

A STUDY OF THE ABUNDANCE, DIVERSITY, AND RECRUITMENT STATUS OF
FRESHWATER MUSSELS IN THE MARAIS DES CYGNES RIVER, KANSAS

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by
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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

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FRESHWATER MUSSELS IN THE MARAIS DES CYGNES RIVER, KANSAS

presented by Megan Bradburn

a candidate for the degree of Master of Science

and hereby certify that in their opinion it is worthy of acceptance.

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A STUDY OF THE ABUNDANCE, DIVERSITY, AND RECRUITMENT STATUS OF FRESHWATER MUSSELS IN THE MARAIS DES CYGNES RIVER, KANSAS

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ABSTRACT

Freshwater mussels have declined rapidly in North America as a result of various factors including commercial exploitation, habitat degradation, impoundment and channelization, alterations of the natural flow regime, and the introduction of exotics (Williams et al. 1993). Many populations may be on the verge of “functional extinction” with little reproduction occurring throughout much of their former range. This study examines the status of a mussel assemblage located on the Marais des Cygnes River at the Marais des Cygnes National Wildlife Refuge in Kansas. The hydrology of the Marais des Cygnes River at the reach where this study takes place has been influenced by several upstream impoundments and historical habitat modifications as many of the prairies and forested riparian areas have been converted to row crop agriculture through the years. The mussel assemblage on the Marais des Cygnes may be influenced by the altered timing and magnitude of high flows as these flows could serve to increase bed scouring and sedimentation through the reach. In addition, mussels that are dependent on low flow conditions and bed stability at the time of reproduction may experience limited recruitment success if the duration and magnitude of floods during the time of the year when certain species are reproducing is altered. Conversely, prolonged dry periods when

water is directly extracted for municipal and industrial uses may lead to physiological stress and high levels of mortality as mussels are stranded. Several species that were historically present on the Marais des Cygnes River are believed to be extirpated from much of their former range.

Four long-term monitoring locations were selected on the river and data were collected at the sites on several parameters that indicate mussel bed quality: density, species richness, extent of the bed, recruitment status, and habitat availability. A stratified, systematic sampling protocol was used which defined the boundaries of each mussel bed in the upstream, downstream, and cross-stream directions and concentrated sampling effort in areas close to the bank where mussels were heavily aggregated. Direct comparisons between mussel beds were made. Total density within the mussel bed at the four sites ranged from 3.0 mussels per m² at Site 2 to 8.9 mussels per m² at Site 4. The threeridge (*Amblema plicata*) was the most abundant species at all four sites with densities ranging from 1.3 mussels per m² to 4.5 mussels per m². At Sites 1-3 pimpleback (*Quadrula pustulosa*) was the next most abundant and washboard (*Megaloniaias nervosa*) was second in abundance at Site 4. Although ranking third among the sites in total mussel abundance and area, the mussel bed at Site 1 had the highest value of species evenness, a greater amount of medium and coarse gravel, and higher numbers of recent recruits in four aged species than the other sites. The mussel bed extended 13 meters in the cross-stream direction. Site 3 and Site 4 consisted of narrow mussel beds that extended approximately 7-8 meters across the stream. Mussels were dense at these sites with the majority of individuals consisting of *Amblema plicata*, *Quadrula pustulosa*, or *Megaloniaias nervosa*. The abundance of recent recruits was

lower than the abundance of mussels greater than 15 year of age at these two sites. It is likely that abundance will gradually decline through the years as the older individuals die and are not replaced at three out of the four mussel beds examined.

By aging a collection of deceased stranded mussels at Site 1 and developing and applying growth models to the lengths of mussels collected during the survey, I determined that annual discharge during the breeding season did not have an impact on recent mussel recruitment of three tachytictic species at the site: *Obliquaria reflexa*, *Quadrula pustulosa*, and *Amblema plicata*. There was no statistical difference among years in recruitment success. In contrast a bradytictic species, *Ellipsaria lineolata*, experienced higher recruitment in years where mean monthly discharge in June through August was low in recent years. There was no evidence of *E. lineolata* recruitment in years when mean monthly discharge was high throughout these months. The timing coincides with the reported time of the year when gravid *E. lineolata* females release glochidia, infect an appropriate host, and metamorphosed juveniles excyst off of the host. The episodic recruitment of butterfly which is closely linked to long periods of lower than average flows during the breeding season, may partly explain the rarity of this species on the river.

Discriminant models were built on mussel microhabitat use at the four sites. Density classes could be distinguished from each other based on depth and substrate variables, but models based on these variables alone were limited in their ability to predict mussel presence or abundance in a given quadrat, particularly in habitats considered favorable. Mussels generally occupied areas with high amounts of medium and coarse gravel and low depth within a particular site but other factors including local

shear velocity and bed stability at higher flows may be limiting their distribution. The local conditions at Site 1 have allowed at least three tachytictic mussel species to successfully recruit every year, while conditions at the three other sites are apparently unfavorable to mussel recruitment in recent years. Future studies on the Marais des Cygnes River should include repeating the survey protocol at the four sites to investigate changes in total abundance, species composition, habitat, and recruitment through time at the four locations and examining the potential causes of successful mussel recruitment at Site 1 compared to the other locations.

Chapter 1. Introduction

Freshwater mussels are located on every continent except Antarctica. The most diverse assemblage of fauna occurs in North America. Freshwater mussels are considered one of the most rapidly declining components of freshwater biodiversity with over 70% of the 300 North American species classified as extinct, endangered, threatened, or a species of special concern (Williams et al. 1993). The decline is attributed to several factors including commercial exploitation, impoundment and channelization, water quality degradation, habitat destruction, water extraction, and the introduction of exotic species (Williams et al 1993). Because mussels are long-lived, a population may seem healthy and diverse but could actually be on the verge of “functional extinction” with little or no reproduction occurring.

A goal stated by the National Native Mussel Conservation Committee (1998) is to “increase knowledge of the status and trends of native mussel populations so that resource managers and administrators can better determine the species and populations most at risk.” (NNMCC, p.1422). Sampling efforts for a particular population should not only focus on estimating abundance and species richness but should also attempt to quantify the age-size structure of the population. Species may be represented by dominant age-classes of individuals born decades ago when historical conditions were favorable to mussel recruitment. Therefore recent impacts that are seemingly benign to abundant freshwater mussel populations may in fact be inhibiting recruitment. Management efforts would then need to focus on reestablishing favorable conditions for natural recruitment and/or attempt to artificially reestablish juveniles into the population.

Otherwise the population would steadily decline through the years as older individuals die.

Remediation requires knowledge about the factors that may have caused the decline in reproductive success over the years and the specific habitat conditions that currently limit the distribution and abundance of individuals.

The goal of this study was to examine the current status of the freshwater mussel population in the Marais des Cygnes River reach that is located on the Marias des Cygnes National Wildlife Refuge property in eastern Kansas by determining appropriate population and ecological characteristics. The four specific research objectives are:

1. Estimate abundance, species richness, and the age/size-structure for several species at four monitoring sites along the Marias des Cygnes River
2. Estimate current mussel recruitment at each site for a selection of tachytictic and bradytictic species by establishing length-at-age relationships based on internal annuli
3. Compare recent recruitment events and annual growth with annual discharge and temperature fluctuations
4. Determine mussel habitat use and selection within each site and between sites

Study Site

The Marais des Cygnes River originates near Eskridge in Wabaunsee County, Kansas and flows east and south through Kansas to connect with the Little Osage River in Bates County, Missouri. The Marais des Cygnes River exists as a sixth order stream and meanders approximately 150 river miles through the Central Irregular Plains ecoregion of Kansas (Obermeyer 2002). This region was historically dominated by open range land, tall grass prairie and bottomland hardwood forest (USFWS 2003). Many of the prairies have been converted to row crop agriculture. The Marais des Cygnes Basin covers approximately 4,304 square miles. Major tributaries include Pottawatomie Creek, Ten Mile Creek, Mulberry Creek, and Big Sugar Creek.

The Marais des Cygnes River has sustained extensive anthropogenic impacts (USFWS 2003). The hydrology of the river is influenced by three reservoirs operated by the Army Corps of Engineers: Ponoma Lake, Melvern Lake, and Hillsdale Lake that control 25 percent of the Marais des Cygnes River Basin in Kansas (Dent et al. 1997) and several impoundments. Only Melvern Lake is situated on the main stem of the Marais des Cygnes River. Direct river withdrawals and floodplain wells sustain dry-weather flows in the upper Osage Basin (Dent et al. 1997). During periods of moderate to high flows, stream bank erosion and sedimentation from agricultural lands cause water quality problems in the river (USFWS 2003).

In Missouri, more than 82 percent of the Marais des Cygnes/Osage River has been impacted by channelization and impoundments (Obermeyer 2000). These impacts include the construction of Lake of the Ozarks, Harry S. Truman Reservoir, and Bates Country Drainage Ditch which diverts flow from approximately 42 miles of the former

channel. It is estimated that less than nine miles of the original channel remains upstream from Truman Reservoir in Missouri. Because of the losses in habitat from the Missouri portion of the Marais des Cygnes, the best opportunity to protect freshwater mussels on the river is in the part that flows through Kansas.

Forty-six species of mussels were documented in the Osage River system both in Kansas and Missouri by Utterback in 1916. The majority of his sites have since been subjected to anthropogenic alterations and it is believed that much of the historic mussel diversity has been lost (Obermeyer 2000).

