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RECRUITMENT AND SURVIVAL OF POST-PARASITIC JUVENILE MUSSELS IN AN EAST TEXAS RIVER

by

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of the requirements for the degree of
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ABSTRACT

Recruitment and Survival of Juvenile Freshwater Mussels in an East Texas River

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July 2013

Freshwater mussels (order Unionoida) are the most imperiled group of fauna in North America. The factors driving this decline chiefly affect juvenile recruitment and survival, rather than adult mortality. However, our present knowledge generally consists of inferences from studies of adult distributions. Juvenile mussels are rarely collected in the field, leaving our understanding of this critical period in the mussel life cycle incomplete. Popular hypotheses, most notably that shear stress during floods scours small mussels from the substrate and largely confines mussel beds to sheltered areas, are supported by circumstantial evidence and have rarely been tested directly. The role of pre-settlement processes such as fish behavior is poorly understood.

I collected adult and juvenile mussels in the Sabine River north of Tyler, Texas. I hand-collected adults from randomly placed 0.25-m^2 quadrats, then collected 10 cm of sediment from the same area, sieved it, and inspected all but the finest portions for bivalves. I collected 468 adult and 137 juvenile mussels representing 14 species. Texas pigtoes outnumbered all other species as both adults and juveniles, while pistolgrips were highly abundant as adults but extremely rare as juveniles. Fragile papershells were common as juveniles but rarely located as adults.

Adult Texas pigtoes, adult Western pimplebacks, juvenile deertoes, and juvenile fragile papershells were most common in the riffle portion of the study area, while adult deertoes and Southern mapleleafs were more abundant and more consistently present in the run portion. Juvenile Texas pigtoes were somewhat denser in the riffle, but not as dramatically as adults; this is consistent with the hypothesis that post-settlement mortality plays a key role in determining mussel distributions. Field measurements of shear stress were not significantly correlated with abundance or presence of most species; Southern mapleleafs, which were more consistently present at low shear, were the sole exception.

I estimate that the young-of-year Texas pigtoes collected excysted around the end of June or later. This coincides with a substantial flood, which may have killed juveniles that excysted earlier in the season. Deertoes and fragile papershells excysted from May to early June and did not continue to excyst later in the season. I suspect that host fish behavior plays a major role in controlling the distribution of these two species, both of which rely exclusively on freshwater drum in the Sabine River.

Overall, I did not collect enough juveniles of most species to draw strong conclusions. Additionally, a single season of data is not necessarily representative of long-term trends. Juvenile mussel sampling is useful for studying post-excystment settling patterns and first-year mortality, but the large amount of labor required for a small amount of data limits its effectiveness as a primary tool.

CHAPTER 1: Freshwater Mussel Ecology

Introduction

Freshwater mussels (Bivalvia: Unionidae) are among the most significant animals in aquatic ecosystems. Historically, they comprised the majority of the benthic biomass in some rivers. As suspension feeders, they improve water quality by removing algae, bacteria, and chemical contaminants from the water (Vaughn 2010, Vaughn and Hakenkamp 2001). Their feces and pseudofeces increase the nutrient content of stream benthos, and they contribute to nutrient cycling between the benthos and water column through bioturbation (Vaughn and Hakenkamp 2001). They also provide prey to diverse animals such as muskrats and bottom-feeding fish (Williams et al. 1993), and their empty shells are used as shelters by small fish and invertebrates.

Unionoid mussels have a unique life cycle with multiple stages (Haag 2012). Males discharge sperm directly into the water column, where females collect it in the course of normal feeding. Fertilized eggs develop into larvae called glochidia, which are carried in their mothers' gill pouches for a period of weeks or months. The glochidia are then expelled into open water, where they must attach parasitically to the gills or fins of a host fish. Some species attract host fish with visual lures and will not discharge glochidia unless a host fish is nearby (Haag 2012). Other species discharge glochidial conglutinates (masses of gelatinous proteins and live glochidia) that resemble invertebrates or fish eggs. Fish that ingest these conglutinates release the glochidia and become infected (Howells et al. 1996). Bottom-feeding fish such as freshwater drum (*Aplodinotus grunniens*) and flathead catfish *(Pylodictis olivaris*) frequently acquire glochidial infections from eating adult mussels directly (Haag 2012); some species actively expose themselves on the streambed while carrying glochidia in order to make themselves accessible to host fish. Drum are particularly significant mussel hosts, as they are capable of surviving intense, high-density glochidial infections without apparent ill effect (Coker et al. 1921) and they serve as host to a wide variety of mussel species (Howells et al. 1996).

Mussels develop many adult structures, but do not grow significantly, during the parasitic stage. After they *excyst*, or detach from the host, they are considered juveniles. Post-parasitic juveniles that come to rest on suitable substrate burrow in and begin feeding on suspended organic material, bacteria, and algae. Juveniles approximately 4 mm or smaller feed by collecting edible particles from sediment or interstitial water with their feet (Yeager et al. 1994, Hanlon 2000), while larger juveniles anchor themselves to the substrate with byssal threads and filter edible material directly from the water column (Hanlon 2000).

Mussels occupy a wide variety of habitats, from swift-flowing, turbulent riffles to still water in lakes, reservoirs, and pools. Any substrate fluid enough to be burrowed into is potential mussel habitat, although stable substrates with coarse material are generally more favorable than sand or silt; specific substrate needs vary by species. Generally speaking, species with light, delicate, smooth shells tolerate or even favor muddy, comparatively unstable substrate, and may occur in shifting sand. Some smooth shelled species, including yellow sandshell (*Lampsilis teres*) and fragile papershell (*Leptodea fragilis*), have been noted to be highly mobile and active compared with other mussels (Howells et al. 1996); this strongly suggests that smooth shells are an adaptation that allows for mobility in unstable substrate, where less mobile individuals could become buried and suffocate. Species with heavier, more sculptured shells are more prevalent in rocky areas, where their elaborately shaped shells may anchor them and keep them stable under scouring forces (Watters 1994, Goodding 2012).

Adult mussels feed on suspended waterborne particles, including algae, bacteria, and organic debris. They collect these particles by drawing water into their incurrent siphons, filtering out particulate material, and expelling the remaining portion. Material that they retain is sorted; some portions are digested, and others are expelled as a mass of mucus and debris called pseudofeces. Their dietary needs and feeding mechanics are poorly known, but they may vary considerably between species. Coker et al. (1921) observed no significant difference in stomach contents between mussel species, while Baker and Levinton (2003) observed that several species appear to sort particles according to size, with *Microcystis* cyanobacteria and similarly sized particles being retained at a higher rate than coarser material. Toxicity also plays a role; Vanderploeg et al. (2001) reviewed several studies that found that invasive zebra mussels (*Dreissena polymorpha*) cannot digest toxic strains of *Microcystis*, whereas the less toxic strains found in the Hudson River are a preferred food source for both native and invasive bivalves (Baker and Levinton 2003, Smith et al. 1998). Prepas et al. (1997), on the other hand, determined that giant floater (*Pyganodon grandis*), a widely distributed species that tolerates silty or turbid water, can feed on toxic cyanobacteria, and can quickly eliminate accumulated toxins when moved to less toxic environments. Gatenby et al. (1996) determined that juvenile rainbow mussels (*Villosa iris*) grow more rapidly in water containing natural river sediment than they do in water with cultivated bacteria and/or phytoplankton, whereas *P. grandis* grow at a steady rate regardless of food type. Juvenile mussels raised in laboratory settings are frequently raised on unialgal cultures, often the oil-rich species *Neochloris oleoabundans* (O'Beirn et al. 1998, Barnhart 2006, Gatenby et al. 1996), although O'Beirn et al. (1998) still found that *V. iris* grows faster and survives at a higher rate in the presence of suspended sediment.

Mussels appear to be less affected by pollutants than other aquatic species, likely owing to their minimal nervous systems. Fish toxicants such as rotenone have little or no effect on mussel health (Howells et al. 1996). Microorganism-borne toxins such as microcystin (Chen and Xie 2007) can accumulate in mussel tissues to the point where their flesh is hazardously toxic to vertebrates, without apparent impact on their own survival. Additionally, some species are able to rapidly eliminate accumulated microcystin from their bodies (Prepas et al. 1997). Juvenile mussels, however, are more susceptible to pollutants, including cyanobacterial toxins (Clearwater et al. 2012) and heavy metals such as copper (Jacobson et al. 1997). Research on toxin accumulation in mussels has generally focused on the risk to vertebrate predators, rather than the impact on the mussels themselves.

Juvenile Recruitment

A single gravid female can hold thousands or millions of glochidia at a time, depending on size and species (Haggerty et al. 2005, Yeager and Neves 1986, Surber 1912), but very few of these will reach maturity. Mortality is extremely high for glochidia and juvenile mussels at every stage of the life cycle. A glochidium must first attach to a suitable host fish, which requires direct contact between the glochidium and the host's fins or gills; individuals that never contact the host, or are ingested by the host, can never attach. Additionally, host-mussel combinations are highly specific; mussels can only metamorphose on select species, and in some cases are fully dependent on a single host species (Coker et al. 1921, Howells et al. 1996, Haag 2012).

Mussels that do attach to suitable hosts must settle in suitable habitat after excystment. Suitable substrate must contain an adequate density of edible particles (Yeager et al. 1994), which requires steady siltation. However, excess silt prevents oxygen exchange between the water column and benthos and prevents juvenile mussels from respiring. Geist and Auerswald (2007) noted that the permeability of the sediment-water interface was the most effective predictor of juvenile freshwater pearl mussel (*Margaritifera margaritifera*) recruitment in European rivers. Thresholds vary by species; smooth, thin-shelled species generally tolerate siltation better than those with heavier or more sculptured shells. Although glochidial settling patterns are not known empirically, it is likely that a significant proportion of juveniles settle in deep silt or other lethal habitats.

Currently, the most widely studied and acknowledged influence on juvenile mussel recruitment is shear stress. Field measurements of baseflow shear stress rarely correlate with mussel abundance, but estimated shear stress during floods and other high-discharge events (conditions which preclude field sampling) is recognized as one of the definitive constraints on mussel distribution (Allen and Vaughn 2010, Daraio et al. 2010, Gangloff and Feminella 2007, Hardison and Layzer 2001, Strayer 1999). The accepted hypothesis is that high shear stress dislodges small, lightweight juveniles from the substrate without displacing the heavier adults. Multiple complex hydraulic models support this idea (Morales et al. 2006, Daraio et al. 2010).

Multiple studies have shown that juvenile mussels are more susceptible to routine environmental stress than adults. Even in the absence of severe shear stress or other recognizable direct threats, newly metamorphosed juveniles have low survival, especially during cold conditions (Hanlon 2000). Waterborne pollutants are also more acutely toxic to juveniles than adults (Lasee 1991, Jacobson et al. 1997).

The close relationship between mussels and their host fish can become quite complex, as demonstrated by Payne and Miller (2000). They observed that ebonyshell mussels (*Fusconaia ebena*) in the lower Ohio River are recruited sporadically, i.e. 1981 and 1990 had strong recruitment, while other years produced almost no juveniles. These two years exhibited strikingly similar patterns in seasonal discharge of water, with high discharge in late May and early June and base flow for the rest of the season. As *F. ebena* release their glochidia during this time period, they hypothesized that high discharge attracted their host fish (skipjack shad, *Alosa chrysochloris*) to mussel beds and promoted mussel-host contact. Such complex interactions may limit the recruitment of species of concern, and may explain their declines in areas where ecologically similar species thrive.

In summary, even a dense and robust bed of adult mussels cannot be considered a “healthy” population unless juvenile recruitment is actively occurring. The conditions necessary for recruitment and survival of juveniles vary by species and are largely unknown. Understanding the ecology of juvenile mussels as well as adults is a necessary prerequisite to effective mussel management. The objective of my research is to 1) assess the rate of juvenile mussel recruitment in the largest river in east Texas, the Sabine River, and 2) examine the conditions associated with the recruitment of the species found in this area.

