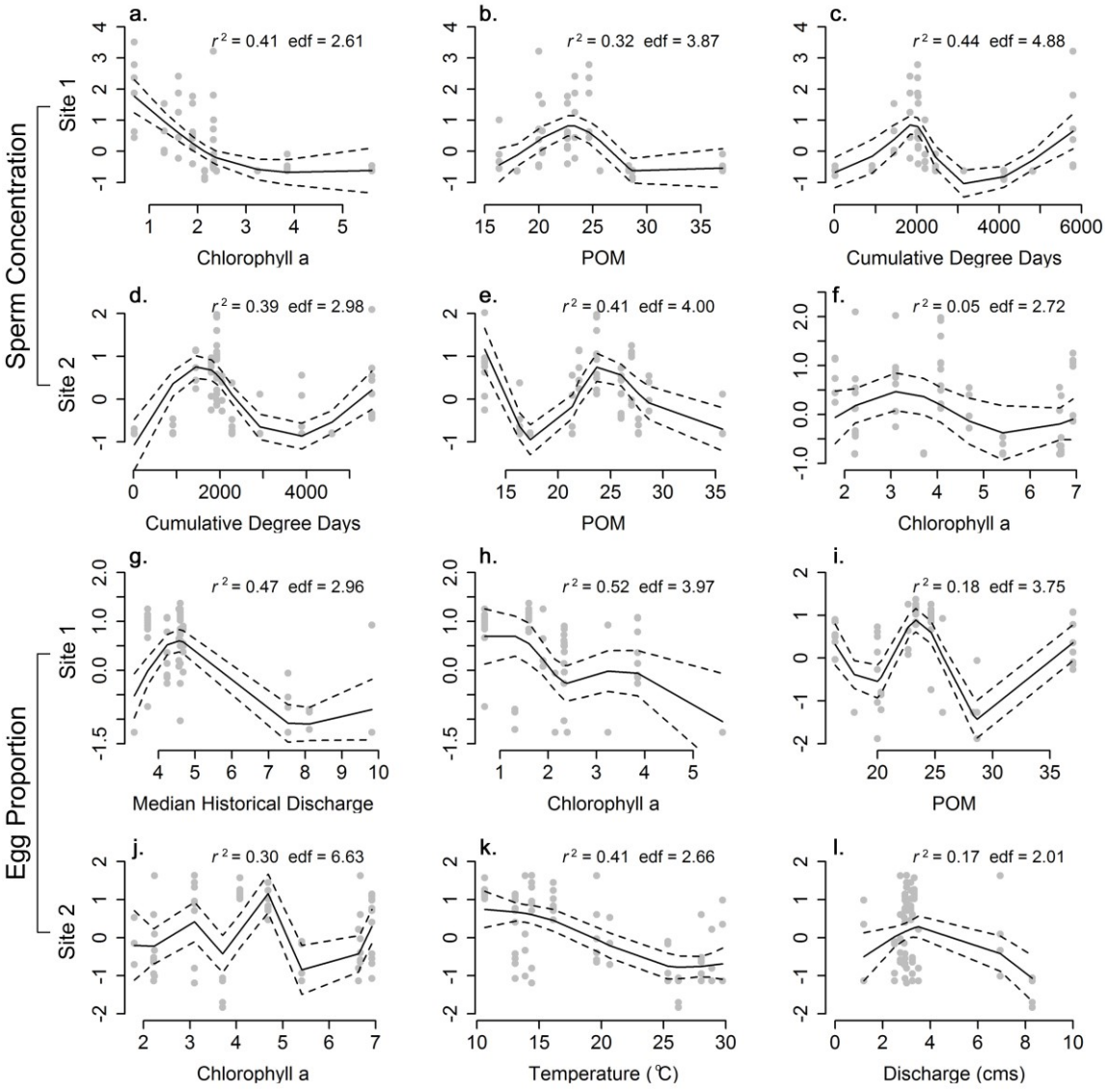

Figure 14. Relationship between sperm concentration (a – f) and egg proportion (g – l) and the top three environmental covariates (based on relative variable importance, Table 12) within each model set for Sites 1 and 2 in the Saba River, Texas.

DISCUSSION

Freshwater mussels and other organisms that growth indeterminately exhibit tradeoffs in key life history traits (e.g., mortality schedules, somatic growth and reproductive effort) as a result of local biotic and abiotic conditions (Jokela 1997), which can, in turn, drive spatial variability in performance and demographic traits (Schaffer 1974, Martone and Micheli 2012). How such traits respond to environmental stimuli may, therefore, differ between populations as a result of genotypic and phenotypic variation through adaptations to local environments (Stearns 1989, Stearns 1992, Kawecki and Ebert 2004). My results suggest performance traits were not significantly constrained by genetic factors when transplanted to novel environments, but these effects depended on the trait and species of mussel in question. Survival probability was most influenced by environmental conditions of the relocation site for both *Quadrula petrina* (smooth pimpleback) and *Quadrula verrucosa* (pistolgrip). This was made evident by similar trends in survival probability between transplant and resident treatments at the site of destination. Survival probability for *Q. verrucosa* actually increased in the transplant treatment from Site 1 → Site 2, mirroring the increase in survival probability of the resident treatment at Site 2. The exception was for the transplant treatment from Site 2 → Site 1, which experienced significantly lower survival probability than the resident treatment at Site 1. This suggests mussels in this transplant treatment (i.e., Site 2 → Site 1) were experiencing stress that might have originated from genetic constraints. *Amblema plicata* (threeridge) and *Quadrula petrina*

(Texas pimpleback) also experienced negative effects resulting from relocation; however, these effects were marginal for *A. plicata*.