This study took place on the 9.5 river-mile portion of the Marais des Cygnes River that flows through the Marais des Cygnes National Wildlife refuge. The refuge was established in 1992 to protect bottomland hardwood habitats along the Marais des Cygnes River in Linn County Kansas and is managed by the U.S. Fish and Wildlife Service. It encompasses 9,300 acres between U.S. Highway 69 and the Missouri State Line and contains various types of habitat including the bottomland oak-hickory forest, native prairie and savannah groves, riparian woodlands, seasonal wetlands, and water-filled mining ponds including Turkeyfoot Lake. The mining ponds contain several species of freshwater mussels.

Background

Previous mussel surveys on the river were conducted by Brian Obermeyer from Stream and Prairie Research in 1999 and 2000 (Obermeyer 2000). He concluded that the reach of the Marais des Cygnes River located on refuge property is an important

refugium for mussels and may be the most diverse and abundant assemblage of mussels remaining in all of the Marais des Cygnes River. He surveyed three sites and examined mussels in exposed mudflats on Turkeyfoot Lake. Refuge personnel confirmed additional extensive mussel beds throughout much of the river located on refuge property (Obermeyer 2000).

There are 31 mussel species on the refuge known from collections of live specimens (Table 1). Fourteen of these species have conservation status in Kansas. In 2003 and 2004, additional surveys found twenty sites containing mussels (Timothy Maloney, personal communication). These sites ranged in quality from areas which contained isolated pockets of a few mussels to extensive mussel beds usually located on point bars. Nineteen species represented by at least one live individual were found at these sites in 2003 (Figure 1).

Table 1. A. Refuge species known from collections of live specimens (incorporates species list from Obermeyer 2000 and recent surveys). B. Refuge mussel species known from weathered or relict shell materials only. C. Additional mussel species known from sites upstream of refuge on the Marais des Cygnes River. Tables show common name, scientific name, and conservation status in Kansas. Species listed as extirpated are presumably extirpated in Kansas. Symbols: SINC=Species in need of conservation, *=candidate, newly discovered in the state, += subfossil materials only

A.

Common Name	Scientific Name	Conservation Status In Kansas
mucket	<i>Actinonaias ligamentina</i>	Endangered
threeedge	<i>Amblema plicata</i>	
flat floater	<i>Anodonta suborbiculata</i>	Endangered
rock pocketbook	<i>Arcidens confragosus</i>	Threatened
purple wartyback	<i>Cyclonaias tuberculata</i>	*
butterfly	<i>Ellipsaria lineolata</i>	Threatened
spike	<i>Elliptio dilatata</i>	SINC
wabash pigtoe	<i>Fusconaia flava</i>	SINC
plain pocketbook	<i>Lampsilis cardium</i>	
yellow sandshell	<i>Lampsilis teres</i>	SINC
white heelsplitter	<i>Lasmigona complanata</i>	

Table 1 (cont.)

fragile papershell	<i>Leptodea fragilis</i>	
black sandshell	<i>Ligumia recta</i>	*
pondmussel	<i>Ligumia subrostrata</i>	
washboard	<i>Megalonaias nervosa</i>	SINC
threehorn wartyback	<i>Obliquaria reflexa</i>	
round pigoe	<i>Pleurobema sintoxia</i>	SINC
pink heelspitter	<i>Potamilus alatus</i>	
pink papershell	<i>Potamilus ohioensis</i>	
giant floater	<i>Pyganodon gradis</i>	
monkeyface	<i>Quadrula metanerva</i>	
wartyback	<i>Quadrula nodulata</i>	SINC
pimpleback	<i>Quadrula pustulosa</i>	
mapleleaf	<i>Quadrula quadrula</i>	
creeper	<i>Strophitus undulatus</i>	SINC
lilliput	<i>Toxolasma parva</i>	
pistolgrip	<i>Tritogonia verrucosa</i>	
fawnsfoot	<i>Truncilla donaciformis</i>	
deertoe	<i>Truncilla truncata</i>	SINC
pondhorn	<i>Unio merus tetralasmus</i>	
paper pondshell	<i>Utterbackia imbecillis</i>	

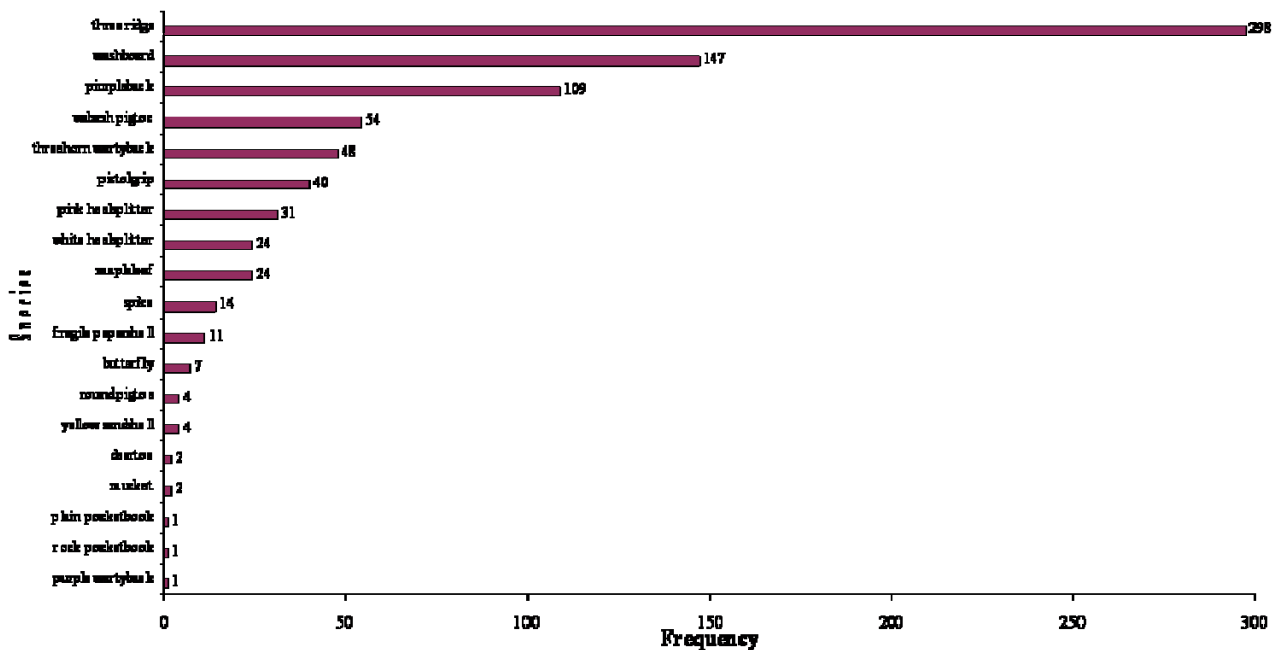
B.

Common Name	Scientific Name	Conservation Status In Kansas
spectaclecase	<i>Cumberlandia monodonta</i>	Extirpated
winged mapleleaf	<i>Quadrula fragosa</i>	Endangered (Federal), Extirpated
mapleleaf form	<i>Quadrula quadrula nobilis</i>	Extirpated

C.

Common Name	Scientific Name	Conservation Status In Kansas
elktoe	<i>Alasmidonta marginata</i>	Endangered
slippershell	<i>Alasmidonta viridis</i>	Extirpated
snuffbox	<i>Epioblasma triquetra</i>	SINC, Extirpated
fatmucket	<i>Lampsilis siliquoidea</i>	SINC

Figure 1. Mussels collected in 2003 by timed searches on the Marais des Cygnes River, at the Marais des Cygnes National Wildlife Refuge (Timothy Maloney, personal communication)



A serious threat to the mussel assemblage at the refuge is the alteration of the natural flow regime. In addition to the impact that higher than average flows during the spawning season may have on recruitment, decreased flows during dry periods due to municipal water extraction can strand mussels particularly those residing in shallow water habitat. Adequate flows (> 25 cubic feet per second) should be maintained on the Marais des Cygnes River during droughts and high water-use demand periods (Obermeyer 2000).

In August 2000, a mussel kill was documented on the refuge when the Kansas City Power and Light Company at La Cygne pumped water from the Marais des Cygnes to replenish its cooling lake which had become depleted due to high summer temperatures and little precipitation. During this period, the discharge fell to about 2.9 cubic feet per second with the mean below 25 cubic feet per second for 21 days in August

(USGS gage, 06916600 Marais des Cygnes River near KS-MO State Line). Bruce Freske, refuge manager at the time, estimated that approximately 2000 mussels were stranded at four sites including Kansas threatened and endangered species such as butterfly (*Ellipsaria lineolata*), mucket (*Actinonaias ligamentina*), and purple pimpleback (*Cyclonaias tuberculata*) (Upchurch 2000). A few dead spectaclecase (*Cumberlandia monodonta*) were also found stranded after this event. Live individuals of this species have not been found recently on the river and *C. monodonta* is believed to be extirpated from Kansas.