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CHAPTER 2: Recruitment and Survival of Post-Parasitic Juvenile Mussels in an East Texas River

Introduction

Freshwater mussels (Unionoida) play a major role in North American riverine ecosystems. They remove bacteria, algae and contaminants from the water column (Vaughn 2010), enhance nutrient cycling between the water column and benthos (Vaughn and Hakenkamp 2001), and provide food to diverse animals including muskrats, raccoons and bottom-feeding fish (Williams et al. 1993). Despite their significance, they are currently the most imperiled animal taxon in North America (Williams et al. 1993). As habitat degradation is believed to be one of the most significant factors driving unionid declines (Williams et al. 1993), numerous studies have investigated their distribution and habitat requirements. However, simple variables, such as water depth, substrate composition, and stream velocity do not reliably correlate with mussel abundance (Hardison and Layzer 2001). More complex variables, most notably shear stress (Hardison and Layzer 2001, Allen and Vaughn 2010, Gangloff and Feminella 2007), have proven to be more effective predictors of mussel distributions.

Habitat variables appear to impact juvenile recruitment more than adult survival or behavior. Multiple studies have found that shear stress during high stream discharge is negatively correlated with mussel abundance (Hardison and Layzer 2001, Allen and Vaughn 2010, Gangloff and Feminella 2007). This holds true across a variety of species, although some (chiefly those with heavy, intricately sculptured shells) tolerate or even favor relatively high shear stress (Watters 1994, Goodding 2012). The leading hypothesis is that juvenile mussels in high-shear areas are dislodged from the substrate and washed downstream during spates.

Other studies have identified other variables that impact juvenile recruitment. Geist and Auerswald (2007) found that freshwater pearl mussels (*Margaritifera margaritifera*) are only recruited in rivers with highly permeable water-sediment interfaces. They hypothesized that juveniles require a constant interchange of oxygen and nutrients between the water column and benthos, and postulated that periodic spates enhance future recruitment by scouring impermeable silt from the riverbed, even if juveniles are scoured as well. Payne and Miller (2000) observed a strong correlation between annual recruitment in ebonyshell mussels (*Fusconaia ebena*) in the Ohio River and seasonal patterns in river discharge. They hypothesized that high discharge during the peak of glochidial release affected host fish behavior, rather than juvenile survival. Notably, both of these studies point to variables that can differ substantially between seasons and are less predictable than flood shear stress.

All of these hypotheses are well supported so far, but testing them directly is challenging as juveniles are difficult to detect. Mussel surveys are most commonly performed by tactile searches, a method that detects large individuals much more efficiently than small ones. Thus, juvenile mussels, especially young-of-year juveniles and those of slow-growing species, are rarely detected (but see Neves and Widlak 1987 and Payne and Miller 2000 for exceptions). Consequentially, most surveys provide severely incomplete data.

Direct detection of juveniles allows for direct investigation of this critical life stage. By analyzing the distribution of juvenile and adult mussels in the Sabine River, I attempted to answer a number of questions. I intended to directly test the “scour-mortality” hypothesis (i.e., that adult mussels are restricted to areas of low shear bankfull shear stress because high shear causes juvenile mortality) by comparing adult and juvenile distributions; if this hypothesis is accurate, then juveniles that have not encountered flood conditions should be present in areas of high bankfull shear stress, where adults are rare. Additionally, if newly excysted juveniles settle more readily in areas of low shear stress, baseflow shear stress and juvenile density should be negatively correlated.

As a secondary objective, I investigated the life histories of the Sabine River's native mussels. Basic ecological information, such as growth rate and timing of reproduction, is unavailable for many mussel species. By monitoring juvenile mussels in their first year of life, I attempted to assess these qualities for common species in the Sabine River.

Materials and Methods

Study Site

All surveys were conducted on the Sabine River within approximately 300 meters downstream from the Farm to Market Road 14 Bridge, directly south of Hawkins, Texas (Figure 1). The Sabine River was chosen for its dense and diverse populations of native mussels, including the state threatened Texas pigtoe (*Fusconaia askewi*) (Ford and Nicholson 2006, Ford et al. 2009), which suggests a highly functional riverine ecosystem with consistent juvenile recruitment. The river flows through several protected areas with floodplains and bottomland hardwood forests, including the Old Sabine Bottom Wildlife Management Area and Little Sandy National Wildlife Refuge. The nearest

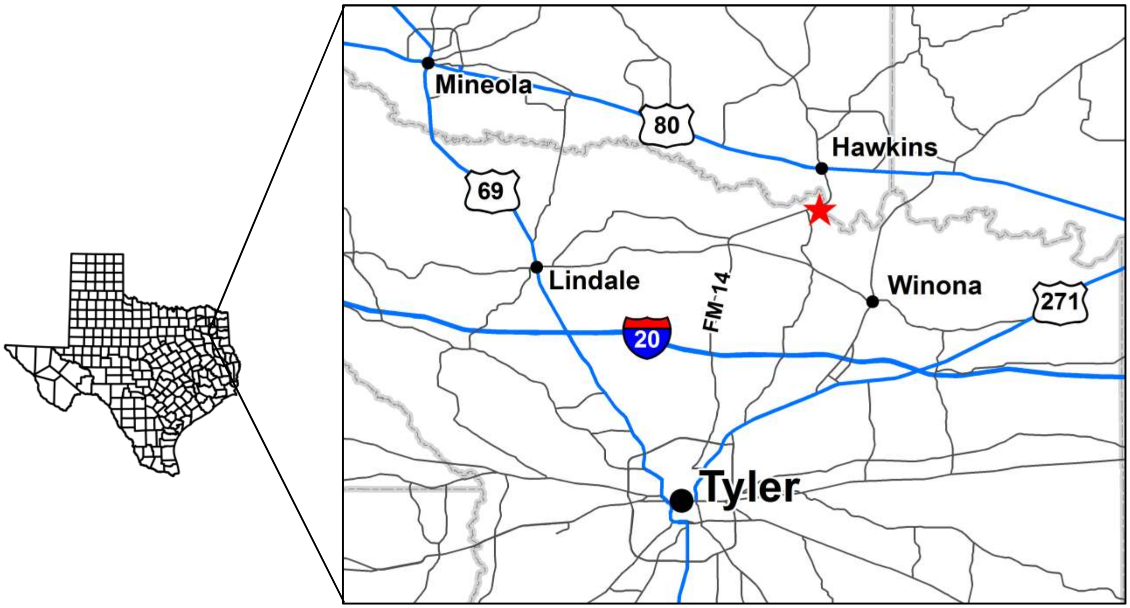

Figure 1. Area map of study site. Location indicated by red star.

Figure 2. Aerial photo of study site. Accessible area is indicated by red outline.

impoundment (Lake Tawakoni) is approximately 30 km upstream of the study site. This section of the Sabine River can be considered relatively “natural” and presents a strong model of high-quality mussel habitat.

The survey area is morphologically heterogeneous, with riffles, pools, and runs in close proximity. Sampling activity was restricted to areas shallow enough to collect substrate without SCUBA equipment (≤1m deep), effectively dividing the sampling area into two plots (Figure 2). The upstream plot covered approximately 600 m^2 on the west/south bank of a channelized run. The downstream section consisted of approximately 1,130 m^2 of shallow riffle with a notably dense mussel population (Ford et al. 2009).

Sample Site Selection and Mapping

Accessible area was estimated through on-site surveys and mapped by hand in ArcGIS 10.0 (Esri, Redlands, CA) from aerial photographs. Target sampling points were designated within the mapped area using the “Create Random Points” tool, with points no less than 5 meters apart. These points were uploaded to a Garmin GPSmap 78 handheld GPS unit and located in the field.

This unit was not consistently accurate enough to be used in analysis, but its use minimized sampling bias by maintaining approximately random site selection. When the GPS was not available, sampling points were chosen subjectively, with effort to sample from a variety of habitats. A total of 54 points, 21 in the upstream plot and 33 in the downstream plot, were surveyed (Appendix C).

The exact position of each point was determined by triangulation. Trees approximately 25m apart were marked with flagging tape, mapped with a Trimble GeoXM 2005 GPS receiver, and used as benchmarks. Two to three magnetic bearings were taken from point locations with a Brunton Sight Master sighting compass and adjusted for declination.

Mussel Sampling

A 0.5 meter by 0.5 meter PVC quadrat was placed on the riverbed at each sampling point. Substrate within the quadrat area was searched by hand until no more bivalves could be detected. Roughly 10 cm of substrate was collected from the surface of the riverbed using a welded steel sampler (Appendix A). The open end of the sampler was driven into the riverbed by one technician, while another loosened hard-packed substrate and removed large obstructions such as buried rocks. The sampler was driven forward at an estimated depth of 10 cm until substrate had been collected from the entire quadrat area. The collected sediment was then transferred into plastic tote bins for sorting.

Sediment was separated with 4.5mm and 2mm sieves. Coarse substrate (anything captured by the 4.5mm sieve) was examined visually to locate bivalves; fine gravel (anything that passed through the 4.5mm sieve but not the 2mm) was spread out on a flat surface and examined under a handheld magnifier. All mussels ~30mm and smaller were preserved in 70% isopropanol. All substrate was returned to the quadrat site, and all adults not retained for analysis or museum collection were returned to the quadrat site in natural posture.

Analysis of Collected Mussels

All small mussels (~25mm or less) were measured along their largest axes. Mussels less than 7.5mm in length were photographed under a dissecting microscope, and the photos were measured using the "Measure" tool in GIMP 2.2 (GNU Image Manipulation Program, www.gimp.org). Larger individuals were measured with digital calipers. Sizes and collection dates were compared to assess species-specific growth rates, and these growth rates were used to estimate year of recruitment for all measured individuals.

I used DNA sequence analysis to identify the species of individuals that did not match any known adult morphology. DNA was extracted from foot tissue of isopropanol-preserved specimens if possible; if not enough foot tissue was present, extractions were performed on partial or entire soft tissues. I used a CTAB protocol modified from Winnepenninckx et al. (1993). Liquid nitrogen tissue disruption was omitted; partially dried tissue was homogenized by mortar & pestle in CTAB buffer, which substituted 1% m/v PVP40 for β-mercaptoethanol.

PCR was used to amplify portions of the ND1 and CO1 mitochondrial genes. My ND1 primers were 5'-TGG CAG AAA AGT GCA TCA GAT TTA AGC-3' and 5'-GCT ATT AGT AGG TCG TAT CG-3', and CO1 primers were 5'-GTT CCA CAA ATC ATA AGG ATA TTG G-3 and 5'-TAC ACC TCA GGG TGA CCA AAA AAC CA-3'. The PCR protocol used was: 92° C for 2 min; 92° C for 40 s, 40° C for 40 s, 72° C for 90 s, ×5; 92°C for 40 s, 50°C for 40 s, 72°C for 90 s, ×25; 72°C for 10min; hold 4°C (from Campbell et al. 2005).

Samples were Sanger sequenced and compared with those from adults of known species. Collected individuals that were not sequenced were assumed to be conspecific with sequenced individuals of similar morphology.

Assessment of Physical Habitat Characteristics

Near-bed shear stress for each quadrat was measured at the time of collection using FST (*Fliesswasserstammtisch*) hemispheres (Statzner and

Müller 1989, Appendix B). Although shear stress at low flow is normally a poor predictor of mussel abundance compared with estimates of flood-level shear stress (Allen and Vaughn 2010, Gangloff and Feminella 2007), I wished to assess the influence of conditions at the time of excystment, which normally occurs in early summer (Kelly and Waters 2010, Neves and Widlak 1988), outside of east Texas's winter flood season, on juvenile settling patterns. To estimate shear stress and other variables during bankfull conditions, I surveyed the river channel for geomorphology in accordance with Harrelson et al. (1996) using a Leica Total Station. Geomorphology data were entered into the STREAMS Reference Reach Survey spreadsheet (Ohio State University, streams.osu.edu) to obtain estimates of shear stress and other complex variables. Current and historical discharge data were provided by the US Geological Survey National Streamflow Information Program.