In contrast to survival probability, shell growth rate and body condition based on Fulton's K index were influenced more by the interactions between environmental and genetic factors. Shell growth of transplanted mussels for *Q. verrucosa* and *Q. petrina* were significantly different from residents at both sites of destination and origin. Shell growth of transplant treatments for *Q. verrucosa* were lower than both resident treatments, at least during the initial months of the study. Although only one transplant treatment was studied for *Quadrula houstonensis* (smooth pimpleback), shell growth was apparently unaffected when compared to the two resident treatments (Sites 1 and 2). Fulton's K index was slightly more difficult to interpret considering treatment effects were supported in all models. Mean values plotted for *Q. petrina* indicate that only the transplant treatment from Site 1 → Site 2 was lower, while the resident treatment at Site 1 was lower and slopes appear to differ among treatments for *Q. verrucosa*. Because I attempted to limit emersion time (≤ 7 min), estimates for Fulton's K index could be influence by the variability in wet weight measurements that might be dependent upon ambient conditions (e.g., air temperature and specific humidity). Finally, sperm concentration in both species seemed to be minimally affected by local environmental conditions, given the support for treatment effects and differences between transplant and resident treatment at the site of destination. Egg proportions in *Q. petrina* was influenced by both genetic and environmental factors; whereas, neither seemed to affect egg proportions in *Q. verrucosa*. However, inferences regarding the effects of gamete

production for *Q. petrina* are likely not reliable given the large number of trematode infestations and, consequently, limited sample sizes.

My results demonstrate that both environmental and genotypic variation create dynamic responses in freshwater mussel performance (i.e., survival, growth and reproduction). Other experimental studies that transplanted mussels to novel environments have comparatively demonstrated mixed trait responses. For example, Jokela and Mutikainen (1995) found maintenance, reproductive effort and growth in transplanted populations of the duck mussel, *Anodonta piscinalis* (Linnaeus 1758), were more similar to resident individuals at the site of destination. However, they also found that some traits in transplanted populations, particularly reproductive output, were more similar to resident populations at the site of origin, suggesting that transplanted mussels were also influenced by genetic factors. In the same species, Englund and Heino (1996) found valve movement behavior was significantly altered in individuals transplanted from lentic to lotic habitats. Hinch et al. (1986) found that shell shape of fatmucket, *Lampsilis radiata* (Barnes 1823), changed when individuals were introduced to novel habitats, but the rate of somatic growth was better explained by the population of origin. In contrast, somatic growth of eastern elliptio, *Elliptio complanata* (Lightfoot 1786), reciprocally transplanted across lakes with variable food resources was influenced most by conditions in the receiving habitat (Kesler et al. (2007).

Although performance of relocated mussels varied, the extent that genetic factors played a role in these responses were clearly less than environmental factors in my study. In fact, magnitudes of treatment effects were not substantial. This suggests that

transplanted mussels, for the most part, acclimated to novel conditions and overall were not severely stressed. Successful acclimation could be attributed to selection of relocations sites within the same river where differences among environmental conditions and genotypic variation among populations were presumable minimal. Indeed, mussel populations occurring within the same river, as oppose to between rivers, have a higher likelihood of being connected by gene flow via host fish dispersal; a mechanism known to reduce genotypic and phenotypic variation among populations (Kawecki and Ebert 2004). Maintenance of genetic diversity is essential for the conservation of endangered mussels (Haag 2012), and relocation as a management strategy increases the risk to losing heterozygosity through founder effects, genetic drift and outbreeding depression (Villella et al. 1998, Jones et al. 2006, Hoftyzer et al. 2008).

Phenotypic variation is also of conservation importance (Weeks et al. 2011). For example, Preston et al. (2010) found distinct ecophenotypes in shell morphology across rivers for the freshwater pearl mussel, *Margaritifera margaritifera* (Linnaeus 1758), and they hypothesized ecophenotypes may hinder relocation success because shell shape of transplanted populations from one set of conditions may be unsuitable for another. Preston et al. (2010) pointed out that past attempts to translocate populations of *M. margaritifera* across rivers led to low survival (50%); whereas, survival in populations translocated within rivers was higher (80%) (as cited in Preston et al. 2010). My results provide evidence that genotypic and phenotypic variation played a role in performance responses even when transplanting mussels within the same river. The implications of this are these effects may be exacerbated if populations are transplanted across rivers or

geographic areas where conditions are drastically different. Therefore, conservationist should treat performance and demographic traits as phenotypes (Stearns 1992) like other more obvious traits (e.g., shell morphology) when considering relocation (Preston et al. 2010). Transplanting mussels between (and even within) rivers could poses serious risk without prior knowledge of how performance or demographic traits initially vary among populations (Fariñas-Franco et al. 2014).

The negative performance responses observed in this study, although modest, suggest mussels were stressed to some degree when relocated to novel environments, and because performance traits were disproportionately affected, a pattern in stress responses was apparent. Survival probability was least effected by relocation and generally conformed to the conditions of novel environments such that few differences were observed among treatments at the destination sites. In contrast, shell growth rate was negatively impacted in some treatments and experienced reduced growth when transplanted to novel sites. Gamete production (i.e., sperm concentration) for *Q. verrucosa* actually increased in both transplanted populations and might reflect stressed-induced response in attempt to maximize reproductive success. Organisms experiencing a state of stress can exhibit energetic trade-offs among important physiological processes (e.g., immune functions, metabolism, reproduction and growth) by diverting energy from one process to another (Stearns 1992, Petes et al. 2008), and priority rules with respect to the importance of certain functions generally exist among taxonomic groups (Stearns 1992, Jokela 1997). Jokela and Mutikainen (1995) found that individuals of duck mussel, *Anodonta piscinalis* (Nilsson 1823), diverted more energy into reproductive

processes than somatic growth under nutrient limitation. Although my study was not designed to explore priority rules in energy allocation, patterns in performance responses could reflect a trade-off between current reproduction and future reproduction based on higher reproductive output (at least for males) and overall reduction of shell growth (Stearns 1992). Growth of freshwater mussels is of evolutionary significance because larger mussels benefit from greater lifetime reproductive output (Sebens 1987, Haag and Rypel 2011). However, performance responses and resulting trade-offs could vary as a function of seasonal conditions and reproductive timing with respect to the time I initially transplanted mussels (i.e., during the summer when gametogenic activity was relatively low) (Jokela 1996).