Several initial research needs to adequately quantify and monitor the mussel population have been recommended (Obermeyer 2000). A more extensive inventory of freshwater mussels and their instream habitat on the refuge was needed. Obermeyer (2000) suggested establishing four long-term monitoring sites where a survey could be repeated at five-year intervals to assess changes in mussel abundance. Recruitment trends should be examined because dense mussel beds may be composed of primarily large, older individuals born decades ago with minimal recruitment taking place. Another high priority was to monitor the river conditions during high water-withdrawal periods to study the effect of low flow on the population and insure that adequate flows are maintained to reduce impacts (Obermeyer 2000).

Chapter 2. Mussel Density, Species Richness, and Distribution on the Marais des Cygnes River

Introduction

There are several standard protocols available for sampling freshwater mussels. The appropriate method depends on the objectives of the study. Studies have been implemented to meet various objectives including estimating presence and species richness, determining the distribution of mussel populations, and estimating density for temporal monitoring. A reconnaissance stage will often precede a more formal sampling protocol (Dunn 2000) and aid in selecting the most appropriate sampling approach. The reconnaissance involves a cursory search of the entire study area to determine mussel presence or absence. The information gathered from this initial study is vital in determining how the sampling will proceed and for choosing the most effective design to implement. Mussels are often patchy in distribution and many or most species aggregate into beds. The density of individuals can be 10 to 100 times higher in areas within mussel beds than in areas outside the bed (Strayer et al. 2004). The researcher may locate mussel beds, determine the range and boundaries of mussel distributions, examine the accessibility and cost of sampling each location, and examine mussel bed habitat. Gathering information on the distribution of mussels in shallow versus deep habitats is particularly useful. This information can determine whether diving is necessary or cost-effective. In a study area that contains many shallow riffle locations containing mussels separated by deeper sections, it may be practical to prioritize locations and rigorously sample a few shallow mussel beds. Mapping the boundaries of the mussel beds and

generating precise baseline density estimates in each location will allow the locations to be monitored through time.

The objectives of this study were to select sites for long-term monitoring, determine the range and distribution of mussels within each site, and generate precise density estimates by employing a cost-effective quantitative sampling regime. The high precision of the density estimates allowed for a comparison of total abundances and species abundances within and between sites, a comparison of age-class abundances, and will be useful for determining changes in abundance through time. Additionally, a study design that was implemented at sites with differences in mussel densities and habitats allowed for the examination of the relationship between microhabitat variables and density (Chapter 5).

Materials and Methods

Mussel surveys on the Marais des Cygnes River were conducted during the summer and fall of 2006. Four locations were selected from prior surveys (Figure 1, Table 1). The locations were selected based on the presence of a mussel bed, heterogeneous depth regimes within the site, and accessibility. The sites ranged from 185-385 meters in length and 4160-8360 m². The final width of each site was determined after a preliminary survey. Site 3 and Site 4 did not include some area off the right downstream bank where no individuals were located during the preliminary survey. The sites were sampled during low flow periods throughout the summer of 2006. The formal survey usually immediately followed the preliminary survey at each site. Because

mussels were highly clustered at each location, a stratified sampling approach was employed to maximize sampling effort in high density areas (Strayer and Smith 2003). A stratified random sampling design is the best design for sampling rare or clustered populations when using quadrats (Christman 2000).

The reconnaissance and preliminary surveys were conducted at each site to define the upstream and downstream boundaries of the mussel distribution at the site, delineate high and low density (strata) within site, and generate rough estimates for density within each stratum. At each location, the central area of the mussel bed was located. Because of low visibility, sampling efforts in the river consisted of tactile searches where an observer gropes and digs through the substrate to locate mussels. To find the extent of each mussel bed, two observers searched in a zigzag pattern in both the upstream and downstream directions from the central location. When density dropped sufficiently and observers were only able to locate one mussel within approximately 5 m, the location was marked with rebar designating the edge of the distribution. GPS coordinates for both the upstream and downstream boundaries were recorded. Additional searches were conducted within 30 m upstream and downstream of this location to verify that the edge was designated correctly.

The high and low density strata were then designated at each site. Because mussels were generally clustered along one bank with density decreasing with distance from the shoreline, I selected a position in the cross-stream direction that could separate the bed into two strata (high and low density). To accomplish this goal, each site was divided into 20 cross-stream transects. Along each transect one 0.25 m² quadrat was sampled every three meters from a random starting point. Surface samples consisted of

groping and removing substrate to a depth of approximately 8 cm. I recorded the cross-stream (X) coordinate, upstream (Y) coordinate, species, height (umbo to posterior edge), and length (anterior to posterior) for each mussel collected. Mussels were measured with calipers to the nearest 1.0 mm.

To delineate the strata, a hypothetical boundary between high and low density strata was tested at each cross-stream (X) position. Several one-way ANOVAs were run to compare the mean number of mussels per sample between the high and low density strata, assuming a different boundary for each ANOVA. The boundary which maximized the difference in density across strata was determined.

Figure 1. Aerial photograph of the survey area and four sites sampled on the Marais des Cygnes River in Linn County, KS in 2006



Table 1. Description of survey sites sampled for mussels on the Marais des Cygnes River, 2006.

Site	Upstream coordinates	Downstream coordinates	Length of Site (m)	Width of Site (m)	Area sampled (m ²)	Dates of preliminary survey	Dates of survey
1	N 38° 13' 15.6" W 94° 38' 58.4"	N 38° 13' 15.3" W 94° 38' 56.7"	210	31	6510	6/14-6/16	6/20-6/29
2	N 38° 14' 12.8" W 94° 39' 15.7"	N 38° 14' 9.6" W 94° 39' 10.6"	185	32	5920	7/1-7/3	7/5-7/18
3	N 38° 13' 16.9" W 94° 38' 40.4"	N 38° 13' 38.9" W 94° 38' 50.5"	380	22	8360	7/24-7/26	7/27-8/4
4	N 38° 13' 28.7" W 94° 37' 49.0 "	N 38° 13' 24.9" W 94° 37' 37.4"	260	16	4160	8/7-8/9	8/15-8/19

Strayer and Smith (2003) outline a method and give an equation for allocating samples among strata defined by varying levels of density: $n_h = nN_h m_h^{0.584} / \sum N_k m_k^{0.584}$; where n is the total number of quadrats at the site, n_h is the number of units that will be sampled in the h th stratum, N_h is the total number of units in the h th stratum, m_h is the mean density in the h th stratum and N_k and m_k denote the total number of units in the entire site and mean density for the entire site (p. 21). Mean density estimates for each stratum were determined from the preliminary survey using the most appropriate boundary. The total number of quadrats (samples) for the formal survey was based on the total density estimated from the preliminary survey. Smith et al. (2001) developed a table of the necessary sample sizes to detect species that occur at low densities (Table 3 in Smith et al. 2001, p.127). The number of quadrats sampled at each mussel bed was based on the recommended sample size for the lowest rough density estimate (of a particular species) from the previous survey and a CV of approximately 0.4.

During the preliminary survey every other quadrat was excavated in order to account for mussels not located within 8 mm of the surface (Strayer and Smith 2000, Smith et al. 2001). I shoveled substrate within the quadrat to a depth of 15 cm, and passed the substrate through a 6-mm hardwire mesh sieve. This size mesh has been shown to perform adequately in detecting juvenile mussels while minimizing costs (Smith et al. 2001). Mussels remaining on the sieve were measured and recorded. According to Smith et al. (2001), the proportion of excavated quadrats that minimized the variance of the density estimate was dependent on the percentage of mussels located at the substrate surface. The proportion of excavated quadrats designated for the formal survey was determined by calculating the percent of mussels found on the surface in the preliminary survey and the following guidelines outlined by Smith et al. (2001): if 60% of the mussels are likely to be detected at the surface then the excavation of 25% of the quadrats will minimize variance; 50 to 60% = excavate 33% of quadrats; 40 to 50% = excavate 50 % of quadrats, and < 40% = excavate 100% of quadrats to minimize variance. At all four sites more than 60% of mussels detected in the preliminary survey were found on the surface (Table 2), so I excavated 25% of quadrats in the formal survey.

After determining the boundary between the high and low density strata, the number of quadrats to be sampled in each stratum, and the necessary proportion of quadrats to be excavated, I employed a systematic sampling regime within each stratum (Strayer and Smith 2003, Smith 2001). Three random X, Y coordinate pairs were generated and the distance interval between quadrats was calculated based on the equation $d = \sqrt{[L*W / (n/k)]}$ where d is the distance interval, L and W are the length and width of the site (or stratum dimensions for this study), n is the sample size for the

stratum, and k is the number of starts (Strayer and Smith 2003, p.14). A quadrat was placed at a random start for the first sample and at intervals of “ d ” upstream and across the channel for subsequent samples. Every fourth quadrat was excavated. All mussels were counted within each quadrat and the length and height were measured to the nearest millimeter with calipers.

Density estimates were calculated from survey data using “Mussel Estimation Program Version 1.5.2 March, 2007” (Dave Smith USGS-LSC, Aquatic Ecology Lab). The program generated density, abundance, standard error, and 90% confidence interval estimates for every species in each stratum. Estimates were checked using formulas outlined in Smith et al. (2001). Asian clam (*Corbicula fluminea*) density was calculated separately as they were only observed in the subsurface of excavated quadrats.