Statistical Analysis

To estimate juvenile recruitment and population-wide reproductive health, I compared total adult and juvenile abundance for all species. Species that were abundant as adults were expected to be abundant as juveniles as well, while disproportionately low juvenile abundance compared with adult abundance was interpreted as a sign of weak recruitment.

A variety of statistical techniques were used to assess the significance of habitat variables on mussel abundance. To assess the influence of broad-scale processes, I compared the upstream run and downstream riffle; to analyze local-scale conditions, I used field measurements of shear stress. I neglected other hydrological variables, including shear velocity and Froude number, because Allen and Vaughn (2010) determined that they were closely correlated with shear stress and that shear stress was a more effective predictor of mussel abundance.

I only performed these analyses for species and ages for which at least ten individuals were located (adult and juvenile Texas pigtoe, *Fusconaia askewi*; adult and juvenile deertoe, *Truncilla truncata*; adult pistolgrip *Quadrula verrucosa*; adult Southern mapleleaf, *Quadrula apiculata*; adult Western pimpleback, *Quadrula mortoni*; adult bankclimber, *Plectomerus dombeyanus*; juvenile fragile papershell, *Leptodea fragilis*; and juvenile yellow sandshell, *Lampsilis teres*). I compared abundance with plot using Aspin-Welch unequal-variance t-tests, and I compared abundance with shear stress using linear regression.

I also performed a similar analysis using logistic regression. Presence and absence of adults and juveniles of all species was compared against plot, shear stress, and both variables in combination. T-tests, linear regressions, and logistic regressions were all performed using NCSS 2007 (www.ncss.com). For all three analyses, a p-value of 0.05 or less was considered highly significant, while anything under 0.1 was considered mildly or marginally significant.

I used non-metric multidimensional scaling to analyze patterns in per-quadrat species composition. Separate analyses were performed for adults and juveniles, and only for relatively numerous species (i.e. species/ages for which I found ≥10 individuals). Fifty runs were used to create a two-axis output. Analysis was performed using PC-ORD 5.0 (http://home.centurytel.net/~mjm/pcordwin.htm).

For species common as juveniles (Texas pigtoes, deertoes, and fragile papershells), I plotted date of collection against shell length. All individuals of a given species were assumed to grow at the same rate. Growth rate was estimated by analyzing the rate at which maximum shell length increased over the course of the season. From this rate, I extrapolated the date at which zero-length juveniles (i.e. freshly excysted) would have first appeared.

Results

Geomorphology

Based on FST measurements, the downstream riffle plot had significantly higher baseflow shear stress than the upstream run (τ_{mean}=0.747 dyn cm^{-2} upstream, 1.57 dyn cm^{-2} downstream; $p<0.001$). No upstream quadrat was under enough shear stress to displace Hemisphere 3 (which moves under 1.17 dyn cm^{-2}). Shear was highly variable for the downstream plot, but usually higher;

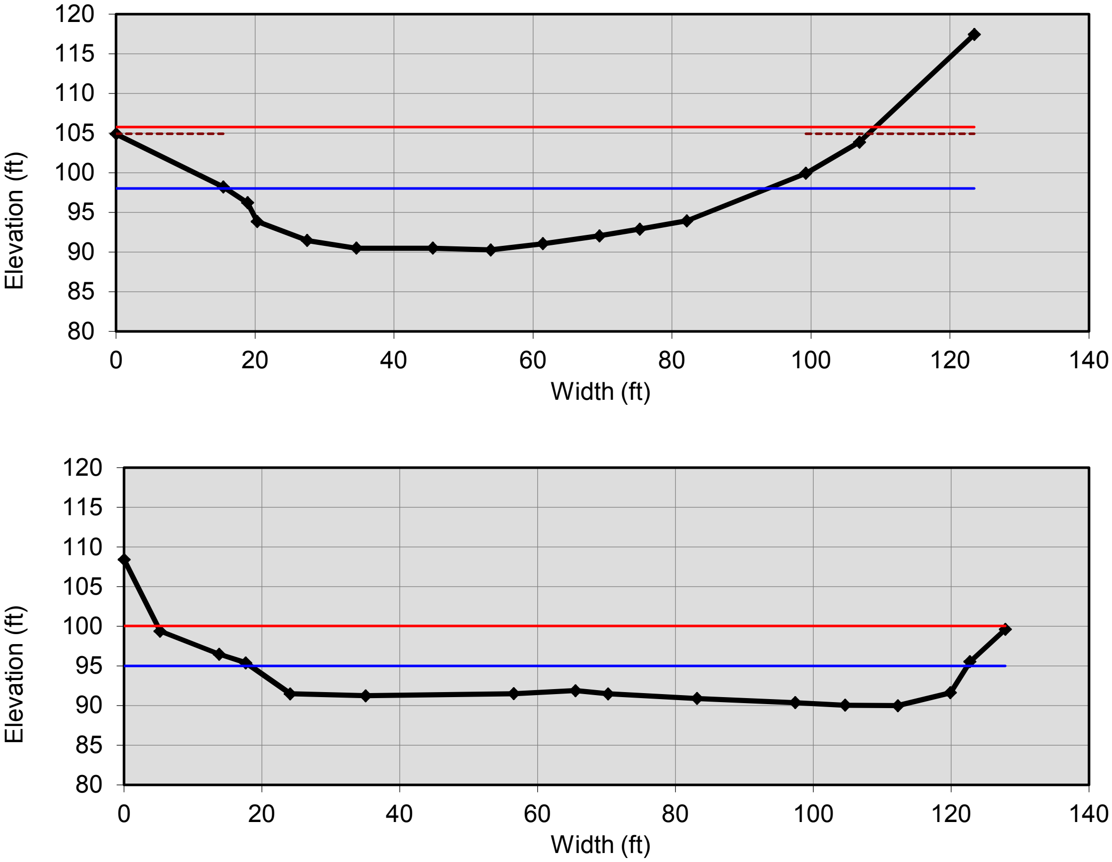

Figure 3. Representative cross-section profiles for upstream (a) and downstream (b) plots.

all hemispheres up to Hemisphere 8 (3.88 dyn cm^{-2}) were displaced at one or more downstream quadrats.

According to STREAMS spreadsheet estimates, the opposite pattern was true of bankfull shear stress. During high water discharge, the upstream run had higher shear stress than the riffle (mean=2.73 dyn cm^{-2} upstream, 1.71 dyn cm^{-2} downstream; p=0.053). Cross sections indicated a generally rounded profile for the upstream plot, and a flat-bottomed, comparatively shallow channel at the downstream area (Figure 3).

Adult and Juvenile Abundance

A total of 468 adult and 137 juvenile mussels were collected representing 14 species (Figure 4, Table 1, Appendix D). Of these, Texas pigtoe (*F. askewi*) was the most common by far, with 176 adults and 77 juveniles. Other highly

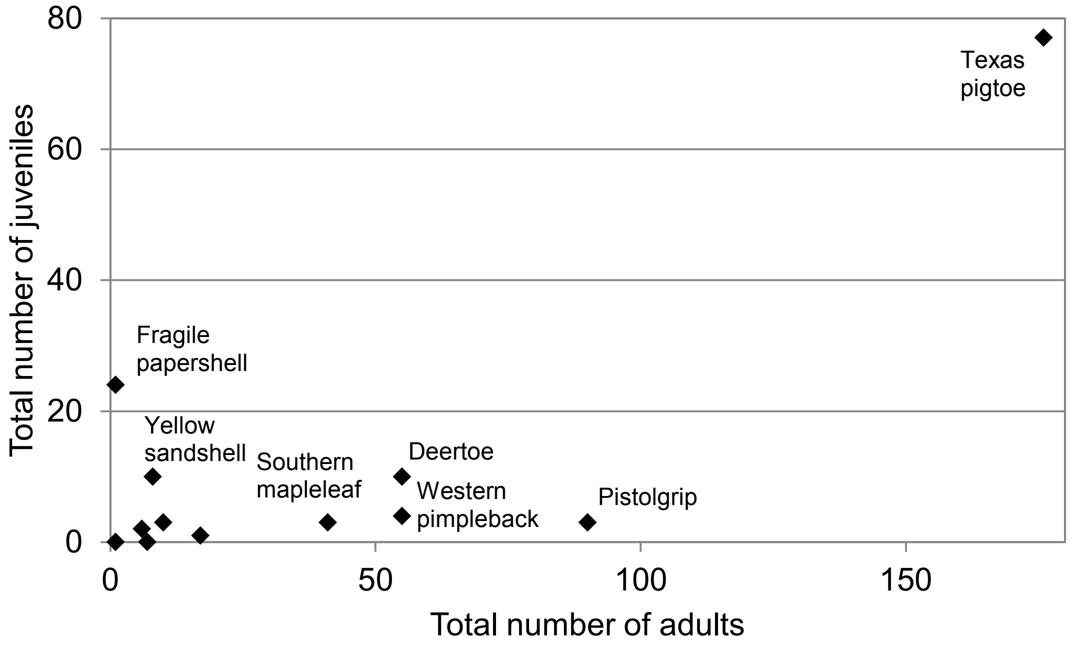

Figure 4. Total counts of adults and juveniles for all species located.

abundant species were pistolgrip (*Quadrula verrucosa*), deertoe (*Truncilla truncata*), Southern mapeleaf (*Quadrula apiculata*), and Western pimpleback (*Quadrula mortoni*).

More than half of juvenile mussels collected were Texas pigtoes, a result that mirrored their adult abundance. Juvenile deertoes were also fairly numerous. Despite their adult abundance, pistolgrip, Southern mapleleaf, and Western pimpleback were scarce or absent as juveniles. Fragile papershells (*Leptodea fragilis*), on the other hand, were numerous as juveniles, while I only collected a single live adult all season. Yellow sandshell (*Lampsilis teres*) juveniles also outnumbered adults.

Influence of Habitat on Mussel Density

Most species were appreciably denser at the downstream riffle than at the upstream run (Table 1). Texas pigtoes and Western pimplebacks in particular were more abundant in the riffle, with close to ten times the per-quadrat density for both species. Most of the other abundant species were more numerous

Table 1. Per-plot adult and juvenile abundance of all species present. P-values are from Welch's t-test (upstream vs. downstream plots) and linear regression (shear stress).

Species	Upstream (run)		Downstream (riffle)		P-value, plot		P-value, shear	
	No. of adults	No. of juv.	No. of adults	No. of juv.	Adult	Juv.	Adult	Juv.
Fusconaia askewi	16	12	160	64	.0024	0.188	0.357	0.763
Quadrula verrucosa	28	1	61	2	0.267	N/A	0.703	N/A
Truncilla truncata	28	1	27	9	0.174	0.079	0.163	0.934
Quadrula mortoni	4	0	37	3	<0.01	N/A	0.288	N/A
Quadrula apiculata	24	3	30	1	0.614	N/A	0.281	N/A
Leptodea fragilis	0	3	1	21	N/A	0.042	N/A	0.965
Plectomerus dombeyanus	6	0	11	1	0.744	N/A	0.196	N/A
Lampsilis teres	5	3	3	7	N/A	0.519	N/A	0.885
Obliquaria reflexa	4	1	5	2	N/A	N/A	N/A	N/A
Megalonaias nervosa	2	0	5	0	N/A	N/A	N/A	N/A
Potamilus purpuratus	1	0	5	2	N/A	N/A	N/A	N/A
Anodonta imbecilis	3	0	3	0	N/A	N/A	N/A	N/A
Arcidens confragosus	1	0	0	0	N/A	N/A	N/A	N/A
Anodonta suborbiculata	1	0	0	0	N/A	N/A	N/A	N/A

downstream, although these differences were rarely statistically significant. Deertoes and Southern mapleleafs were actually denser at the upstream plot.