While differences in treatment responses are interpreted as having either stronger genetic basis in the responses of performance traits, stronger environmental basis, or an interaction among the two, there are several sources of variation that could confound the main effects found in this study. First, there was no significant difference observed in survival probability between resident handled versus resident unhandled treatments, implying that handling and transplanting mussels into the experimental plots did not negatively affect survival probability. Growth in the undisturbed treatment, however, was not assessed; consequently, the effects of handling on shell growth rate, body condition and reproduction in resident mussels cannot be ruled out. Handling associated with marking and recapturing could have affected mussel growth (Waller et al. 1999, Haag 2009, Wilson et al. 2011); however, all mussels studied where marked with PIT tags and differences among resident and transplanted treatments were discernable.

Second, transplant treatments were handled more than resident treatments because of the added handling time incurred traveling between sites. However, travel time between sites was short (< 45 min) and multiple precautions were taken to minimize stress (following recommendations of Waller et al. 1995, Chen et al. 2001, Yusufzai et al. 2010). If mussels were stressed from transport, it is likely these effects were ameliorated in the early stages of the study. Finally, I accounted for size-specific differences in mussel performance by including initial shell length as an additive covariate to increase precision of the main effects. Differences in mean initial shell length could have confounded treatment effects, but this is unlikely because some treatments with marginally higher mean shell length actually grew faster or had lower survival, despite the negative relationship between shell length and growth rate and positive relationship between shell length and survival (Figure 1, Table 1). Thus, the main effects observed likely originated from environmental and genetic sources.

Physiological processes of freshwater mussels are regulated, in part, by biotic and abiotic properties within local aquatic environments (Cummings and Graf 2009). Mussels are ectothermic filter feeders where water temperature strongly influences filtration, O_2 consumption and respiration rates (Ganser et al. 2015). The effects of temperature on metabolic rates typically varies by species and along thermal gradients (Spooner and Vaughn 2008), but metabolic rates and resource assimilation generally increase with increasing temperatures (Huey and Kingsolver 1989, Cummings and Graf 2009). Freshwater mussels assimilate fine particulate organic matter (POM; < 250µm) suspended within the water column and benthic organic matter (BOM) from substrata

(Raikow and Hamilton 2001, Christian et al. 2004), and acquisition of C and N resources comes mainly from bacteria attached to POM and BOM (Raikow and Hamilton 2001). Although considerably less research has identified the effects of flow on mussel performance, riverine species are highly adapted to flow regimes (e.g., life history, behavior and morphological) and flow regimes may influence current demographic trends (Lytle and Poff 2004). For example, Inoue et al. (2014) linked survival probability to discharge in a long-term mark-recapture experiment with Texas hornshell, *Popenaias popeii* (Lea 1857).

Several of the environmental variables investigated were consistently more important than others in explaining performance of freshwater mussels. Cumulative degree days, the summation of optimal heat conditions for growth and development of an organism over time, has been used to explain thermal relationships of aquatic ectotherms (Ward and Stanford 1982) and was among the most supported variables found explaining mussel performance (i.e., growth, Fulton's K and sperm concentration). Overall, shell growth declined through time, but the point at which growth increased on a seasonal bases corresponded to when rate heat accumulation was highest; during mid fall prior to declining temperatures. Fulton's K index increased linearly with cumulative degree days, which could translate to increased metabolic rates and assimilation of food. However, the limited unique independent values for growth rate and Fulton's K index, coupled with the strong positive relationship between cumulative degree days and time ($r^2 = 0.96$), may partially explain its importance. In contrast, cumulative degree days significantly explained sperm concentration, such that

peak sperm concentration corresponded to lower rates of heat accumulation. Mean temperature also was relatively important and negatively varied with egg proportion. Galbraith and Vaughn (2009) similarly found gamete production was correlated with cumulative degree days and suggested that cooler temperatures could function as a "trigger" mechanism for gametogenesis and spawning.

Chlorophyll *a* and BOM were also included in the top variables explaining variability in mussel performance. Interestingly, chlorophyll *a* was negatively correlated with survival and growth, positively correlated with Fulton's K index and inversely correlated with gamete production; peak gamete production occurred during low values of chlorophyll *a*. The negative relationship between food availability with survival and growth is somewhat contradictory to higher nutritional needs for increased performance. However, BOM was not statistically significant across seasons and may not have varied enough to discern meaningful relationships with survival and growth. Shell growth did generally increase with increasing values of BOM but the strength of this relationship was relatively weak. Moreover, the mixed results observed between food available and performances traits also could be attributed to within season energetic trade-offs. For example, Jokela (1996) found that although mussels responded positively to sites with higher productivity, energy allocated to somatic growth was highest post-reproduction. This might explain why shell growth rate in my study increased in the fall, following reproduction which presumably occurred winter (spawning) through spring and summer (brooding, host-infection). Therefore, detecting the effects of seasonal environments on mussel performance, at least for survival, growth and body condition, may not be

detectable using regression techniques because of the effects of other endogenous factors (e.g., within season energy allocation) (Jokela 1996).