Individual stratum estimates were combined to calculate total estimates of density and abundance at each site. Diversity Indices including Species richness, Simpson’s Index (Simpson 1949), Margalef Index, Shannon-Weiner Index (Shannon 1948), and Shannon Evenness Index were calculated for each site (Friedrich et al. 1992, Gordon et al. 2004). The various indices reflect different properties of species composition. Simpson’s Index is a measure of dominance in a sample and is not sensitive to rare species and the Margalef Index is not sensitive to evenness (Gordon et al. 2004). The Shannon-Weiner Index is sensitive to the number of rare species and species evenness. SAS 9.1 (SAS Institute Inc. Cary, NC 2002-2003) was used for further analysis. Non-parametric one-way tests were conducted to compare density estimates between sites. Non-parametric Wilcoxon two-sample and Kruskal-Wallis (> 2 samples) tests were used to examine for significant differences between sites rather than general linear models or

ANOVA because of the non-normality of the data and high number of quadrats with a count equal to zero.

Additionally, the densities of subsurface mussels and *Corbicula fluminea* were calculated separately by dividing the counts by the total number of quadrats excavated at each site and within each stratum. Nonparametric tests were then employed to determine whether there were any differences between *C. fluminea* density and mussel density in the subsurface at the four sites.

To examine if size had an influence on whether an individual was found in the subsurface or surface, I divided all individuals into 10-mm height categories at each site. Surface density was calculated by dividing the count of each size group by the total number of quadrats irrespective of stratum while subsurface density was calculated by only dividing the counts by the number of excavated quadrats.

Finally, I examined whether cross-stream (X) position had an influence on density at each site. The mussel distribution from the systematic survey was plotted with representative symbols for each count. I generated summary statistics of density at each X-position and used the respective means in a regression analysis. I tested whether there was a statistical relationship between mean count and cross-stream position.

Results

Preliminary Survey Results

The number of 0.25 m² quadrats surveyed at each site along transects varied according to the area of each site (Table 2). At Site 1, over 87% of mussels collected

were located on the surface of quadrats and in Sites 2-4, over 95% of mussels were located on the surface. The high percentage of mussels located on the surface may have been attributed to the thoroughness of surface tactile searches and/or a lack of mussels in the smallest size categories at each site. For maximum efficiency during the formal survey, I scheduled roughly 25% of quadrats for excavation based on the high surface detectability and recommendation in Smith et al. (2001). Density estimates (per 0.25 m²) from the preliminary survey were similar and within the margin of error for Sites 1, 2, and 3 (Table 3). Mussel density was greatest at Site 4.

Individuals tended to be clustered along one bank (Table 4). The one-way ANOVA results indicated the separation boundaries between high and low density strata at each site. I tested all reasonable cross-stream boundaries and selected the boundary that maximized the difference in density between strata. At Site 1 this boundary was located at x=13, at Site 2 the boundary was at x=16, at Site 3 the boundary was at x=8, and at Site 4 the boundary was located at x=7.5. These positions represented the cross-stream distance in m from the left downstream bank. The mean densities in the “high density stratum” were significantly higher than mean densities in the “low density stratum” at all four sites ($P < 0.001$).

Table 2. Number of samples and mussel counts from preliminary surveys at four sites. Percent to be excavated refers to the percentage of quadrats scheduled for excavation in the next stage of sampling based on the surface detectability of mussels at the sites

Site	Surface quadrats	No. mussels (surface)	Excavated quadrats	No. mussels (subsurface)	Ratio (surface: total) %	Percent to be excavated (%)
1	167	54	84	8	87.1	25
2	136	55	67	2	96.5	25
3	232	149	110	7	95.5	25
4	110	150	50	3	98.0	25

Table 3. Summary statistics from initial surveys of mussels collected from 6/14 to 8/9 in 2006 at four sites. Mean is the number of mussels per sampling unit (0.25 m²).

Statistic	Site 1	Site 2	Site 3	Site 4
Mean	0.323	0.404	0.642	1.364
Standard Error	0.071	0.073	0.099	0.243
Minimum	0	0	0	0
Maximum	6	6	14	15
Mussel Count	54	55	149	150
Number of Quadrats	167	136	232	110
Confidence Level (95%)	0.141	0.145	0.195	0.483

Table 4. Single factor analysis of variance for stratification based on density at all sites. Horizontal (x) position determined by maximizing the difference in means between the high and low density strata.

Site 1: ANOVA Single Factor (x=13)

SUMMARY

Groups	Number of Quadrats	Number of Mussels	Mean (per 0.25 m ²)	Variance
High Density	55	48	0.873	2.039
Low Density	112	6	0.054	0.051

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	24.751	1	24.751	35.271	1.649E-08	3.898
Within Groups	115.788	165	0.701			
Total	140.539	166				

Site 2: ANOVA Single Factor (x=16)

SUMMARY

Groups	Number of Quadrats	Number of Mussels	Mean (per 0.25 m ²)	Variance
High Density	87	53	0.609	1.008
Low Density	49	4	0.081	0.118

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8.724	1	8.724	12.653	5.189E-4	3.912
Within Groups	92.386	134	0.689			
Total	101.110	135				

Site 3 ANOVA Single Factor (x=8)

SUMMARY

Groups	Number of Quadrats	Number of Mussels	Mean (per 0.25 m ²)	Variance
High Density	74	104	1.405	5.532
Low Density	158	45	0.285	0.371

Table 4 (cont.)

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	63.285	1	63.285	31.504	5.703E-08	3.882
Within Groups	462.021	230	2.009			
Total	525.306	231				

Site 4: ANOVA: Single Factor (x=7.5)**SUMMARY**

<i>Groups</i>	<i>Number of Quadrats</i>	<i>Number of Mussels</i>	<i>Mean (per 0.25 m²)</i>	<i>Variance</i>
High Density	52	125	2.404	10.834
Low Density	58	25	0.431	0.916

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	106.711	1	106.711	19.057	2.918E-05	3.929
Within Groups	604.743	108	5.599			
Total	711.454	109				

Systematic Survey Results

Based on preliminary survey results and species densities a sample size was determined for each site (Table 5). The samples were proportioned among strata based on averages calculated from the preliminary survey with the high density stratum containing more samples than the low density stratum. The distance between quadrats was smaller in the high density stratum. Using three randomly generated starting points and the calculated appropriate distance between quadrats, coordinated positions for each quadrat to be sampled were assigned (see Strayer and Smith 2004). I sampled 542 mussels at Site 1, 302 mussels at Site 2, 512 mussels at Site 3, and 666 mussels at Site 4 (Tables 6, 7, 8, 9).

Species-specific density and abundance estimates were generated for each location (Tables 10, 11, 12, 13; Figure 2). The most abundant species at each site was *Amblema plicata* (three-ridge). At Site 1, *A. plicata* comprised approximately 33% of individuals in the high-density stratum and entire site. The *A. plicata* density estimate was 2.16 individuals per m² in the high density stratum. The next most abundant species was *Quadrula pustulosa* (pimple-back) followed by *Obliquaria reflexa* (three-horn warty-back). *Fusconaia flava* (wabash pigtoe), *Pleurobema sintoxia* (round pigtoe), *Quadrula quadrula* (maple-leaf), and *Ellipsaria lineolata* (butterfly) were intermediate in abundance at Site 1. The rarest species included *Potamilus ohiensis* (pink papershell), *Lasmigona costata* (white heelsplitter), *Leptodea fragilis* (fragile papershell), and *Truncilla truncata* (deertoe). *Megaloniais nervosa* (washboard) was relatively rare at this site compared to the other locations.

Table 5. Survey design and site delineation for systematic surveys conducted from 6/20 to 8/19, 2006.

Site	Total number of quadrats (0.25 m ²)	Area (m ²)	High density count	High density area (m ²)	Distance between quadrats (m)	Low density count	Low density area (m ²)	Distance between quadrats (m)
1	518	6510	378	2730	4.5	140	3780	9
2	550	5920	410	2960	4.5	140	2960	8
3	420	8360	252	3040	6	168	5320	9
4	440	4160	290	1820	4.5	150	2340	7

At Site 2, *A. plicata* comprised over 40% of the high density stratum and total site but was less abundant overall than at the other sites. *Q. pustulosa* was the second most abundant species, followed distantly by *M. nervosa*. Site 2 did not contain as many

species as Site 1. *E. lineolata*, *T. truncata*, and *T. donaciformis* (fawnsfoot) were absent from Site 2. *Elliptio dilatata* (spike) was located at this site and not at Site 1. Many of the rarer species at Site 1 were even less abundant at Site 2.

Site 3 was the largest in area and contained more mussels than the other sites. In the high density stratum, 60.9% of all mussels collected were *A. plicata* which was estimated at 4.51 individuals per m². *Q. pustulosa* was the next highest in density, comprising 13.3% of the total mussels collected in the high density stratum and 0.99 mussels per m².

Table 6. Field counts of mussels by species at Site 1, sampled from 6/20 - 6/29, 2006 during the systematic survey. Surface, excavated, and total counts are displayed for the high and low density strata (sample sizes for 0.25 m² quadrats: high density stratum, n=378, low density stratum n=140, total=518).