Adult and juvenile density did not follow consistent patterns. Adult Texas pigtoes and Western pimplebacks were significantly more abundant downstream (p=0.024 and <0.001 respectively), whereas juvenile pigtoes were more evenly distributed between the plots (p=0.188). Juvenile deertoes and fragile papershells, on the other hand, were more common at the downstream riffle (deertoe, p=0.079; fragile papershell, p=0.042), while adult deertoes were more evenly distributed (p=0.174).

Shear stress was insignificant for all species and ages. The most noteworthy association was a very weak correlation with abundance of adult deertoes (p=0.163), which were slightly more common at low shear stress.

Influence of Habitat on Presence and Absence

Interaction between shear stress and plot were consistently non-significant (Table 2). Only juvenile deertoes showed significant interaction (p<0.001), and this result was invalid as the rarity of juvenile deertoes led to quasi-complete separation. Thus, only the results of one-way analyses are presented here.

Patterns in presence and absence of mussels were inconsistent for both adults and juveniles. Plot was individually significant for Western pimplebacks (p=0.003) while shear was fairly significant (p=0.062); pimplebacks were more consistently present in the downstream plot and at high shear stress. Plot was significant for deertoes (p=0.030), which were more consistently present at the upstream plot. Southern mapleleafs responded somewhat to shear stress; they were more common in areas of low shear. This was stronger than their response to plot, though neither was significant (p=0.129 shear, 1.00 plot). Neither variable was significant for juveniles of any species, although plot came relatively close for deertoes (p=0.159) and fragile papershells (p=0.147).

Table 2. Logistic regression P-values for plot (upstream vs. downstream), shear stress (from FST hemispheres), and interaction, as run individually and in combination.

Species	Shear	Plot	Shear | Plot	Shear | Plot | Interaction
Adults				
F. askewi	0.829	0.144	0.448 | 0.099	0.313 | 0.968 | 0.970
Q. verrucosa	0.419	0.836	0.338 | 0.586	0.308 | 0.432 | 0.491
T. truncata	0.137	0.030	0.453 | 0.302	0.368 | 0.972 | 0.972
Q. mortoni	0.062	0.003	0.757 | 0.009	0.705 | 0.976 | 0.975
Q. apiculata	0.129	1.00	0.077 | 0.161	0.069 | 0.125 | 0.210
P. dombeyanus	0.262	0.404	0.065 | 0.038	0.058 | 0.261 | 0.471
Juveniles				
F. askewi	0.357	0.665	0.477 | 0.910	0.494 | 0.795 | 0.809
T. truncata	0.716	0.159	0.219 | 0.049	0.574 | <0.01 | <0.01[1]
L. fragilis	0.448	0.147	0.716 | 0.058	0.750 | 0.977 | 0.976

[1] This model was invalid because of quasi-complete separation.

If both variables were used in the model, plot was significant for adult Western pimplebacks, while shear lost its significance entirely (p=0.009 and 0.757 respectively). Texas pigtoes were more consistently present upstream, although the statistical significance was marginal (p=0.099). Southern mapleleafs were rare in high shear areas (p=0.077), while both variables were at least mildly significant for adult bankclimbers (p=0.065 shear, 0.038 plot). Plot was marginal for juvenile deertoes (p=0.067) and significant for juvenile fragile papershells (p=0.041); shear was insignificant for juveniles of all species.

Community Composition

Non-metric multidimensional scaling showed few differences between the two plots (Figure 4). Species vectors were consistently minor compared with variation between quadrats. Adult pistolgrips and deertoes were relatively rarely found together, while Western pimplebacks and Texas pigtoes were closely correlated. Although points did not form distinct clusters, upstream quadrats chiefly mapped to the right side of the biplot (Figure 5a), while downstream quadrats were largely to the left. The vectors for Texas pigtoe and Western pimpleback both had strongly negative Axis 1 values, reflecting their greater density at the downstream plot.

No distinct patterns in juvenile density could be observed (Figure 5b). Many quadrats had identical juvenile abundances and plotted to identical coordinates (e.g. six separate quadrats, each with a single Texas pigtoe, plotted to 1.01937 -0.59059). Deertoe and Texas pigtoe vectors were strongly opposed, while Texas pigtoe and fragile papershell were somewhat correlated.

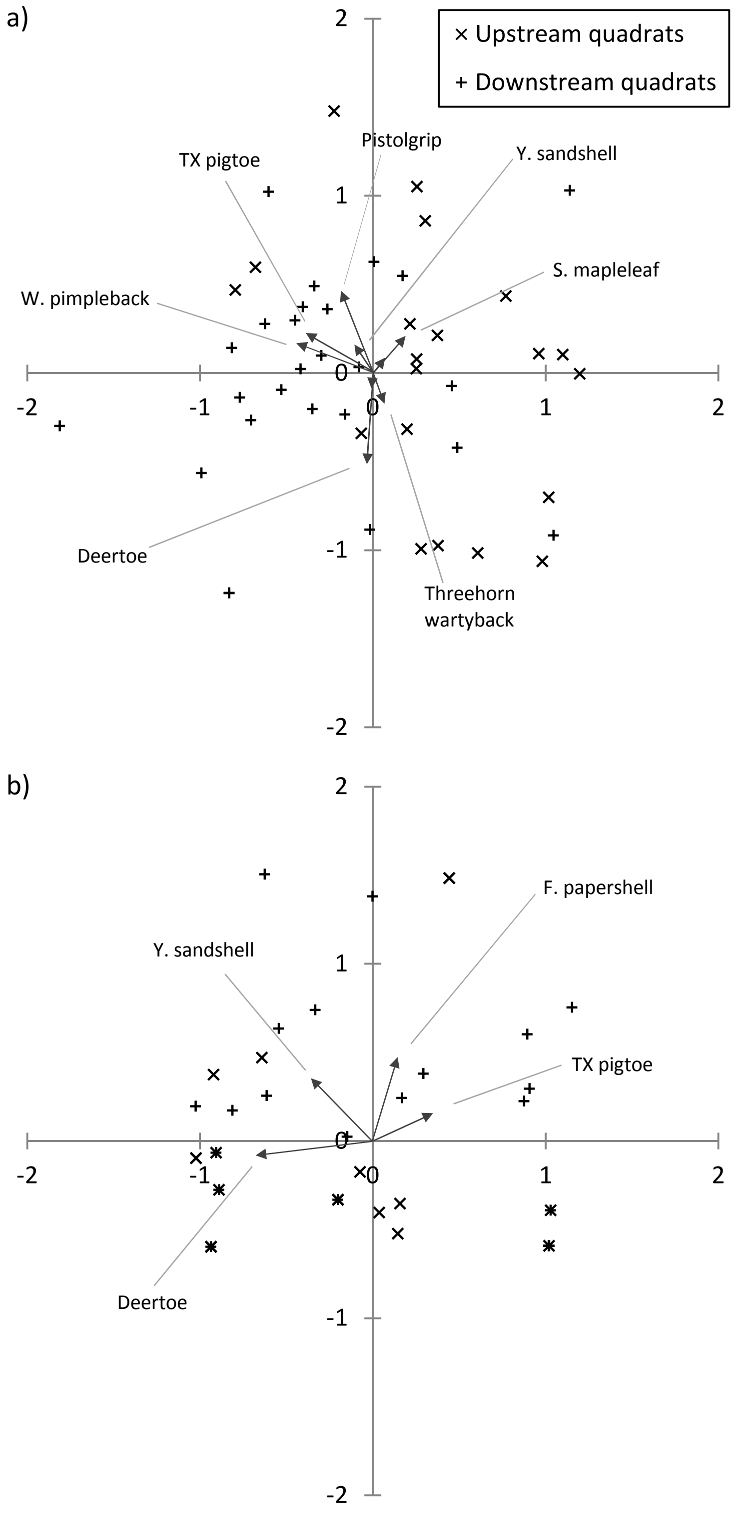

Figure 5. NMDS biplots of adult (a) and juvenile (b) mussel community composition.

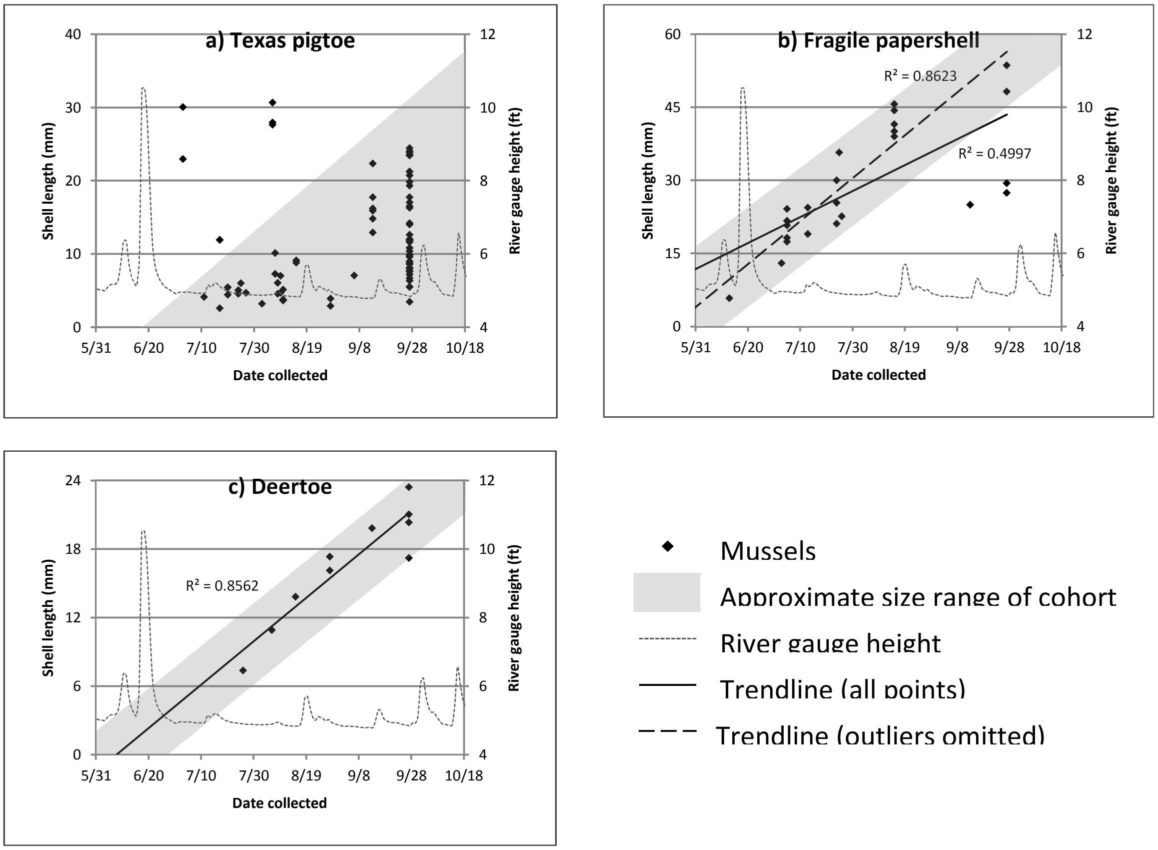

Figure 6. Size versus date of collection for all small Texas pigtoes, fragile papershells, and deertoes located.

Growth Rate and Timing of Excystment

Small Texas pigtoes, some barely large enough to be retained by the 2mm sieve, were collected starting in early July (Figure 6a). The individuals I collected likely excysted shortly before this, in late June. This point in time coincides with a substantial flood, during which the river height rose to approximately two meters above base flow. By the last round of field collection, on September 27, young-of-year pigtoes had achieved a maximum size of approximately 25 mm. I believe that the five pigtoes >20 mm collected in July and early August were recruited in 2011. Small pigtoes were located throughout the season.