The relationships between gamete production and environmental variables were more meaningful and easier to interpret because of the greater number of samples collected throughout a year. In addition to cumulative degree days and temperature (described above), an inverse relationship was found between gamete production and chlorophyll *a*. While the evidence supports cooler temperatures might play a role in the timing of gamete production in this study, it is unlikely the negative relationship found between chlorophyll *a* and gamete production was causal. However, in one case, egg proportion was slightly higher at intermediate values, but this trend was not clear. More interestingly, historical discharge was negatively correlated with egg proportion, which might reflect adaptations by freshwater mussels to spawn during lower seasonal flows. Studies have demonstrated that seasonal discharge events can function as important spawning cues for benthic invertebrates (e.g., Bunn 1988) and freshwater fishes (e.g., Paragamian and Wakkinen 2011). It is conceivable that freshwater mussels would cast sperm into the water column during relatively low flows to maximize female capture and fertilization successful; however, studies regarding the effects of discharge on reproduction processes in unregulated streams is scant. In a comprehensive report, Haley et al. (2007) failed to find conclusive relationships between reproductive timing (i.e., spawning, glochidia release and juvenile excystment) and pulse flows in California streams, in part, because many of the species examined were asynchronous spawners. Adaptations to seasonal flows is more likely to occur in streams with predictable flows

and synchronous spawners. Therefore, other factors may play a critical role in the timing and success of mussel reproduction and future research should explore these patterns across spatiotemporal scales.

CHAPTER IV

MUSSEL RELOCATION: CONCLUSIONS

Relocation has become a popular strategy for the conservation of species (Griffith et al. 1989, Chauvenet et al. 2013). The efficacy of this strategy, however, remains subject to debate largely due to the low rates of success (Fischer and Lindenmayer 2000, Massei et al. 2010), lack of consensus on acceptable criteria for relocation success (Chauvenet et al. 2013), and its potential genetic and evolutionary consequences (Weeks et al. 2011). This is also true for freshwater mussels where the success of relocations has been limited (Cope and Waller 1995, Haag and Williams 2014). Although recent advances have improved the outcome of mussel relocations (Dunn et al. 1999, Cope et al. 2003), there is still a wide knowledge gap concerning the factors contributing to relocation success and how mussel traits vary in response to relocation. Through reciprocal transplant experimentation, I examined the effects relocation has on individual and population performance of freshwater mussels. Three main points regarding mussel relocations can be taken from the results in this study: (1) performance of relocated mussels may differ among populations because of genotypic and phenotypic variation; (2) measuring multiple physiological responses, such as survival, growth and reproduction, are needed to detect meaningful changes to mussel performance and physiological traits; and (3) ascertaining how habitat characteristics affect relocated mussels is essential for understanding relocation success.

In this study, I show that mussels generally acclimated to the environmental conditions of the sites such that performance was not greatly affected, but some negative impacts were incurred even when controlling for handling and relocating mussels to sites within the same river and with hydraulic refugia. Thus, some variability in performance appeared to stem from the interaction between environmental and genetic factors, despite the limited differences detected in habitat between the study sites. These effects highlight the implications phenotypic variation among populations can have on relocation success. Studies on the genetic structure of freshwater mussel populations suggest that genetic differentiation can be high, depending upon host fish vagility, in smaller streams and occur randomly across relatively small spatial scales (Berg et al. 2007). Thus, genotypic-induced responses resulting from transplantation to novel environments could stress mussels (Hinch et al. 1986, Jokela and Mutikainen 1995, Englund and Heino 1996, Kesler et al. 2007), and even subtle ecological and genetic differences between populations could hinder population performance and persistence (Preston et al. 2010, Fariñas-Franco et al. 2014). Ultimately, the ramifications of mixing evolutionary divergent populations is poor performance, disruption of natural gene flow dynamics and limited evolutionary potential (Villella et al. 1998). Resource managers and conservations, therefore, must be cognizant of both genotypic and phenotypic variation when contemplating the suitability of relocation (Villella et al. 1998, Weeks et al. 2011, Pérez et al. 2012). Minimizing geographical and ecological distances among populations might help alleviate genotypic-induced responses and maintain natural gene flow among populations (IUCN 1998, Fariñas-Franco et al. 2014). Finally, while the

results found in my study indicate that mussels generally responded positively to relocation, they should not be considered transferable between rivers, perhaps even large distances within rivers, because forces of natural selection vary across riverscapes.

Cope and Waller (1995) argued that mussel relocation studies must look beyond crude end-points, such as survival, to infer relocation success. While studies have investigated effects of relocation at the sublethal level (e.g., Newton et al. 2001, Peck 2010), the manner in which stress-induced responses translates to individual and population performance have been poorly studied. Inferences regarding relocation success based solely on survival provides little detail on performance. For example, under stressful conditions energy allocated to maintenance will override energy allocated to growth and reproduction (Jokela and Mutikainen 1995, Jokela 1997). Thus, populations could persist in suboptimal conditions in the short-term, but impacts sustained to growth and reproduction will preclude long-term persistence. In an ideal scenario, age-or stage-structured population models could be developed to assess the demography of relocated populations (e.g., Jones et al. 2012); however, this requires years of mark-recapture data that unfortunately come with high cost. Assessing multiple physiological or demographic traits (e.g., survival, somatic growth, reproduction, and immune responses) to understand how traits respond and interact among themselves can be useful to gain a more complete understanding of relocation success. Looking to life history theory could then be used to interpret such data to make informed management and conservation decisions. I echo arguments made by Berg et al. (2008) that most

studies on freshwater mussels are species-centered and researchers must steer towards the development of general conservation guidelines grounded in ecological theory.