Species	High Density Count			Low Density Count			Site Total
	surface	excavated	total	surface	excavated	total	
<i>Amblema plicata</i>	194	0	194	4	0	4	198
<i>Ellipsaria lineolata</i>	14	0	14	0	0	0	14
<i>Fusconaia flava</i>	18	2	20	0	0	0	20
<i>Lasmigona costata</i>	2	0	2	0	0	0	2
<i>Leptodea fragilis</i>	2	0	2	1	0	1	3
<i>Megalonaias nervosa</i>	4	0	4	0	0	0	4
<i>Obliquaria reflexa</i>	90	6	96	3	0	3	99
<i>Pleurobema sintoxia</i>	13	2	15	0	0	0	15
<i>Potamilus alata</i>	4	0	4	0	0	0	4
<i>Potamilus ohioensis</i>	1	0	1	0	0	0	1
<i>Quadrula pustulosa</i>	137	4	141	3	0	3	144
<i>Quadrula quadrula</i>	22	0	22	1	1	2	24
<i>Truncilla donaciformis</i>	4	1	5	0	1	1	6
<i>Truncilla truncata</i>	3	0	3	0	0	0	3
<i>Tritogonia verrucosa</i>	4	0	4	1	0	1	5
Total	512	15	527	13	2	15	542

Table 7. Field counts of mussels by species at Site 2, sampled from 7/5 – 7/18, 2006 during the systematic survey. Surface, excavated, and total counts are displayed for the high and low density strata (sample sizes for 0.25 m² quadrats: high density stratum, n=410, low density stratum n=140, total=550).

Species	High Density Count			Low Density Count			Site Total
	surface	excavated	total	surface	excavated	total	
<i>Amblema plicata</i>	133	0	133	5	0	5	138
<i>Elliptio dilatata</i>	3	1	4	1	0	1	5
<i>Fusconaia flava</i>	14	1	15	0	0	0	15
<i>Lasmigona costata</i>	1	0	1	0	0	0	1
<i>Leptodea fragilis</i>	1	0	1	0	0	0	1
<i>Megalonaias nervosa</i>	23	0	23	1	0	1	24
<i>Obliquaria reflexa</i>	18	0	18	0	1	1	19
<i>Pleurobema sintoxia</i>	2	0	2	0	0	0	2
<i>Potamilus alata</i>	3	0	3	0	0	0	3
<i>Potamilus ohioensis</i>	1	0	1	0	0	0	1
<i>Quadrula pustulosa</i>	76	2	78	5	0	5	83
<i>Quadrula quadrula</i>	1	0	1	0	0	0	1
<i>Tritogonia verrucosa</i>	9	0	9	0	0	0	9
Total	285	4	289	12	1	13	302

Table 8. Field counts of mussels by species at Site 3, sampled from 7/27 – 8/4, 2006 during the systematic survey. Surface, excavated, and total counts are displayed for the high and low density strata (sample sizes for 0.25 m² quadrats: high density stratum, n=252, low density stratum n=168, total=420).

	High Density Count			Low Density Count			Site Total
	surface	excavated	total	surface	excavated	total	
<i>Actinonaias ligamentina</i>	1	0	1	0	0	0	1
<i>Amblema plicata</i>	264	1	265	33	0	33	298
<i>Arcidens confragosus</i>	1	0	1	0	0	0	1
<i>Elliptio dilatata</i>	5	0	5	0	0	0	5
<i>Ellipsaria lineolata</i>	9	0	9	4	0	4	13
<i>Fusconaia flava</i>	24	0	24	6	0	6	30
<i>Lampsilis teres</i>	1	0	1	0	0	0	1
<i>Leptodea fragilis</i>	5	0	5	0	0	0	5
<i>Megalonaias nervosa</i>	28	0	28	0	0	0	28
<i>Obliquaria reflexa</i>	21	0	21	9	1	10	31
<i>Pleurobema sintoxia</i>	6	1	7	1	1	2	9
<i>Potamilus alatus</i>	8	0	8	2	0	2	10
<i>Potamilus ohioensis</i>	1	0	1	0	0	0	1
<i>Quadrula pustulosa</i>	56	1	57	11	0	11	68
<i>Quadrula quadrula</i>	4	0	4	1	0	1	5
<i>Truncilla donaciformis</i>	3	0	3	0	0	0	3
<i>Tritogonia verrucosa</i>	2	0	2	1	0	1	3
Total	439	3	442	68	2	70	512

Table 9. Field counts of mussels by species at Site 4, sampled from 8/15-8/19, 2006 during the systematic survey. Surface, excavated, and total counts are displayed for the high and low density strata (sample sizes for 0.25 m² quadrats: high density stratum, n=290, low density stratum n=150, total=440).

	High Density Count			Low Density Count			Site Total
	surface	excavated	total	surface	excavated	total	
<i>Actinonaias ligamentina</i>	1	0	1	0	0	0	1
<i>Amblema plicata</i>	317	0	317	26	0	26	343
<i>Elliptio dilatata</i>	6	0	6	1	0	1	7
<i>Ellipsaria lineolata</i>	4	0	4	1	0	1	5
<i>Fusconaia flava</i>	13	0	13	0	0	0	13
<i>Lasmigona costata</i>	2	0	2	2	0	2	4
<i>Lampsilis teres</i>	2	0	2	0	0	0	2
<i>Leptodea fragilis</i>	8	1	9	0	0	0	9
<i>Megalonaias nervosa</i>	160	0	160	5	0	5	165
<i>Obliquaria reflexa</i>	14	2	16	2	0	2	18
<i>Pleurobema sintoxia</i>	8	0	8	1	0	1	9
<i>Potamilus alatus</i>	7	0	7	1	0	1	8
<i>Potamilus ohioensis</i>	2	0	2	0	0	0	2
<i>Quadrula pustulosa</i>	56	2	58	1	0	1	59
<i>Quadrula quadrula</i>	5	0	5	3	0	3	8
<i>Truncilla donaciformis</i>	7	1	8	1	0	1	9
<i>Tritogonia verrucosa</i>	3	0	3	1	0	1	4
Total	615	6	621	45	0	45	666

Table 10. Species-specific and total estimates of density and abundance within high (A) and low (B) density strata and for the entire site at Site 1. Number of quadrats sampled in each area: high density stratum (n=378), low density stratum (n=140), and site total (n=518).

A.

Species	Relative Abundance (%)	Density (per m ²)	SE	CV	Abundance	SE	90% CL
<i>Amblema plicata</i>	33.47	2.161	0.196	0.09	5900	535.14	5082-6849
<i>Ellipsaria lineolata</i>	2.33	0.150	0.085	0.57	410	233.27	161-1045
<i>Fusconaia flava</i>	4.88	0.315	0.104	0.33	861	283.50	501-1480
<i>Lasmigona costata</i>	0.33	0.021	0.025	1.17	59	68.40	9-400
<i>Leptodea fragilis</i>	0.33	0.021	0.021	0.98	59	57.54	12-295
<i>Megaloniais nervosa</i>	0.66	0.043	0.025	0.58	117	68.40	45-306
<i>Obliquaria reflexa</i>	20.67	1.334	0.196	0.15	3643	535.86	2860-4640
<i>Pleurobema sintoxia</i>	4.03	0.260	0.092	0.35	710	250.69	397-1269
<i>Potamilus alata</i>	0.66	0.043	0.025	0.58	117	68.40	45-306
<i>Potamilus ohioensis</i>	0.16	0.011	0.011	0.98	29	28.77	6-147
<i>Quadrula pustulosa</i>	26.82	1.732	0.189	0.11	4728	516.10	3951-5658
<i>Quadrula quadrula</i>	3.65	0.236	0.065	0.28	644	177.35	409-1013
<i>Truncilla donaciformis</i>	0.83	0.054	0.028	0.52	146	76.12	62-344
<i>Truncilla truncata</i>	0.50	0.032	0.025	0.78	88	68.40	24-316
<i>Tritogonia verrucosa</i>	0.66	0.043	0.021	0.49	117	57.54	52-263
Total		6.456	0.4737	0.07	17628	1293.25	15624-19889

B.

Species	Relative Abundance (%)	Density (per m ²)	SE	CV	Abundance	SE	90% CL
<i>Amblema plicata</i>	26.63	0.116	0.076	0.66	438	288.56	148-1294
<i>Leptodea fragilis</i>	6.69	0.029	0.029	1.00	110	109.06	21-563
<i>Obliquaria reflexa</i>	20.00	0.087	0.050	0.58	329	188.90	128-846
<i>Quadrula pustulosa</i>	20.00	0.087	0.087	1.00	329	327.19	64-1690
<i>Quadrula quadrula</i>	13.31	0.058	0.029	0.50	219	109.06	97-497
<i>Truncilla donaciformis</i>	6.69	0.029	0.029	1.00	110	109.06	21-563
<i>Tritogonia verrucosa</i>	6.69	0.029	0.029	1.00	110	109.06	21-563
Total		0.435	0.218	0.50	1645	823.42	721-3747

C.