Fragile papershells were among the first juveniles located, starting in the middle of June (Figure 6b). Only one individual, the first one located, was less than 10mm in length. The largest individuals collected on the last day of sampling exceeded 50 mm. Throughout the season, papershell size and date of collection were closely correlated, except for a cluster of unusually small outliers located in September that I suspect represented a separate recruitment event. I estimate that excystment of this species peaked in middle to late May, and ended before the late-June flood.

The first juvenile deertoe collected, slightly over 7 mm in length, was located in late July (Figure 6c). Deertoes were consistently larger in size the later they were collected, reaching a maximum size of close to 25 mm by late September. This species evidently excysted *en masse* in the middle of June, around the time of the flood, and shortly after the main cohort of fragile papershells.

Discussion

Remarks

In a summer of research, I surveyed 50 quadrats and collected slightly more than 600 mussels. Less than a quarter were young-of-year, and almost half of the young-of-year were Texas pigtoes. Juveniles of all other species were rare. This small sample size, coupled with the fact that this study only covers a single season at a single site, makes statistically rigorous analyses challenging and makes well-supported conclusions difficult to draw. The results of this study should not be assumed to be representative of mussels nationwide, or even throughout east Texas, and the conclusions herein are largely speculative.

Nevertheless, some patterns were recognizable. Differences in observed recruitment success were substantial, and some ecological qualities could be inferred for the three species most common as juveniles (Texas pigtoe, deertoe, and fragile papershell).

Recruitment and Population Health

Based on the large number of juveniles collected, Texas pigtoes appear to have had high reproductive success in the Sabine River in 2012. I also collected adults of all sizes, indicating that reproductive success for this species has been high in past years as well. Deertoes also had high recruitment in 2012, albeit by a smaller margin than Texas pigtoes.

Pistolgrips and Western pimplebacks were numerous as adults but very rare as juveniles. I did not collect enough juveniles of either species to estimate growth rate, so I cannot say with confidence that all of the “juveniles” I collected were in fact young-of-year. The near absence of juvenile pistolgrips in particular contrasts starkly with their considerable adult density; this species recruited poorly in the 2012 season. This may be a long-term pattern; adults were typically large (>10 cm) with weathered shells, indicative of age, while smaller individuals were rarely located.

Superficially, this suggests that this species is in decline, despite its present abundance. However, I lack estimates of adult mortality and cannot gauge the overall rate of population change. If adult mortality is low, juvenile recruitment does not need to be rapid. Additionally, a single season of data may not accurately represent long-term trends. Payne and Miller (2000) determined that ebonyshell mussels in the lower Ohio River only recruit under highly specific river conditions, but recruitment under these conditions is high enough to offset weak recruitment in most years. Likewise, pistolgrip recruitment may require specific conditions that were not met in 2012.

Fragile papershells were more common as juveniles than as adults. There are no indications that this species is climbing in number; unless recruitment was unusually high in 2012, juvenile mortality must be high to compensate for this level of fecundity. The explanation may lie in their reproductive ecology. Freshwater drum (*Aplodinotus grunniens*) is the only known papershell host (Howells et al. 1996), and they presumably acquire glochidial infections by eating gravid females (Haag 2012). Papershells can potentially achieve reproductive maturity in under a year (Haag 2012), and as I located young-of-year papershells approaching and exceeding the 53 mm size at maturity identified by Littleton (referenced in Howells et al. 1996), I believe they do so in the Sabine River. High fecundity and high first-year mortality are both likely results of heavy seasonal predation by drum. I suspect that female papershells in the Sabine River rarely live over a year because of predation, and are effectively a semelparous species.

Role of Habitat Variables

I anticipated that local patterns in baseflow shear stress would affect settling of juveniles, either by promoting particle settling or providing favorable microhabitat to host fish. While this variable has not correlated with mussel abundance in previous studies (Allen and Vaughn 2010, Daraio et al. 2010, Gangloff and Feminella 2007, Hardison and Layzer 2001), I predicted different results with regards to juvenile abundance, as juveniles that have not yet encountered bankfull conditions have not been affected by flood-level scour. This hypothesis was not corroborated by my data, which found no relationship between juvenile abundance and shear stress.

I found that adult presence/abundance was more strongly correlated with baseflow shear stress than juvenile presence/abundance, which contradicts most prior studies as well as my predictions. However, in most cases where shear stress was significant, plot was substantially more so, and I suspect that these instances reflect the consistently higher base-flow shear stress at the downstream plot. Species that were more common in the riffle for any reason would have been more common at high shear stress as a result.

Southern mapleleafs were consistently absent from areas of high baseflow shear stress, although this variable was only significant in the full-model logistic regression (p=0.069 for logistic regression, p=0.281 for linear regression). I did not observe this pattern for any other species. This suggests that Southern mapleleafs are more susceptible to pre-settlement processes than most species. The host fish for this species is not known, although other *Quadrula*, including the closely related mapleleaf (*Quadrula quadrula*), metamorphose on catfish

(Ictaluridae) or, more rarely, sunfish (Centrarchidae). Assuming Southern mapleleafs require catfish as hosts, host fish behavior is an unlikely explanation, as pistolgrips (which are entirely reliant on catfish) did not exhibit the same pattern. Newly excysted Southern mapleleafs may drift with the stream current and settle in deposition areas, although I did not collect enough juvenile Southern mapleleafs to evaluate this hypothesis directly.

Adult Texas pigtoes were significantly more common in the downstream riffle than the upstream run, while juveniles were more evenly distributed between the two plots. This is broadly consistent with the "juvenile-scour" hypothesis; the channelized upstream plot has higher estimated bankfull shear stress than the downstream run. It is plausible that juveniles that settle in the upstream run are less likely to survive floods than those in the more protected habitat downstream.

Size data also lend conditional support to this hypothesis. Most of the juvenile pigtoes located evidently excysted after a substantial flood in late June. I do not know if excystment occurred prior to this date, but it occurred steadily throughout the research season, and could well have started before the flood. If juveniles had excysted early in the year, they would have been highly susceptible to scour during the flood. The scarcity of juveniles that excysted earlier may have been the result of a mass mortality event.

Deertoes followed the opposite pattern; adults were slightly denser in the upstream run, while juveniles were overwhelmingly located in the downstream riffle. While I cannot conclusively identify what factors drove this pattern, distribution of juvenile deertoes was strikingly similar to that of juvenile fragile papershells. Both species were significantly more prevalent in the downstream riffle than the upstream run; both species exhibited a similar response to shear stress; and both species excysted *en masse* around the same time.

The NMS analysis does not support this interpretation, as it shows a mild negative correlation between deertoe and papershell abundance. However, all species vectors in this analysis were weak, and presumably resulted from the generally low density of juveniles. A larger analysis with more sites may have revealed more significant patterns.

Freshwater drum is the only host for either species found in the Sabine River, and I propose that host behavior is responsible for the observed similarities. If drum favored the downstream area between late May and the middle of June, around the same time I estimate that papershells and deertoes were excysting, these species would have settled frequently in this area. Alternatively, if mussel growth rate is rapid immediately following excystment and slows in later weeks, both species may have excysted during the late-June flood. If so, the flood may have driven drum to the relatively sheltered downstream plot and promoted settling in the same area. Severe mortality in the upstream section could also explain the pattern, as previously hypothesized for Texas pigtoe, but this does not explain the rarity of excystment later in the season. Feeding behavior of drum in Lake Erie has pronounced seasonal patterns, with different food items predominating at different times of year (Griswold and Tubb 1977). If drum in the Sabine River follow seasonal feeding patterns as well, they probably feed heavily on bivalves in late spring.

Review of Sampling Methods

The methods used here were highly labor-intensive compared with conventional adult-only time and quadrat surveys. In addition to searching the quadrat by hand for adults, I had to collect riverbed material (depending on substrate makeup, this could take as much as an hour), run it through a coarse sieve, run the fine material through a 2 mm sieve, and finally search the 2 mm portion for bivalves. Completing a single 0.25-m^2 quadrat usually took two to three hours, compared with as little as ten minutes for a tactile survey. As a result, I completed fewer than 50 quadrats over the season and did not collect enough juveniles of most species to conduct robust statistical analyses. However, it did result in collection of young-of-year juveniles as small as 3 mm, which is not a feasible goal if using conventional survey methods.

The usefulness of the methods used in this thesis depends on the goal of the study. For a detailed analysis of a single season's recruitment, including settling patterns and mortality events, thorough and ongoing sampling is necessary. This requires extensive manpower and considerable time, if a statistically relevant sample size is desired. This type of study has the potential to reveal details of species-specific life history, such as first-season growth rates and timing of excystment. It may also reveal season-specific events such as flood-related mortality. Other methods may be more practical for exploring these questions, however. Life history and survival are more easily studied using artificial populations (including "seeded" mussel beds in natural rivers) than naturally occurring ones. Natural post-excystment settling patterns can only be investigated by intensive field sampling, but the degree of labor involved makes this an impractical topic for study.

Population health is best evaluated over the course of years; a single season's data are unlikely to be representative of long-term trends. To measure long-term population growth and identify conditions conducive to recruitment, adult age distributions are more informative than single-season juvenile surveys. Adult surveys should incorporate shell measurements and attempt to identify the year in which each collected mussel was recruited.

If collection of small mussels is required (for instance, to estimate first-year winter mortality), sieving is necessary. Tactile detection of mussels is most effective for large individuals and infeasible for individuals smaller than approximately 20-30 mm, depending on substrate composition. However, juveniles may be collected by simpler protocols than I used. If precise quantitative measurements (i.e. exact and directly comparable per-quadrat counts) are not required, running handfuls of substrate through a 10 mm sieve could improve detection of small mussels without substantially more per-quadrat effort than a conventional tactile survey requires. Young-of-year juveniles over 10 mm were present at my site by August, so field collection of recently recruited mussels is possible by this method if conducted late in the season.

Future Directions

Mussel conservation depends on understanding the ecological needs of juveniles. Adult presence does not confirm active reproduction, so merely replicating or preserving conditions under which adults are currently found is not sufficient to protect mussel populations.

As evidenced by the differences in distribution between the species at the study site, different species have different requirements. Identifying these requirements should be a priority in mussel conservation; even common, secure species may be useful in identifying patterns that could be applied to threatened or endangered species. The influence of physical variables such as shear stress could be studied effectively by introducing captive-raised juveniles to either artificial habitat (e.g. raceways in fish hatcheries, as in Hanlon 2000) or plots in natural rivers. Manually introducing mussels to specific habitats would isolate the direct effects of physical conditions on juvenile survival from host fish behavior and other pre-settlement processes.

This study adds to the growing body of evidence that host fish behavior plays a critical and poorly understood role in mussel recruitment success. This topic is not so easily investigated through captive studies. Monitoring host-fish behavior and microhabitat selection, potentially by tagging host fish and tracking their movement, could be one effective means of investigating this difficult question. Comparing host-fish movement to late-season young-of-year mussel distribution could clarify the role that host fish play in determining post-excystment settling patterns.

Regardless of methods used, future studies should examine the specific conditions under which mussel recruitment occurs in addition to broad-scale, long-term factors such as land use and cover. Conservation measures that only promote adult survival will not delay the ongoing declines of unionoid populations; reproduction and juvenile survival are necessary to ensure the preservation of this undervalued resource.