The results found in my study indicated performance of relocated mussels was generally more similar to performance of resident mussels at the relocation sites, meaning that performance was most effected by ecological conditions at the sites. However, limited differences were detected in environmental conditions between sites, and few of the variables examined were important in explaining seasonal variation in performance traits (i.e., survival, growth and body conditions). Habitat requirements for freshwater mussels have long perplexed ecologists (Strayer 1981). Research on habitat of mussels have focused on factors shaping distribution and abundance (Strayer 2008), structure of species assemblages (Haag and Warren 1998) and, more recently, patch dynamics (Newton et al. 2008). Considerably less research has been aimed towards quantifying habitat quality for mussels; that is, factors that contribute to survival probability, reproductive success and future reproductive output (Van Horne 1983), which may not be positively related to species density. Therefore, identifying ecological factors that enhance the reproductive success of freshwater mussels (e.g., food availability and quality, and host fish quality), in contrast to those factors that only explain mussel presence, will be more useful for understanding performance and demography. Future research aimed at identifying habitat quality could be used to advance our understanding of relocation success and, ultimately, the conservation of freshwater mussels.

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APPENDIX A

Appendix A. Candidate models used to estimate recapture and recovery probabilities across the two study sites in the San Saba River, Texas.

Model	k	QAICc	ΔQAICc	w_i
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p_{(\text{Time} + \text{Site})}\ r.\ F_1$	13	699.04	0.00	0.49516
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p_{(\text{Time} + \text{Site})}\ r_{(\text{Time})}\ F_1$	14	699.63	0.59	0.36893
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p_{(\text{Time} + \text{Site})}\ r_{(\text{Time} + \text{Site})}\ F_1$	15	701.62	2.59	0.13586
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p_{(\text{Time})}\ r.\ F_1$	12	719.96	20.92	0.00001
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p_{(\text{Time})}\ r_{(\text{Time})}\ F_1$	13	720.59	21.56	0.00001
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p_{(\text{Site})}\ r.\ F_1$	12	721.14	22.10	0.00001
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p_{(\text{Site})}\ r_{(\text{Time})}\ F_1$	13	721.74	22.70	0.00001
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p_{(\text{Time})}\ r_{(\text{Site})}\ F_1$	13	721.94	22.90	0.00001
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p_{(\text{Time})}\ r_{(\text{Time} + \text{Site})}\ F_1$	14	722.59	23.55	0.00000
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p_{(\text{Site})}\ r_{(\text{Site})}\ F_1$	13	723.12	24.09	0.00000
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p_{(\text{Site})}\ r_{(\text{Time} + \text{Site})}\ F_1$	14	723.74	24.70	0.00000
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p.\ r.\ F_1$	11	740.52	41.48	0.00000
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p.\ r_{(\text{Time})}\ F_1$	12	741.16	42.12	0.00000
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p.\ r_{(\text{Site})}\ F_1$	12	742.50	43.46	0.00000
$S_{(\text{Treat} \times \text{Time} \times \text{Site} + \text{Length})}\ p.\ r_{(\text{Time} + \text{Site})}\ F_1$	13	743.15	44.11	0.00000

APPENDIX B

Appendix B. Candidate models used to estimate survival probabilities across the two study sites in the San Saba River, Texas. Site by destination consisted of a set of models in which the transplant treatment was assigned to the site of destination, whereas site by origin considered of a set of models in which the transplant treatment was assigned to the site of origin.