Species	Relative Abundance (%)	Density (per m ²)	SE	CV	Abundance	SE	90% CL
<i>Amblema plicata</i>	32.89	0.974	0.093	0.10	6338	607.98	5413-7421
<i>Ellipsaria lineolata</i>	2.13	0.063	0.036	0.57	410	233.27	161-1045
<i>Fusconaia flava</i>	4.47	0.132	0.044	0.33	861	283.50	501-1480
<i>Lasmigona costata</i>	0.31	0.009	0.011	1.22	59	68.40	9-397
<i>Leptodea fragilis</i>	0.88	0.026	0.019	0.73	169	123.31	51-561
<i>Megaloniais nervosa</i>	0.61	0.018	0.011	0.61	117	68.40	45-306
<i>Obliquaria reflexa</i>	20.61	0.610	0.087	0.14	3972	568.18	3139-5026
<i>Pleurobema sintoxia</i>	3.68	0.109	0.039	0.36	710	250.69	397-1269
<i>Potamilus alata</i>	0.61	0.018	0.011	0.61	117	68.40	45-306
<i>Potamilus ohioensis</i>	0.15	0.004	0.004	1.00	29	28.77	6-148
<i>Quadrula pustulosa</i>	26.24	0.777	0.094	0.12	5057	611.08	4145-6169
<i>Quadrula quadrula</i>	4.48	0.133	0.032	0.24	863	208.20	580-1283
<i>Truncilla donaciformis</i>	1.33	0.039	0.020	0.51	256	133.00	109-602
<i>Truncilla truncata</i>	0.46	0.014	0.011	0.79	88	68.40	25-316
<i>Tritogonia verrucosa</i>	1.18	0.035	0.019	0.54	227	123.31	93-555
Total		2.961	0.236	0.08	19273	1533.14	16909-21967

Table 11. Species-specific and total estimates of density and abundance within high (A) and low (B) density strata and for the entire site at Site 2. Number of quadrats sampled in each area: high density stratum (n=410), low density stratum (n=140), and site total (n=550).

A.

Species	Relative Abundance (%)	Density (per m ²)	SE	CV	Abundance	SE	90% CL
<i>Amblema plicata</i>	43.43	1.298	0.211	0.16	3841	623.22	2941-5016
<i>Elliptio dilatata</i>	2.42	0.072	0.046	0.64	214	136.94	75-613
<i>Fusconaia flava</i>	6.06	0.181	0.064	0.35	536	190.15	299-960
<i>Lasmigona costata</i>	0.33	0.01	0.010	0.98	29	28.37	6-145
<i>Leptodea fragilis</i>	0.33	0.01	0.010	0.98	29	28.37	6-145
<i>Megaloniaias nervosa</i>	7.51	0.224	0.048	0.22	664	142.99	466-946
<i>Obliquaria reflexa</i>	5.88	0.176	0.035	0.20	520	102.16	376-718
<i>Pleurobema sintoxia</i>	0.66	0.02	0.019	0.96	58	55.16	12-278
<i>Potamilus alata</i>	2.28	0.068	0.043	0.62	202	126.08	72-564
<i>Potamilus ohioensis</i>	0.33	0.01	0.010	0.98	29	28.37	6-145
<i>Quadrula pustulosa</i>	27.51	0.822	0.131	0.16	2433	388.17	1871-3163
<i>Quadrula quadrula</i>	0.33	0.01	0.010	0.98	29	28.37	6-145
<i>Tritogonia verrucosa</i>	2.94	0.088	0.033	0.38	260	98.38	139-484
Total		2.989	0.308	0.10	8844	912.74	7463-10480

B.

Species	Relative Abundance (%)	Density (per m ²)	SE	CV	Abundance	SE	90% CL
<i>Amblema plicata</i>	35.73	0.143	0.102	0.72	423	303.12	130-1375
<i>Elliptio dilatata</i>	7.18	0.029	0.028	0.99	85	84.07	16-434
<i>Leptodea fragilis</i>	7.18	0.029	0.028	0.99	85	84.07	16-434
<i>Megaloniaias nervosa</i>	7.18	0.029	0.028	0.99	85	84.07	16-434
<i>Obliquaria reflexa</i>	7.18	0.029	0.028	0.99	85	84.07	16-434
<i>Quadrula pustulosa</i>	35.73	0.143	0.102	0.72	423	303.12	130-1375
Total		0.400	0.316	0.79	1184	936.16	323-4347

C.

Species	Relative Abundance (%)	Density (per m ²)	SE	CV	Abundance	SE	90% CL
<i>Amblema plicata</i>	42.52	0.720	0.051	0.07	4264	304.15	3792-4795
<i>Elliptio dilatata</i>	2.98	0.051	0.014	0.27	299	84.88	187-477
<i>Fusconaia flava</i>	5.35	0.091	0.002	0.02	536	13.79	514-559
<i>Lasmigona costata</i>	0.29	0.005	0.001	0.20	29	5.33	21-39
<i>Leptodea fragilis</i>	1.14	0.019	0.014	0.74	114	84.24	34-384
<i>Megaloniaias nervosa</i>	7.47	0.127	0.014	0.11	749	84.92	622-903
<i>Obliquaria reflexa</i>	6.03	0.102	0.014	0.14	605	84.68	481-762
<i>Pleurobema sintoxia</i>	0.58	0.010	0.001	0.10	58	7.43	47-72
<i>Potamilus alata</i>	2.01	0.034	0.002	0.06	202	11.23	184-221
<i>Potamilus ohioensis</i>	0.29	0.005	0.001	0.20	29	5.33	21-39
<i>Quadrula pustulosa</i>	28.48	0.482	0.051	0.11	2856	303.76	2398-3402
<i>Quadrula quadrula</i>	0.29	0.005	0.001	0.20	29	5.33	21-39
<i>Tritogonia verrucosa</i>	2.59	0.044	0.002	0.05	260	9.92	244-277
Total		1.694	0.158	0.09	10028	936.65	8600-11693

Table 12. Species-specific and total estimates of density and abundance within high (A) and low (B) density strata and for the entire site at Site 3. Number of quadrats sampled in each area: high density stratum (n=252), low density stratum (n=168), and site total (n=420).

A.

Species	Relative Abundance (%)	Density (per m ²)	SE	CV	Abundance	SE	90% CL
<i>Actinonaias ligamentina</i>	0.21	0.016	0.016	0.99	48	47.75	9-246
<i>Amblema plicata</i>	60.93	4.509	0.465	0.10	13706	1414.76	11566-16242
<i>Arcidens confragosus</i>	0.21	0.016	0.016	0.99	48	47.75	9-246
<i>Elliptio dilatata</i>	1.07	0.079	0.039	0.50	241	119.85	107-546
<i>Ellipsaria lineolata</i>	1.93	0.143	0.082	0.57	434	248.12	170-1112
<i>Fusconaia flava</i>	5.15	0.381	0.076	0.20	1158	230.31	835-1606
<i>Lampsilis teres</i>	0.21	0.016	0.016	0.99	48	47.75	9-246
<i>Leptodea fragilis</i>	1.07	0.079	0.031	0.40	241	95.50	126-463
<i>Megaloniais nervosa</i>	6.01	0.444	0.076	0.17	1351	230.31	1021-1788
<i>Obliquaria reflexa</i>	4.50	0.333	0.067	0.20	1013	202.20	730-1407
<i>Pleurobema sintoxia</i>	1.50	0.111	0.031	0.28	338	95.50	212-538
<i>Potamilus alatus</i>	1.72	0.127	0.016	0.12	386	47.75	315-473
<i>Potamilus ohioensis</i>	0.21	0.016	0.039	2.48	48	119.85	1-2870
<i>Quadrula pustulosa</i>	13.33	0.986	0.140	0.14	2998	426.67	2372-3789
<i>Quadrula quadrula</i>	0.86	0.063	0.039	0.62	193	119.85	70-536
<i>Truncilla donaciformis</i>	0.64	0.048	0.039	0.83	145	119.85	37-565
<i>Tritogonia verrucosa</i>	0.43	0.032	0.031	0.99	97	95.50	19-491
Total		7.399	0.539	0.07	22493	1638.23	19953-25356

B.

Species	Relative Abundance (%)	Density (per m ²)	SE	CV	Abundance	SE	90% CL
<i>Amblema plicata</i>	47.14	0.786	0.310	0.39	4180	1649.83	2184-8001
<i>Ellipsaria lineolata</i>	5.72	0.095	0.063	0.66	507	333.80	171-1497
<i>Fusconaia flava</i>	8.57	0.143	0	0	760	0	760-760
<i>Obliquaria reflexa</i>	14.29	0.238	0.024	0.10	1267	126.17	1075-1492
<i>Pleurobema sintoxia</i>	2.85	0.048	0.024	0.50	253	126.17	112-575
<i>Potamilus alatus</i>	2.85	0.048	0.024	0.50	253	126.17	112-575
<i>Quadrula pustulosa</i>	15.71	0.262	0.063	0.24	1393	333.80	940-2066
<i>Quadrula quadrula</i>	1.43	0.024	0.024	1.00	127	126.17	25-652
<i>Tritogonia verrucosa</i>	1.43	0.024	0.024	1.00	127	126.17	25-652
Total		1.667	0.291	0.17	8867	1550.35	6651-11821

C.