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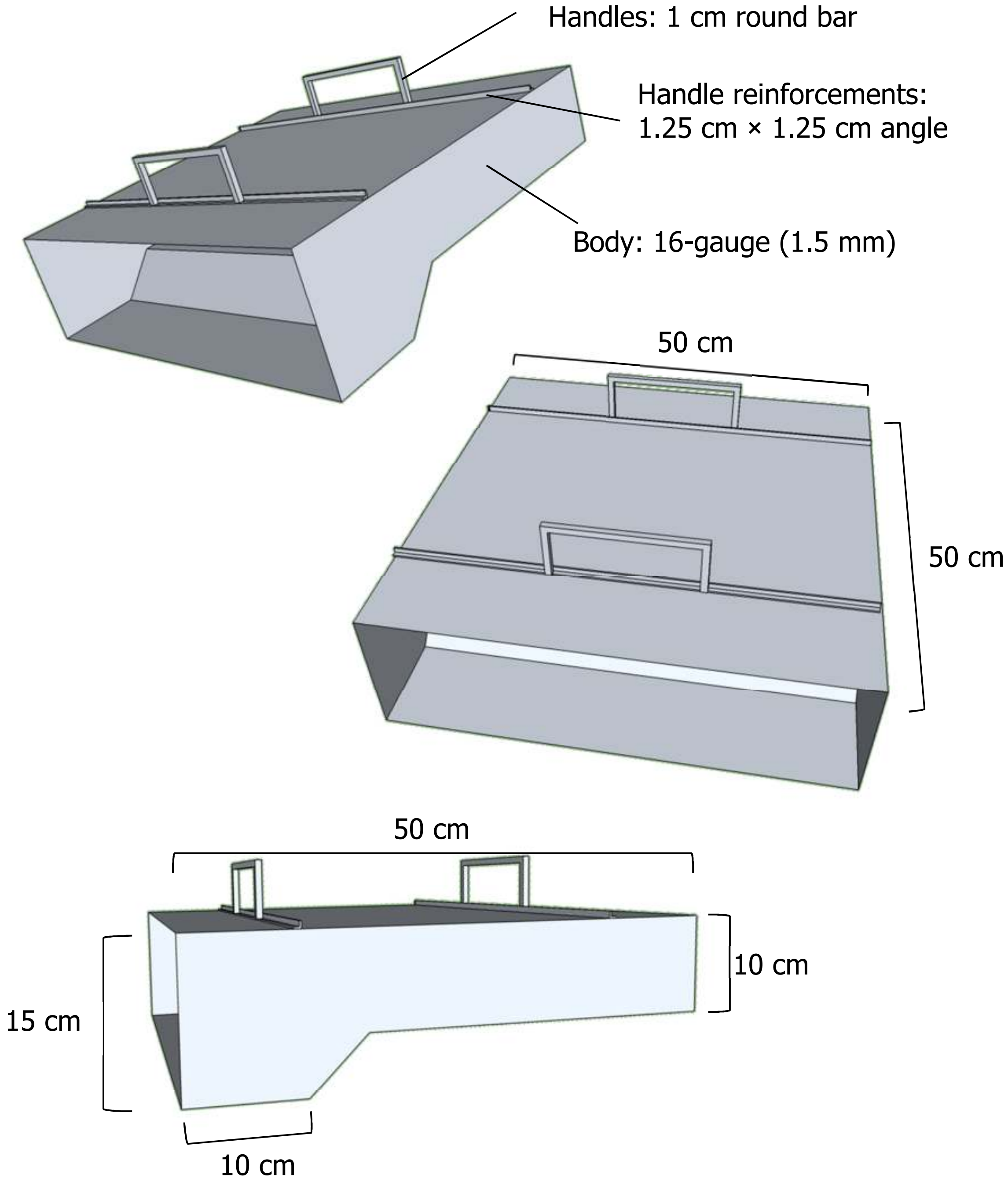
APPENDIX A. Materials and construction of sediment sampler



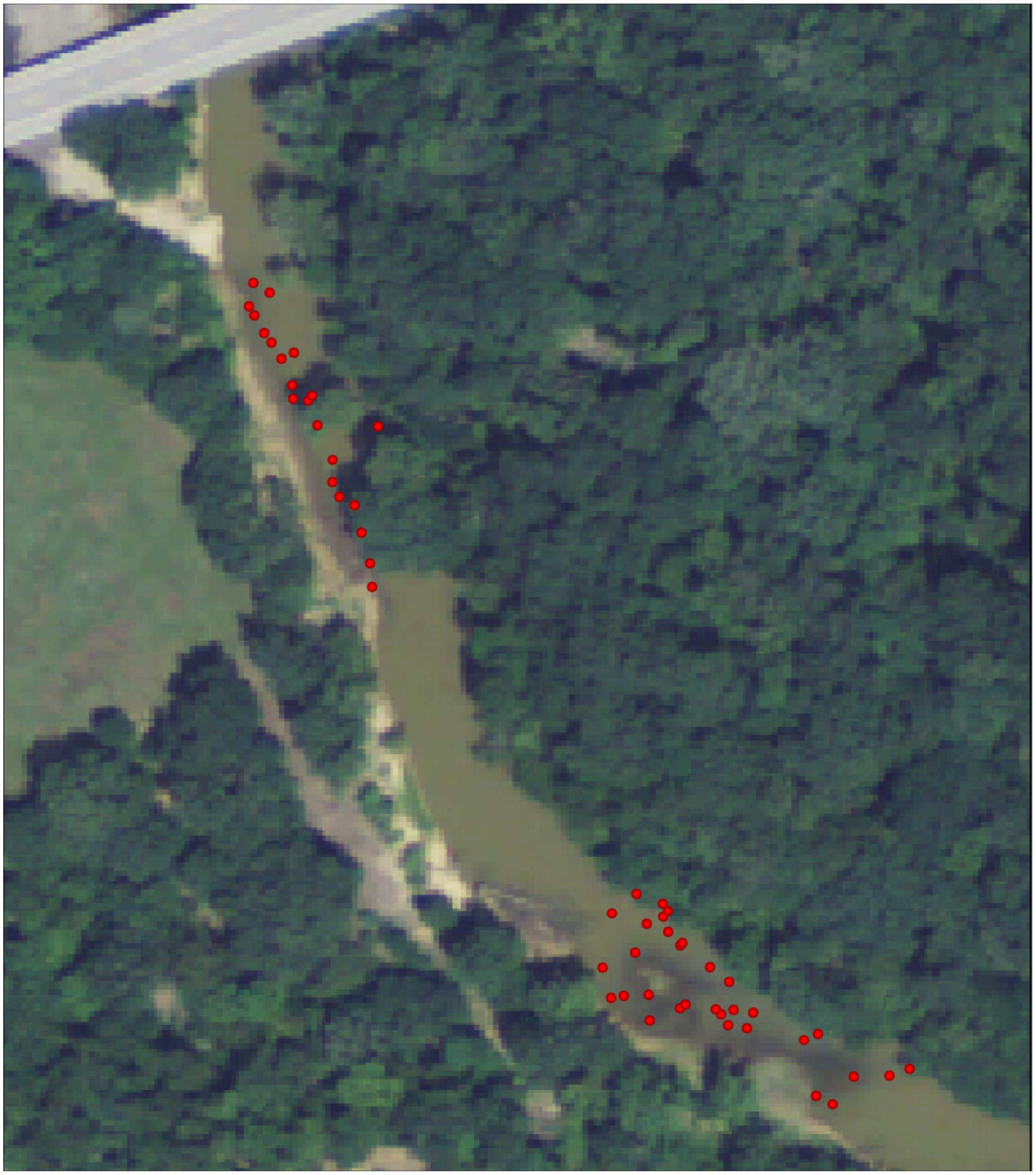
APPENDIX B. Details of FST hemispheres

Hemisphere number	Mass (g)	Density (g cm^{-3})	Shear stress (dyn cm^{-2})
1	14.6	1.016006	0.837161
2	14.82	1.031315	0.949228
3	15.26	1.061935	1.173361
4	15.75	1.096033	1.422965
5	16.22	1.12874	1.66238
6	17.24	1.199722	2.181962
7	18.35	1.276966	2.74739
8	20.57	1.431454	3.878246
9	24.25	1.687543	5.752818
10	26.37	1.835073	6.832735
11	30.38	2.114127	8.875407
12	34.64	2.410578	11.04543
13	37.4	2.602644	15.27328
14	43.79	3.047321	23.94235
15	48.09	3.346555	31.2683
16	54.24	3.77453	44.0615
17	61.67	4.29158	63.52709
18	69.3	4.822547	88.58046
19	78.15	5.438413	124.7659
20	90.2	6.276966	187.753
21	100.02	6.960334	252.0552
22	113.47	7.896312	361.1264

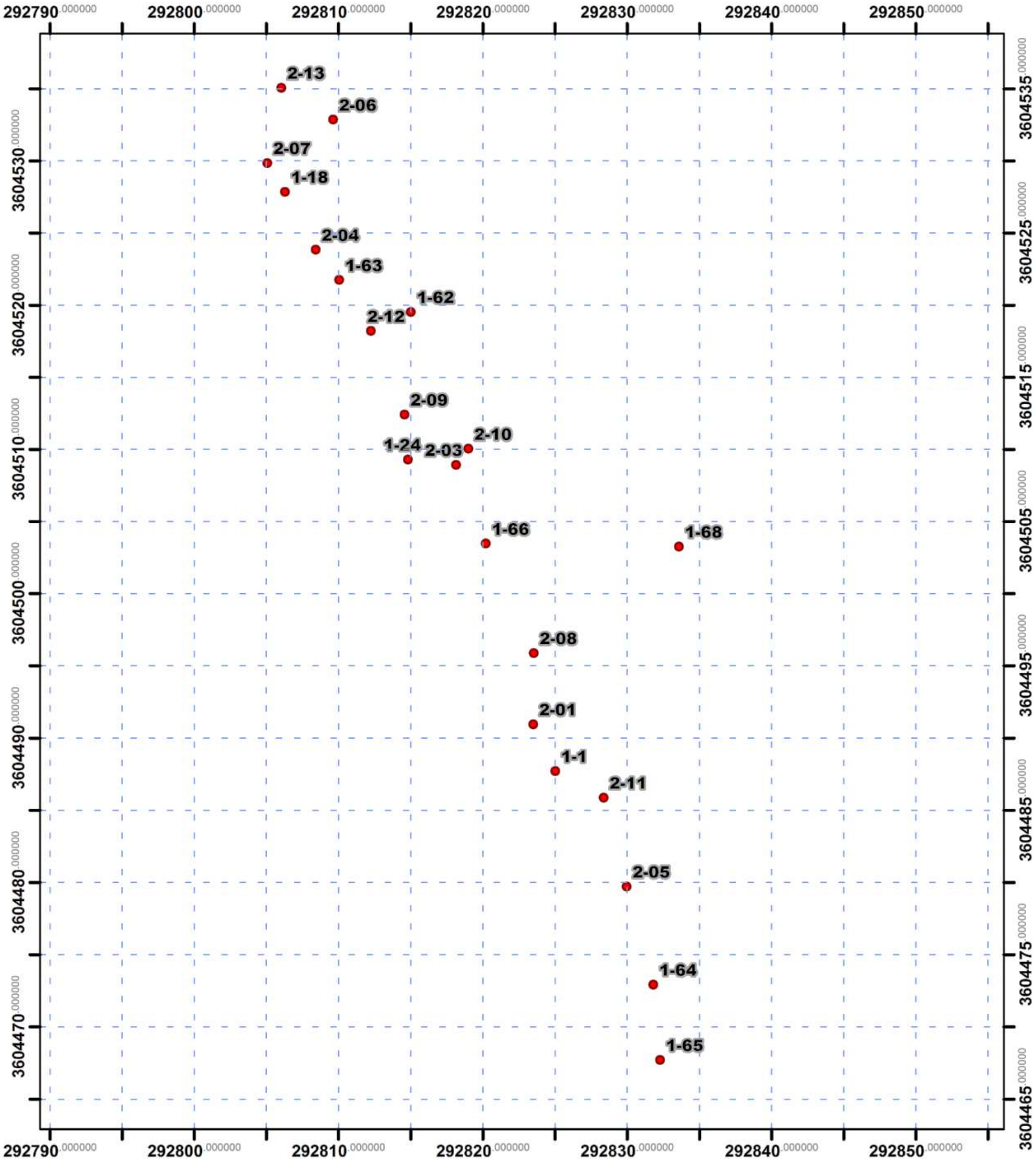
FST hemispheres (based on Statzner and Müller 1989) consisted of halved 38mm table-tennis balls filled with EpoxAcast 650 epoxy casting resin and medium hardener (Smooth-On, Easton, PA). Hemispheres less dense than epoxy alone contained internal voids; hemispheres denser than epoxy contained lead-alloy or tungsten shotgun pellets as ballast. Both the hemispheres and the acrylic-topped baseplate were coated with Rust-Oleum aerosol paint.