	Site by Destination				*Site* by Origin			
Model	**k**	**QAICc**	**ΔQAICc**	w_i	**k**	**QAICc**	**ΔQAICc**	w_i
Amblema plicata								
1	4	48.35	0.75	0.25				
Length	5	50.38	2.77	0.09				
Time	5	49.85	2.25	0.12				
Time + Length	6	51.89	4.29	0.04				
Treat	**4**	**47.60**	**0.00**	**0.36**				
Treat × Time + Length	6	50.97	3.37	0.07				
Treat + Time + Length	6	50.97	3.37	0.07				
Quadrula houstonensis								
1	5	200.86	2.73	0.08	5	200.86	2.73	0.09
Length	6	201.22	3.09	0.07	6	201.22	3.09	0.07
Site	6	201.48	3.35	0.06	6	201.54	3.41	0.06
Site × Time + Length	9	202.55	4.42	0.03	9	206.03	7.90	0.01
Site + Time	7	202.64	4.51	0.03	7	202.69	4.56	0.04
Site + Time + Length	8	200.83	2.70	0.08	8	204.21	6.08	0.02
Time	6	202.05	3.93	0.04	6	202.05	3.93	0.05
Treat	**6**	**198.13**	**0.00**	**0.31**	**6**	**198.13**	**0.00**	**0.34**
Treat × Time × Site + Length	11	205.83	7.70	0.01	11	205.83	7.70	0.01
Treat × Time + Length	9	202.44	4.31	0.04	9	202.44	4.31	0.04
Treat + Site	7	200.17	2.04	0.11	7	200.17	2.04	0.12
Treat + Time + Length	8	200.41	2.28	0.10	8	200.41	2.28	0.11
Treat + Time + Site + Length	9	202.13	4.01	0.04	9	202.13	4.01	0.05
Quadrula petrina								
1	5	372.66	8.01	0.01	5	372.66	3.07	0.07
Length	6	374.67	10.02	0.00	6	374.67	5.08	0.02
Site	6	368.35	3.70	0.09	6	373.32	3.73	0.05
Site × Time + Length	9	368.21	3.56	0.10	9	372.49	2.90	0.07
Site + Time	**7**	**364.65**	**0.00**	**0.59**	7	370.03	0.44	0.24
Time	6	369.59	4.94	0.05	**6**	**369.59**	**0.00**	**0.30**
Treat	6	374.52	9.87	0.00	6	374.52	4.93	0.03
Treat × Time × Site + Length	13	372.41	7.76	0.01	13	372.41	2.82	0.07
Treat × Time + Length	9	374.91	10.26	0.00	9	374.91	5.32	0.02
Treat + Length	7	376.54	11.89	0.00	7	376.54	6.95	0.01
Treat + Site	7	370.34	5.69	0.03	7	375.16	5.57	0.02
Treat + Time + Length	8	373.49	8.84	0.01	8	373.49	3.90	0.04
Treat + Time + Site + Length	9	368.36	3.71	0.09	9	372.97	3.38	0.06
Quadrula verrucosa								
1	5	324.78	8.40	0.01	5	482.13	13.11	0.00
Length	6	322.89	6.51	0.02	6	478.29	9.26	0.01
Site	6	322.79	6.41	0.02	6	484.12	15.10	0.00
Site × Time + Length	**9**	**316.38**	**0.00**	**0.41**	9	474.78	5.75	0.04
Site + Time	7	319.52	3.15	0.09	7	478.24	9.21	0.01
Site + Time + Length	8	317.72	1.34	0.21	8	473.45	4.42	0.07
Time	6	321.52	5.14	0.03	6	476.24	7.21	0.02
Treat	6	326.50	10.12	0.00	6	483.71	14.68	0.00
Treat × Time × Site + Length	13	321.47	5.10	0.03	**13**	**469.03**	**0.00**	**0.65**
Treat × Time + Length	9	320.99	4.62	0.04	9	472.40	3.38	0.12
Treat + Length	7	324.86	8.48	0.01	7	480.23	11.20	0.00
Treat + Site	7	324.50	8.12	0.01	7	485.70	16.68	0.00
Treat + Time + Length	8	321.11	4.73	0.04	8	473.59	4.56	0.07
Treat + Time + Site + Length	9	319.45	3.07	0.09	9	475.38	6.35	0.03

APPENDIX C

Appendix C. Candidate models used to estimate yearly proportional shell growth across the two study sites in the San Saba River, Texas. Site by destination consisted of a set of models in which the transplant treatment was assigned to the site of destination, whereas site by origin considered of a set of models in which the transplant treatment was assigned to the site of origin.

	Site by Transplant			Site by Origin		
Model	**AIC**	**ΔAIC**	w_i	**AIC**	**ΔAIC**	w_i
Amblema plicata						
Time	-248.64	80.50	0.00			
Time + Length	-275.47	53.67	0.00			
Time + Treat	-285.88	43.25	0.00			
Time + Treat + Length	-291.78	37.36	0.00			
Time × Treat	-311.60	17.54	0.00			
Time × Treat + Length	**-329.14**	**0.00**	**1.00**			
Quadrula houstonensis						
Time	-444.61	0.41	0.21	**-444.61**	**0.00**	**0.27**
Time + Length	-443.16	1.86	0.10	-443.16	1.45	0.13
Time + Length + Site	-443.21	1.81	0.10	-441.30	3.31	0.05
Time + Length + Site + Treat	-441.78	3.24	0.05	-441.78	2.82	0.07
Time + Length + Treat	-441.91	3.11	0.05	-441.91	2.70	0.07
Time + Site	**-445.02**	**0.00**	**0.25**	-443.29	1.31	0.14
Time + Site + Treat	-443.02	2.00	0.09	-443.02	1.58	0.12
Time + Treat	-443.12	1.90	0.10	-443.12	1.49	0.13
Time × Site	-440.37	4.65	0.02	-437.98	6.62	0.01
Time × Site + Length	-438.60	6.42	0.01	-435.99	8.62	0.00
Time × Treat	-435.27	9.75	0.00	-435.27	9.34	0.00
Time × Treat + Length	-434.05	10.97	0.00	-434.05	10.55	0.00
Quadrula petrina						
Time	-519.34	12.11	0.00	-519.34	11.11	0.00
Time + Length	-520.55	10.91	0.00	-520.55	9.90	0.00
Time + Length + Site	-521.37	10.09	0.00	-526.28	4.17	0.04
Time + Length + Site + Treat	-523.90	7.56	0.01	-529.88	0.57	0.25
Time + Length + Treat	-523.09	8.37	0.01	-523.09	7.36	0.01
Time + Site	-518.04	13.41	0.00	-527.26	3.19	0.07
Time + Site + Treat	-520.81	10.64	0.00	**-530.45**	**0.00**	**0.34**
Time + Site × Treat	-529.26	2.20	0.16	-529.26	1.19	0.19
Time + Site × Treat + Length	-527.92	3.53	0.08	-527.92	2.53	0.10
Time + Treat	-522.08	9.38	0.00	-522.08	8.37	0.01
Time × Site	-518.93	12.52	0.00	-515.53	14.92	0.00
Time × Site × Treat	**-531.45**	**0.00**	**0.49**	-517.30	13.15	0.00
Time × Site × Treat + Length	-529.95	1.50	0.23	-516.22	14.23	0.00
Time × Treat	-515.55	15.91	0.00	-515.55	14.90	0.00
Quadrula verrucosa						
Time	-376.22	71.16	0.00	-376.22	74.58	0.00
Time + Length	-374.28	73.11	0.00	-374.28	76.52	0.00
Time + Length + Site	-394.53	52.86	0.00	-386.55	64.25	0.00
Time + Length + Site + Treat	-427.10	20.29	0.00	-429.33	21.47	0.00
Time + Length + Treat	-405.68	41.71	0.00	-405.68	45.12	0.00
Time + Site	-391.35	56.04	0.00	-386.76	64.05	0.00
Time + Site + Treat	-427.95	19.44	0.00	-421.52	29.28	0.00
Time + Site × Treat	**-447.39**	**0.00**	**0.60**	-447.39	3.41	0.09
Time + Site × Treat + Length	-446.57	0.82	0.40	-446.57	4.24	0.06
Time + Treat	-406.47	40.92	0.00	-406.47	44.33	0.00
Time × Site	-382.49	64.90	0.00	-385.26	65.54	0.00
Time × Site × Treat	-437.51	9.88	0.00	**-450.80**	**0.00**	**0.51**
Time × Site × Treat + Length	-436.62	10.77	0.00	-450.01	0.79	0.34
Time × Treat	-423.46	23.93	0.00	-423.46	27.34	0.00