Species	Relative Abundance (%)	Density (per m ²)	SE	CV	Abundance	SE	90% CL
<i>Actinonaias ligamentina</i>	0.15	0.006	0.006	1.00	48	47.75	9-247
<i>Amblema plicata</i>	57.03	2.14	0.260	0.12	17886	2173.36	14645-21844
<i>Arcidens confragosus</i>	0.15	0.006	0.006	1.00	48	47.75	9-247
<i>Elliptio dilatata</i>	0.77	0.029	0.014	0.48	241	119.85	106-546
<i>Ellipsaria lineolata</i>	3.00	0.112	0.050	0.45	941	415.92	455-1947
<i>Fusconaia flava</i>	6.12	0.23	0.028	0.12	1918	230.31	1574-2337
<i>Lampsilis teres</i>	0.15	0.006	0.016	2.67	48	134.90	0-4888
<i>Leptodea fragilis</i>	0.77	0.029	0.019	0.66	241	158.24	82-710
<i>Megaloniais nervosa</i>	4.31	0.161	0.031	0.19	1351	262.61	981-1860
<i>Obliquaria reflexa</i>	7.27	0.273	0.029	0.11	2280	238.34	1920-2708
<i>Pleurobema sintoxia</i>	1.88	0.071	0.019	0.27	591	158.24	380-918
<i>Potamilus alatus</i>	2.04	0.077	0.016	0.21	639	134.90	452-904
<i>Potamilus ohioensis</i>	0.15	0.006	0.014	2.33	48	119.85	1-2918
<i>Quadrula pustulosa</i>	14.00	0.525	0.065	0.12	4391	541.73	3584-5739
<i>Quadrula quadrula</i>	1.02	0.038	0.021	0.55	320	174.02	131-783
<i>Truncilla donaciformis</i>	0.46	0.017	0.014	0.82	145	119.85	37-565
<i>Tritogonia verrucosa</i>	0.71	0.027	0.019	0.70	224	158.24	70-716
Total		3.751	0.270	0.07	31360	2255.523	27861-35299

Table 13. Species-specific and total estimates of density and abundance within high (A) and low (B) density strata and for the entire site at Site 4. Number of quadrats sampled in each area: high density stratum (n=290), low density stratum (n=150), and site total (n=440).

A.							
Species	Relative Abundance (%)	Density (per m ²)	SE	CV	Abundance	SE	90% CL
<i>Actinonaias ligamentina</i>	0.16	0.014	0.014	1.00	25	24.60	5-126
<i>Amblema plicata</i>	49.17	4.372	0.448	0.10	7958	816.14	6722-9420
<i>Elliptio dilatata</i>	0.93	0.083	0.023	0.28	151	42.61	95-240
<i>Ellipsaria lineolata</i>	0.62	0.055	0.036	0.65	100	65.08	35-292
<i>Fusconaia flava</i>	2.01	0.179	0.046	0.26	326	83.74	214-498
<i>Lasmigona costata</i>	0.32	0.028	0.014	0.49	50	24.60	22-112
<i>Lampsilis teres</i>	0.32	0.028	0.033	1.19	50	59.84	7-357
<i>Leptodea fragilis</i>	2.17	0.193	0.094	0.48	351	170.33	158-780
<i>Megalonaias nervosa</i>	24.82	2.207	0.241	0.11	4017	439.24	3355-4808
<i>Obliquaria reflexa</i>	4.07	0.362	0.130	0.36	659	236.21	366-1189
<i>Pleurobema sintoxia</i>	1.24	0.110	0.033	0.30	201	59.84	123-328
<i>Potamilus alatus</i>	1.09	0.097	0.014	0.14	176	24.60	140-221
<i>Potamilus ohioensis</i>	0.32	0.028	0.033	1.19	50	59.84	7-357
<i>Quadrula pustulosa</i>	10.30	0.916	0.187	0.20	1668	339.49	1193-2331
<i>Quadrula quadrula</i>	0.78	0.069	0.064	0.92	126	115.88	27-573
<i>Truncilla donaciformis</i>	1.24	0.110	0.060	0.53	201	107.22	83-483
<i>Tritogonia verrucosa</i>	0.46	0.041	0	0	75	0	75-75
Total		8.892	0.703	0.08	16184	1279.40	14210-18432
B.							
Species	Relative Abundance (%)	Density (per m ²)	SE	CV	Abundance	SE	90% CL
<i>Amblema plicata</i>	57.78	0.693	0.322	0.46	1622	753.02	756-3481
<i>Elliptio dilatata</i>	2.22	0.027	0.027	0.99	62	61.90	12-319
<i>Ellipsaria lineolata</i>	2.22	0.027	0.027	0.99	62	61.90	12-319
<i>Lasmigona costata</i>	4.44	0.053	0.053	0.99	125	123.80	24-638
<i>Megalonaias nervosa</i>	11.11	0.133	0.070	0.52	312	163.77	132-740
<i>Obliquaria reflexa</i>	4.44	0.053	0.027	0.50	125	61.90	55-282
<i>Pleurobema sintoxia</i>	2.22	0.027	0.027	0.99	62	61.90	12-319
<i>Potamilus alatus</i>	2.22	0.027	0.027	0.99	62	61.90	12-319
<i>Quadrula pustulosa</i>	2.22	0.027	0.027	0.99	62	61.90	12-319
<i>Quadrula quadrula</i>	6.67	0.080	0.046	0.57	187	107.21	73-480
<i>Truncilla donaciformis</i>	2.22	0.027	0.027	0.99	62	61.90	12-319
<i>Tritogonia verrucosa</i>	2.22	0.027	0.027	0.99	62	61.90	12-319
Total		1.200	0.510	0.42	2805	1193.85	1395-5651
C.							
Species	Relative Abundance (%)	Density (per m ²)	SE	CV	Abundance	SE	90% CL
<i>Actinonaias ligamentina</i>	0.13	0.006	0.006	1.00	25	24.60	5-126
<i>Amblema plicata</i>	50.44	2.303	0.267	0.12	9580	1110.46	7917-11592
<i>Elliptio dilatata</i>	1.13	0.052	0.018	0.35	213	75.15	119-381
<i>Ellipsaria lineolata</i>	0.86	0.039	0.022	0.56	162	89.82	65-403
<i>Fusconaia flava</i>	1.72	0.078	0.020	0.26	326	83.74	214-497
<i>Lasmigona costata</i>	0.92	0.042	0.030	0.71	175	126.22	53-573
<i>Lampsilis teres</i>	0.27	0.012	0.014	1.17	50	59.84	7-358
<i>Leptodea fragilis</i>	1.85	0.084	0.041	0.49	351	170.33	158-780
<i>Megalonaias nervosa</i>	22.79	1.040	0.113	0.11	4329	468.78	3623-5173
<i>Obliquaria reflexa</i>	4.12	0.188	0.059	0.31	784	244.19	1309-470
<i>Pleurobema sintoxia</i>	1.39	0.063	0.021	0.33	263	86.10	153-451
<i>Potamilus alatus</i>	1.26	0.058	0.016	0.28	238	66.61	150-377
<i>Potamilus ohioensis</i>	0.27	0.012	0.014	1.17	50	59.84	7-358
<i>Quadrula pustulosa</i>	9.11	0.416	0.083	0.20	1730	345.09	1246-2402
<i>Quadrula quadrula</i>	1.65	0.075	0.037	0.49	313	157.87	137-718
<i>Truncilla donaciformis</i>	1.39	0.063	0.030	0.48	263	123.81	121-571
<i>Tritogonia verrucosa</i>	0.73	0.033	0.015	0.45	137	61.90	65-288
Total		4.565	0.421	0.09	18989	1749.9	16318-22097

F. flava, *O. reflexa*, and *M. nervosa* were intermediate in abundance. The rarest species at the site were *Actinonaias ligamentina* (mucket), *Arcidens confragosus* (rock pocketbook), *Lampsilis teres* (yellow sandshell), and *Potamilus ohiensis* (pink papershell).

The species composition at Site 4 was dominated by two species, *A. plicata* and *M. nervosa*. *Q. pustulosa*, *O. reflexa*, and *F. flava* were also abundant. The density of *Leptodea fragilis* (papershell) was higher at Site 4 than the other three sites. *Actinonaias ligamentina* was the rarest species at the site. Other less abundant species included *Lasmigona costata* (white heelsplitter), *L. teres*, and *Potamilus ohiensis*.

Site Density and Diversity

The mussel density at Site 2 was significantly lower than the density at the other sites ($P < 0.0001$) (Table 14, Figure 3). The density at Site 1 was lower than the density at Sites 3 and 4 but was only significantly lower than the density at Site 4 ($P < 0.001$). The density at Site 3 was slightly lower than the density at Site 4 but the difference was not significant.

Site 3 and Site 4 both contained 17 species (Table 15). They also both ranked higher than the other sites according to Simpson's Index of Dominance and Margalef Diversity index which is not sensitive to species evenness. These sites ranked lower than Sites 1 and 2 according to the Shannon-Weiner index and Shannon Evenness index. Site 1 ranked higher than all the sites according to the Shannon-Weiner index, exhibiting greater evenness in the distribution of individuals among species.

Figure 2. Comparison of density estimates and 90 % confidence intervals for each species in the high density strata at four sites (Site 1=dark grey bar, Site 2=diagonal line bar, Site 3=light grey bar, Site 4=dotted bar). Density estimates of the five most abundant species (A) are shown separate from the rest of the species at the sites (B).