Shear stress necessary to initiate movement of stationary hemispheres was estimated differently depending on mass. For hemispheres 1-12, I used the formula $\tau=7.32d-6.60$, where d is the density of the hemisphere in g cm^{-3}. For all others, I used $\tau=d^{2.85}$ (Statzner et al. 1991).

APPENDIX C. Maps of quadrat locations

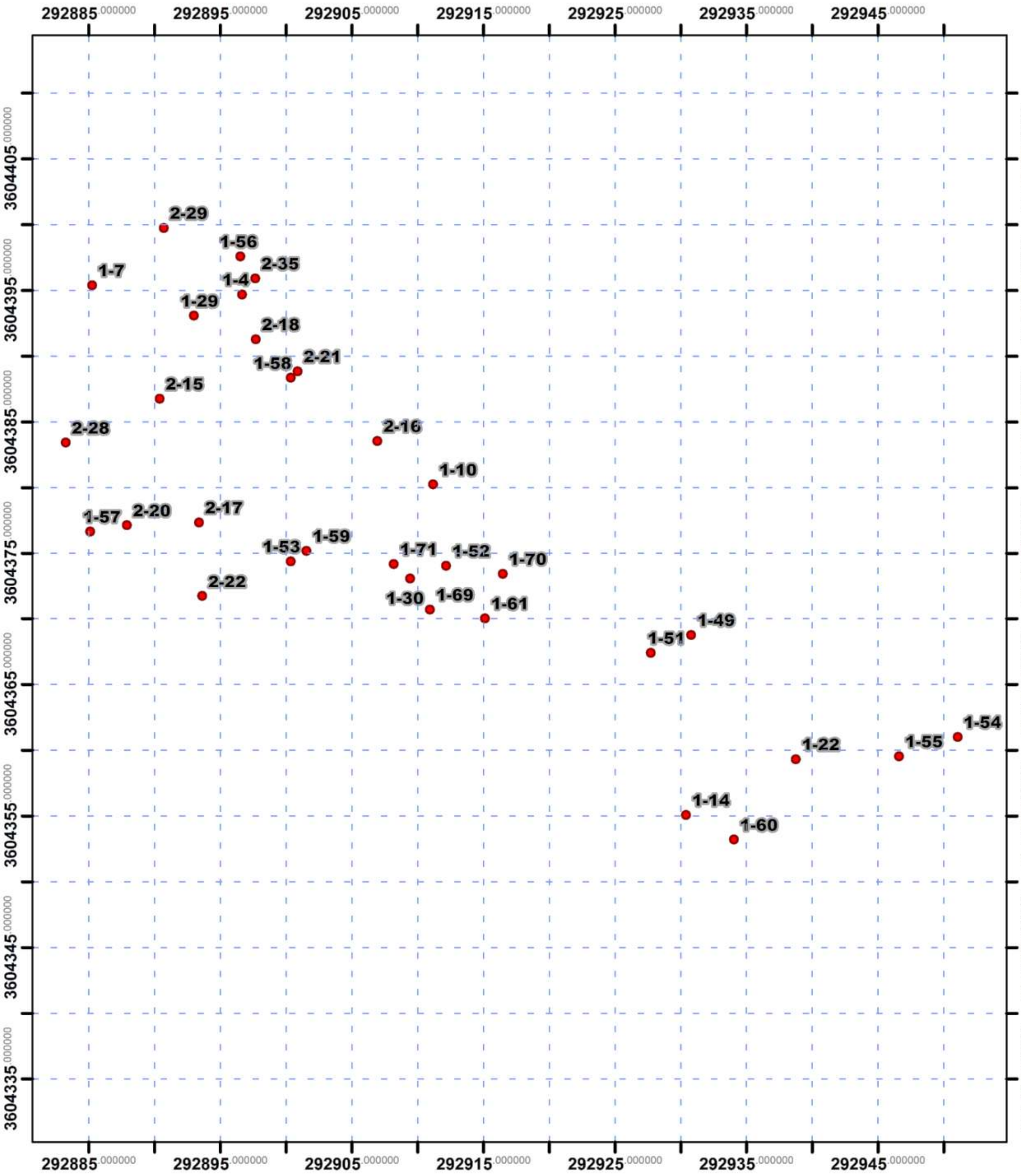


C-1. Overview of survey area. FM-14 bridge visible at top of photo.



UTM 15N, NAD 1983

C-2. Locations of quadrats at the upstream run plot, with 5-meter UTM grid.



UTM 15N, NAD 1983

C-3. Locations of quadrats at the downstream riffle plot.

APPENDIX D. Per-quadrat counts of adults and juveniles

Quadrat ID	Date	Shear (dyn cm^{-2})	*Fusconaia askewi*	*Quadrula verrucosa*	*Truncilla truncata*	*Quadrula mortoni*	*Quadrula apiculata*	*Leptodea fragilis*	*Plectomerus dombeyanus*	*Potamilus purpuratus*	*Lampsilis teres*	*Obliquaria reflexa*
1-7	6/22	N/A	0	0	0	0	0	0	0	0	0	0
1-4	6/22	N/A	0	0	0	0	0	0	0	0	0	0
1-29	6/22	N/A	0	0	0	0	0	0	0	0	1	0
1-30	7/3	1.173	0	0	0	0	0	0	1	0	0	0
1-49	7/3	1.423	0	0	0	0	0	0	0	0	0	0
1-10	7/3	3.878	2	0	0	0	0	1	0	0	0	1
1-14	7/5	0.837	0	0	0	0	0	0	0	0	2	0
1-22	7/5	0.949	0	0	0	0	0	5	0	0	0	0
1-51	7/11	1.662	1	0	0	0	0	0	0	0	0	0
1-52	7/13	1.662	0	0	0	0	0	1	0	0	1	0
1-53	7/13	1.662	0	0	0	0	0	1	0	0	1	0
1-55	7/17	1.423	1	0	0	0	0	0	0	0	0	0
1-54	7/17	2.747	1	0	0	0	0	0	0	0	0	0
1-56	7/18	1.662	0	0	0	0	0	0	0	0	0	0
1-58	7/18	1.662	0	0	0	0	0	0	0	0	1	0
1-57	7/20	0.837	2	0	0	0	0	0	0	0	0	0
1-59	7/20	1.173	0	0	0	0	0	0	0	0	0	0
1-60	7/24	0.700[1]	0	0	0	0	0	1	0	0	0	0
1-61	7/24	1.662	2	0	0	0	0	2	0	1	0	0
1-69	8/6	0.949	1	1	0	0	0	0	0	0	0	0
1-71	8/6	0.949	0	0	1	0	1	0	0	0	0	0
1-70	8/6	1.662	2	0	0	0	0	0	0	1	1	0
2-15	8/15	0.949	2	0	1	0	0	5	0	0	0	0
2-29	8/15	1.662	0	0	0	0	0	0	0	0	0	0
2-18	8/28	1.662	1	0	2	0	0	0	0	0	0	0
2-35	8/28	2.182	1	0	0	1	0	0	0	0	0	0
2-28	9/9	0.700[1]	0	0	0	0	0	0	0	0	0	0
2-21	9/9	1.662	1	0	0	0	0	0	0	0	0	0
2-20	9/13	0.700[1]	6	0	1	0	0	1	0	0	0	0
2-16	9/13	3.878	0	1	0	0	0	0	0	0	0	0
2-17	9/27	1.173	8	0	1	1	0	2	0	0	0	1
2-22	9/27	1.423	33	0	3	1	0	2	0	0	0	0

[1] If river current was too slow to move Hemisphere 1, 0.700 dyn cm^{-2} was entered as a placeholder value (Statzner et al. 1991).

D-1. Number of juveniles of all species per 0.25 m^2 quadrat in downstream (riffle) plot.

Quadrat ID	*Fusconaia askewi*	*Quadrula verrucosa*	*Truncilla truncata*	*Quadrula mortoni*	*Quadrula apiculata*	*Leptodea fragilis*	*Plectomerus dombeyanus*	*Lampsilis teres*	*Obliquaria reflexa*	*Megalonaias nervosa*	*Potamilus purpuratus*	*Anodonta imbecilis*	*Arcidens confragosus*	*Anodonta suborbiculata*
1-7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1-29	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1-30	5	3	0	1	3	0	1	0	0	0	1	0	0	0
1-49	24	2	3	5	2	0	0	1	1	3	0	0	0	0
1-10	3	0	0	1	0	0	0	0	0	0	0	0	0	0
1-14	3	3	3	3	0	0	1	0	0	0	0	0	0	0
1-22	5	2	2	2	2	0	0	0	0	0	0	0	0	0
1-51	17	4	0	2	1	1	0	0	0	0	1	0	0	0
1-52	0	2	0	1	0	0	0	0	0	0	0	0	0	0
1-53	7	5	1	2	1	0	0	0	0	0	0	0	0	0
1-55	1	0	2	3	0	0	0	0	0	0	0	0	0	0
1-54	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1-56	0	0	0	1	0	0	0	0	0	0	0	0	0	0
1-58	28	4	2	2	4	0	0	0	1	0	0	0	0	0
1-57	0	0	0	0	1	0	0	0	0	0	0	0	0	0
1-59	2	1	0	0	2	0	1	0	0	0	0	0	0	0
1-60	2	0	2	0	1	0	0	0	0	0	1	1	0	0
1-61	2	2	0	0	1	0	0	0	0	0	0	1	0	0
1-69	6	1	0	2	1	0	0	1	0	1	0	0	0	0
1-71	4	6	0	2	2	0	2	0	0	0	0	0	0	0
1-70	16	7	5	2	2	0	1	0	0	0	1	0	0	0
2-15	14	1	1	1	3	0	1	0	0	0	0	1	0	0
2-29	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2-18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2-35	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2-28	0	0	2	0	0	0	0	0	1	0	0	0	0	0
2-21	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2-20	6	8	3	2	0	0	2	1	1	0	0	0	0	0
2-16	0	2	1	1	0	0	0	0	0	0	1	0	0	0
2-17	7	4	0	0	1	0	1	0	1	0	0	0	0	0
2-22	6	4	0	4	3	0	1	0	0	1	0	0	0	0

D-2. Number of adults of all species per 0.25 m^2 quadrat at downstream (riffle) plot.

Quadrat ID	Date	Shear (dyn cm^{-2})	*Fusconaia askewi*	*Quadrula verrucosa*	*Truncilla truncata*	*Quadrula mortoni*	*Quadrula apiculata*	*Leptodea fragilis*	*Plectomerus dombeyanus*	*Potamilus purpuratus*	*Lampsilis teres*	*Obliquaria reflexa*
1-1	6/13	0.700[1]	0	0	0	0	0	1	0	0	1	0
1-18	6/25	0.700[1]	0	0	0	0	0	0	0	0	0	0
1-24	6/25	0.700[1]	0	0	0	0	0	0	0	0	0	0
1-62	7/25	0.700[1]	1	0	0	0	0	0	0	0	0	0
1-63	7/25	0.700[1]	0	0	0	0	0	1	0	0	0	0
1-65	7/26	0.700[1]	0	0	0	0	0	1	0	0	0	0
1-64	7/26	0.949	0	0	1	0	1	0	0	0	0	0
1-66	8/2	0.700[1]	1	0	0	0	0	0	0	0	0	0
1-68	8/2	0.700[1]	1	0	0	0	0	0	0	0	0	0
2-13	8/7	0.949	1	0	0	0	0	0	0	0	1	0
2-06	8/7	0.949	1	1	0	0	0	0	0	0	0	0
2-07	8/8	0.700[1]	2	0	0	0	0	0	0	0	0	0
2-04	8/8	0.700[1]	0	0	0	0	0	0	0	0	1	0
2-12	8/9	0.700[1]	2	0	0	0	0	0	0	0	0	0
2-09	8/9	0.700[1]	0	0	0	0	0	0	0	0	0	0
2-03	8/9	0.700[1]	0	0	0	0	0	0	0	0	0	0
2-10	8/10	0.700[1]	0	0	0	0	0	0	0	0	0	0
2-08	8/10	0.700[1]	0	0	0	0	0	0	0	0	0	0
2-01	8/10	0.700[1]	3	0	0	0	0	0	0	0	0	1
2-05	8/13	0.700[1]	0	0	0	0	0	0	0	0	0	0
2-11	8/13	0.949	0	0	0	0	2	0	0	0	0	0

D-3. Number of juveniles of all species per 0.25 m^2 quadrat at upstream (run) plot.