APPENDIX D

Appendix D. Candidate models used to estimate Fulton's K body condition index across the two study sites in the San Saba River, Texas. Site by destination consisted of a set of models in which the transplant treatment was assigned to the site of destination, whereas site by origin considered of a set of models in which the transplant treatment was assigned to the site of origin.

	Site by Transplant			Site by Origin		
Model	**AIC**	**ΔAIC**	w_i	**AIC**	**ΔAIC**	w_i
Amblema plicata						
Time	511.47	46.98	0.00			
Time + Length	482.14	17.65	0.00			
Time + Treat	511.21	46.72	0.00			
Time + Treat + Length	472.66	8.17	0.02			
Time × Treat	510.04	45.55	0.00			
Time × Treat + Length	**464.49**	**0.00**	**0.98**			
Quadrula houstonensis						
Time	850.06	57.35	0.00	850.06	57.35	0.00
Time + Length	847.98	55.27	0.00	847.98	55.27	0.00
Time + Site	823.28	30.57	0.00	842.11	49.39	0.00
Time + Site + Length	820.87	28.16	0.00	841.36	48.65	0.00
Time + Site + Treat	824.68	31.97	0.00	824.68	31.97	0.00
Time + Site + Treat + Length	792.76	0.04	0.49	792.76	0.04	0.49
Time + Treat	848.30	55.59	0.00	848.30	55.59	0.00
Time + Treat + Length	843.70	50.99	0.00	843.70	50.99	0.00
Time × Site	824.47	31.75	0.00	841.45	48.74	0.00
Time × Site + Length	821.79	29.08	0.00	840.93	48.21	0.00
Time × Site × Treat	825.46	32.74	0.00	825.46	32.74	0.00
Time × Site × Treat + Length	**792.71**	**0.00**	**0.51**	**792.71**	**0.00**	**0.51**
Time × Treat	849.70	56.99	0.00	849.70	56.99	0.00
Time × Treat + Length	844.75	52.03	0.00	844.75	52.03	0.00
Quadrula petrina						
Time	1233.56	145.22	0.00	1233.56	142.31	0.00
Time + Length	1182.96	94.63	0.00	1182.96	91.71	0.00
Time + Site	1224.60	136.27	0.00	1171.67	80.42	0.00
Time + Site + Length	1120.01	31.67	0.00	1170.35	79.09	0.00
Time + Site + Treat	1215.05	126.72	0.00	1156.21	64.95	0.00
Time + Site + Treat + Length	**1088.34**	**0.00**	**0.81**	1153.21	61.95	0.00
Time + Treat	1224.95	136.61	0.00	1224.95	133.69	0.00
Time + Treat + Length	1166.16	77.82	0.00	1166.16	74.91	0.00
Time × Site	1225.81	137.47	0.00	1173.42	82.17	0.00
Time × Site + Length	1118.64	30.30	0.00	1172.25	81.00	0.00
Time × Site × Treat	1141.33	53.00	0.00	1141.33	50.08	0.00
Time × Site × Treat + Length	1091.25	2.92	0.19	**1091.25**	**0.00**	**1.00**
Time × Treat	1226.83	138.50	0.00	1226.83	135.58	0.00
Time × Treat + Length	1167.97	79.63	0.00	1167.97	76.71	0.00
Quadrula verrucosa						
Time	1020.05	49.83	0.00	7237.91	44.93	0.00
Time + Length	1017.45	47.22	0.00	7239.20	46.22	0.00
Time + Site	1018.82	48.59	0.00	7201.41	8.43	0.01
Time + Site + Length	1013.41	43.19	0.00	7203.22	10.24	0.00
Time + Site + Treat	1013.74	43.51	0.00	7194.46	1.48	0.22
Time + Site + Treat + Length	1010.08	39.86	0.00	7196.08	3.10	0.10
Time + Treat	1015.15	44.92	0.00	7233.67	40.69	0.00
Time + Treat + Length	1013.90	43.68	0.00	7235.12	42.14	0.00
Time × Site	1019.57	49.34	0.00	7199.27	6.29	0.02
Time × Site + Length	1013.04	42.81	0.00	7201.07	8.10	0.01
Time × Site × Treat	**970.23**	**0.00**	**0.73**	**7192.98**	**0.00**	**0.46**
Time × Site × Treat + Length	972.18	1.95	0.27	7194.86	1.89	0.18
Time × Treat	1016.78	46.55	0.00	7235.67	42.69	0.00
Time × Treat + Length	1015.47	45.24	0.00	7237.12	44.14	0.00

APPENDIX E

Appendix E. Candidate models used to estimate sperm concentration and proportion of eggs within the 80th percentile (Egg Proportion) across the two study sites in the San Saba River, Texas. Site by destination consisted of a set of models in which the transplant treatment was assigned to the site of destination, whereas site by origin considered of a set of models in which the transplant treatment was assigned to the site of origin.