Quadrat ID	*Fusconaia askewi*	*Quadrula verrucosa*	*Truncilla truncata*	*Quadrula mortoni*	*Quadrula apiculata*	*Leptodea fragilis*	*Plectomerus dombeyanus*	*Lampsilis teres*	*Obliquaria reflexa*	*Megalonaias nervosa*	*Potamilus purpuratus*	*Anodonta imbecilis*	*Arcidens confragosus*	*Anodonta suborbiculata*
1-1	0	0	2	0	0	0	0	0	0	0	0	0	0	0
1-18	0	0	1	0	1	0	0	0	0	0	0	0	0	1
1-24	0	2	0	0	1	0	0	0	0	0	0	0	0	0
1-62	0	0	2	1	0	0	0	0	0	0	0	1	0	0
1-63	0	3	0	0	2	0	0	0	1	0	0	0	0	0
1-65	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1-64	1	0	1	0	0	0	0	0	1	0	0	0	0	0
1-66	0	1	1	0	1	0	0	0	0	0	0	0	0	0
1-68	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2-13	3	1	1	0	4	0	2	2	0	0	0	0	0	0
2-06	2	2	2	0	5	0	0	0	0	1	0	0	0	0
2-07	3	4	0	0	0	0	0	2	0	0	0	1	0	0
2-04	0	0	1	0	3	0	2	0	0	1	0	0	0	0
2-12	0	0	1	0	2	0	0	0	0	0	0	1	0	0
2-09	1	4	0	1	0	0	0	0	0	0	0	0	1	0
2-03	0	0	3	0	0	0	1	0	2	0	1	0	0	0
2-10	1	3	2	0	0	0	0	0	0	0	0	0	0	0
2-08	1	2	1	0	1	0	1	0	0	0	0	0	0	0
2-01	1	3	2	2	0	0	0	1	0	0	0	0	0	0
2-05	1	0	4	0	0	0	0	0	0	0	0	0	0	0
2-11	2	2	4	0	4	0	0	0	0	0	0	0	0	0

D-4. Number of adults of all species per 0.25 m^2 quadrat at upstream (run) plot.

APPENDIX E. Catalog of collected juvenile mussels

Quadrat ID	Species	Shell length (mm)
1-01	Fragile papershell	5.86
	Yellow sandshell	10.6
1-29	Yellow sandshell	8.7
1-49	Threehorn wartyback	40.9
1-10	Fragile papershell	13
	Texas pigtoe	30[1]
		22.9[1]
	Threehorn wartyback	33.4
1-30	Bankclimber	39
1-14	Yellow sandshell	12.8
		11.3
1-22	Fragile papershell	24.1
		21.7
		20.8
		18.2
		17.4
1-51	Texas pigtoe	4.14
1-52	Fragile papershell	19
	Yellow sandshell	14.8
1-53	Fragile papershell	24.4
	Yellow sandshell	11.8
1-54	Texas pigtoe	11.9
1-55	Texas pigtoe	2.59
1-58	Yellow sandshell	11.2
1-57	Texas pigtoe	5.45
		4.42
1-60	Fragile papershell	25.4
	Paper pondshell[2]	15.5
1-61	Bleufer	30
	Fragile papershell	30
		21.1
	Paper pondshell[2]	11.5
	Texas pigtoe	5.05
		4.57
1-62	Paper pondshell[2]	32.5
	Texas pigtoe	6
1-63	Fragile papershell	35.7
1-64	Deertoe	7.35
	Southern mapleleaf	20.8
1-65	Fragile papershell	22.6
1-66	Texas pigtoe	4.68
1-67	Texas pigtoe	3.21
1-69	Pistolgrip	3.42
	Texas pigtoe	27.6
1-70	Bleufer	35.6
	Texas pigtoe	30.6[1]
		27.9[1]
	Yellow sandshell	10.9
1-71	Deertoe	10.9
	Southern mapleleaf	30.3
2-06	Pistolgrip	14.9
	Texas pigtoe	7.28[3]
2-13	Texas pigtoe	10.1[3]
	Yellow sandshell	13.2
2-04	Yellow sandshell	13.5
2-07	Paper pondshell[2]	32.5
	Texas pigtoe	6.05
		4.57
2-12	Paper pondshell[2]	25
	Texas pigtoe	7
		4.71
2-01	Texas pigtoe	5.14
		3.79
		3.63[3]
	Threehorn wartyback	7.53
	Yellow sandshell	31.1[3]

[1] These large Texas pigtoes most likely recruited in 2011.

[2] Age could not be estimated for paper pondshells, so all individuals collected are listed here.

[3] Species was confirmed genetically for these individuals.

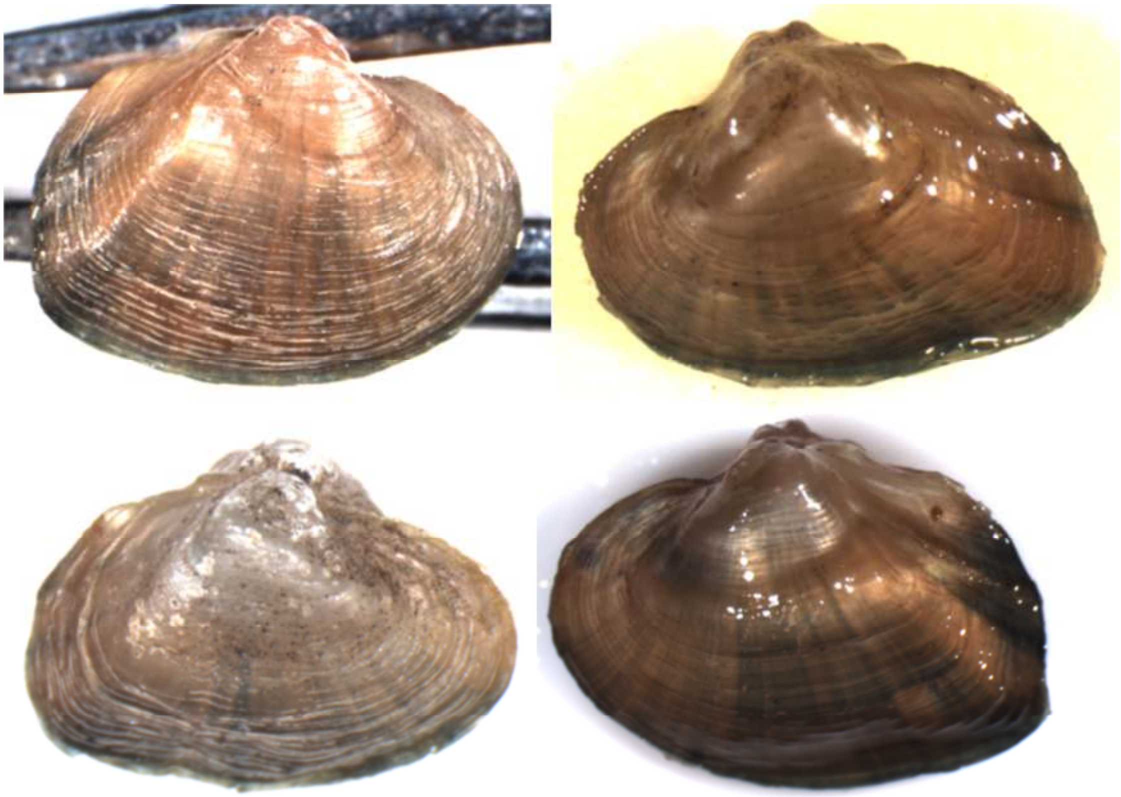
[4] Species identification is uncertain for these individuals.

(Continued on next page)

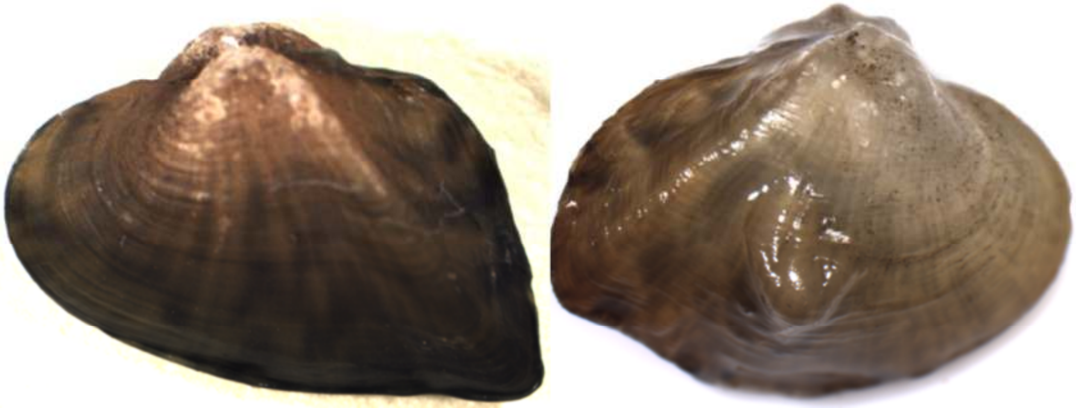
(Appendix E, continued)

Quadrat ID	Species	Shell length (mm)
2-15	Deertoe	13.8
	Fragile papershell	45.6
		44.3
		41.5
		40
		39
	Paper pondshell[2]	12
	Texas pigtoe	9.1
		8.8
2-35	Texas pigtoe	3.89
	Western pimpleback	24.1
2-18	Deertoe	17.3
		16.1
	Texas pigtoe	2.91
2-21	Texas pigtoe	7.05
2-16	Pistolgrip	23
2-20	Deertoe	19.8
	Fragile papershell	25
	Texas pigtoe	22.3
		17.7
		16.2
		15.9
		14.8[4]
		12.9
2-17	Deertoe	23.4
	Fragile papershell	53.6
		48.2
	Texas pigtoe	23.8
		21.2
		21.2
		11.8
		11.6
		10.4
		6.35
		5.52
	Threehorn wartyback	9.5
	Western pimpleback	10.4
	Undetermined	21.3
2-22	Deertoe	21
		20.3
		17.2
	Fragile papershell	29.4
		27.4
	Texas pigtoe	24.4
		24
		23.5
		23.4
		20.7
		19.8
		19.3
		17.7
		17
		16.5
		16.3
		14.2
		14
		12.6
		12
		12
		11.8
		10.8
		9.9
		9.8
		9.6
		9
		8.7
		8.6
		8.1
		8
		7.7
		7.6
		7.2
		6.7
		5.5
		3.48[4]
	Western pimpleback	35.4

APPENDIX F. Photos of selected juvenile mussels



Texas pigtoes (*Fusconaia askewi*), shell lengths 5.45 mm, 4.57 mm, 3.63 mm, 6.05 mm.



Deertoe (*Truncilla truncata*), 7.35 mm, and threehorn wartyback (*Obliquaria reflexa*), 7.53 mm.