	Analysis by Destination			Analysis by Origin		
Model	**AIC**	**ΔAIC**	w_i	**AIC**	**ΔAIC**	w_i
Sperm Concentration						
Quadrula petrina						
Time	71.30	12.42	0.00	71.30	12.42	0.00
Time + Length	71.53	12.65	0.00	71.53	12.65	0.00
Time + Length + Site	73.56	14.67	0.00	72.25	13.36	0.00
Time + Length + Site + Treat	73.62	14.74	0.00	73.62	14.74	0.00
Time + Length + Treat	72.03	13.15	0.00	72.03	13.15	0.00
Time + Site	73.26	14.37	0.00	73.26	14.37	0.00
Time + Site + Treat	75.00	16.11	0.00	75.00	16.11	0.00
Time + Treat	72.95	14.06	0.00	72.95	14.06	0.00
Time × Site	67.36	8.47	0.01	67.45	8.57	0.01
Time × Site + Length	69.26	10.37	0.01	65.18	6.29	0.04
Time × Site × Treat	66.90	8.01	0.02	67.64	8.75	0.01
Time × Site × Treat + Length	67.52	8.63	0.01	66.05	7.16	0.02
Time × Treat	66.35	7.46	0.02	66.35	7.46	0.02
Time × Treat + Length	**58.89**	**0.00**	**0.92**	**58.89**	**0.00**	**0.88**
Quadrula verrucosa						
Time	527.95	1.32	0.11	527.95	39.33	0.00
Time + Length	526.99	0.36	0.18	526.99	38.37	0.00
Time + Length + Site	528.64	2.01	0.08	525.32	36.70	0.00
Time + Length + Site + Treat	528.27	1.64	0.10	524.54	35.92	0.00
Time + Length + Treat	**526.63**	**0.00**	**0.22**	526.63	38.01	0.00
Time + Site	529.60	2.97	0.05	528.79	40.17	0.00
Time + Site + Treat	530.49	3.86	0.03	529.77	41.15	0.00
Time + Treat	528.84	2.21	0.07	528.84	40.22	0.00
Time × Site	529.75	3.12	0.05	494.77	6.15	0.04
Time × Site + Length	528.45	1.82	0.09	**488.62**	**0.00**	**0.95**
Time × Site × Treat	531.48	4.85	0.02	498.38	9.76	0.01
Time × Site × Treat + Length	532.93	6.31	0.01	500.76	12.14	0.00
Time × Treat	536.05	9.42	0.00	536.05	47.43	0.00
Time × Treat + Length	534.36	7.73	0.00	534.36	45.74	0.00

Appendix E. (continued)

Model	Analysis by Destination			Analysis by Origin		
	AIC	ΔAIC	w_i	AIC	ΔAIC	w_i
Egg Proportion (80th percentile)						
Quadrula petrina						
Time	-50.80	1.57	0.11	-50.80	0.45	0.17
Time + Length	-49.83	2.53	0.07	-49.83	1.41	0.11
Time + Length + Site	-49.41	2.95	0.06	-49.29	1.95	0.08
Time + Length + Site + Treat	-47.92	4.44	0.03	-47.92	3.32	0.04
Time + Length + Treat	-47.88	4.49	0.03	-47.88	3.36	0.04
Time + Site	-51.25	1.12	0.14	**-51.24**	**0.00**	**0.21**
Time + Site + Treat	-49.90	2.46	0.07	-49.90	1.34	0.11
Time + Treat	-48.86	3.51	0.04	-48.86	2.38	0.06
Time × Site	**-52.37**	**0.00**	**0.24**	-49.18	2.06	0.08
Time × Site + Length	-50.10	2.27	0.08	-47.21	4.03	0.03
Time × Site × Treat	-50.30	2.07	0.09	-47.32	3.92	0.03
Time × Site × Treat + Length	-48.44	3.93	0.03	-45.45	5.79	0.01
Time × Treat	-46.65	5.72	0.01	-46.65	4.59	0.02
Time × Treat + Length	-44.95	7.42	0.01	-44.95	6.30	0.01
Quadrula verrucosa						
Time	-111.81	7.03	0.01	-111.81	7.03	0.01
Time + Length	**-118.84**	**0.00**	**0.44**	**-118.84**	**0.00**	**0.40**
Time + Length + Site	-117.74	1.09	0.25	-118.14	0.70	0.28
Time + Length + Site + Treat	-115.74	3.09	0.09	-116.14	2.70	0.10
Time + Length + Treat	-116.84	2.00	0.16	-116.84	2.00	0.15
Time + Site	-110.51	8.33	0.01	-112.69	6.15	0.02
Time + Site + Treat	-109.30	9.54	0.00	-111.39	7.44	0.01
Time + Treat	-110.58	8.26	0.01	-110.58	8.26	0.01
Time × Site	-102.78	16.05	0.00	-105.22	13.61	0.00
Time × Site + Length	-112.10	6.74	0.02	-112.14	6.70	0.01
Time × Site × Treat	-103.01	15.83	0.00	-101.82	17.01	0.00
Time × Site × Treat + Length	-109.75	9.08	0.00	-108.60	10.23	0.00
Time × Treat	-102.23	16.61	0.00	-102.23	16.61	0.00
Time × Treat + Length	-109.63	9.21	0.00	-109.63	9.21	0.00