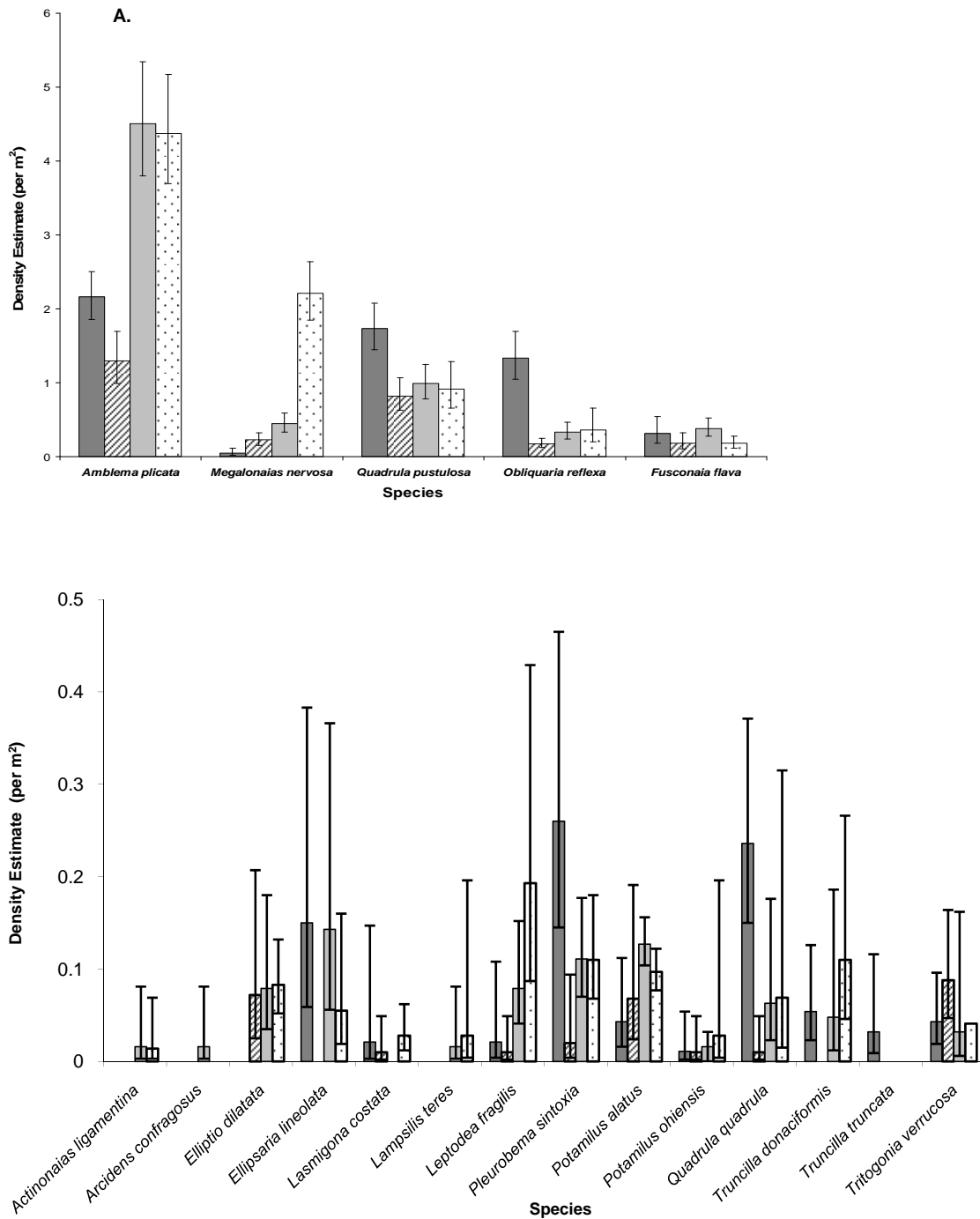


Figure 3. Total site mussel density estimates at four sites with 90% confidence intervals (A) and mussel density estimates in the high density (solid bars) and low density (horizontal line bars) strata (B).

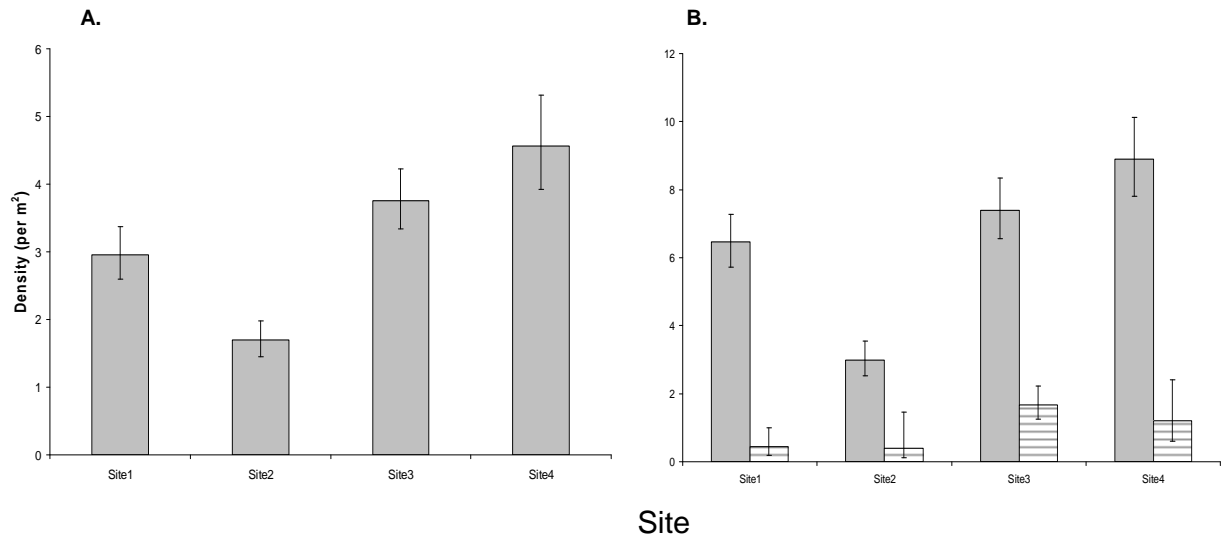


Table 14. Density comparisons between sites and significant differences determined for site density estimates by Wilcoxon two-sided nonparametric tests

Site	Density Estimate (per m ²)	SE	90% CL	High Density Strata Estimate (per m ²)	SE	90% CL	Site Significant Differences
1	2.960	0.055	2.60-3.37	6.456	0.474	5.72-7.28	2 (P<0.0001), 4 (P<0.001)
2	1.695	0.158	1.46-1.98	2.989	0.308	2.52-3.54	
3	3.751	0.073	3.33-4.22	7.399	0.539	6.56-8.34	2 (P<0.0001)
4	4.565	0.177	3.92-5.31	8.892	0.703	7.81-10.13	1 (P<0.001), 2 (P<.0001)

Table 15. Diversity indices calculated from systematic survey data at the four sites

Site	Number of Species	Simpson	Margalef	Shannon-Weiner	Evenness
1	15	0.228	1.419	1.787	0.661
2	13	0.276	1.303	1.651	0.644
3	17	0.357	1.545	1.573	0.555
4	17	0.318	1.624	1.627	0.574

Subsurface Density

Corbicula fluminea was only found in the subsurface of excavated quadrats.

There was no significant difference in subsurface *C. fluminea* density or subsurface mussel density among the four sites ($P > 0.05$, Table 16). At Sites 1 and 2, the density of *C. fluminea* was significantly higher than the density of subsurface mussels (Site 1: $P < 0.05$, Site 2: $P < 0.01$). There were slightly higher numbers of *C. fluminea* than mussels in the subsurface at Sites 3 and 4 but the differences were not significant.

Table 16. Number of excavated quadrats and density estimates of subsurface freshwater mussels and Asian clams (*Corbicula fluminea*) at four sites

Site	Number of Excavated Quadrats	Subsurface Mussel Density (per m ²)	Subsurface <i>Corbicula fluminea</i> Density (per m ²)
1	124	0.548	0.968
2	127	0.157	0.63
3	64	0.438	0.50
4	73	0.384	1.096

Influence of Mussel Size on Surface Detectability

At all four sites the majority of mussels and *Corbicula fluminea* less than 20 mm in height were located in the subsurface by excavation (Figure 4). The mussels in these height categories found on the surface were typically very young individuals attached to surface mussels with byssal threads. The smallest mussels located on the surface by tactile searches alone were located at Site 1, in the 20.1-30 mm size category. A mussel of this size was more prone to be found by excavation. At Sites 3 and 4, mussels in the 30.1-40.0 mm size category were slightly more likely to be detected by excavation but this was not the case at Site 1. Typically, excavation would primarily uncover individuals less than 50 mm in length but a few larger individuals were also located by this method. The largest size category (above 150.1) was made up entirely of washboard and was greatest at sites containing higher numbers of this species.

Influence of Cross-stream Position on Density

At all four sites, the majority of mussels were located near the left downstream bank. As distance from that bank increased, density would typically decline.

At Site 1, the highest average counts per quadrat were found at x=2.5, 3, and 4.5 (Figure 5, Table 17). As distance increased, the density steadily declined. Although the mussel bed was believed to stretch 210 m in the upstream direction, comparatively few mussels were found above the 150 m mark and no quadrats uncovered greater than 2 mussels past this mark. The relationship between density and cross-stream position

became non-existent past the 150 meter mark. The overall relationship between density and cross-stream position was strong at this site (density= $2.061 - 0.086 x$, $R^2 = 0.74$).

The distributional pattern at Site 2 (Figure 6, Table 17) did not show increasing density with cross-stream position. The high density stratum was cut off from the low density stratum at $x=15$ and density was very low past this point except in the upper portion of the site.

Within the high density strata, the zero position had very few mussels and was essentially located immediately adjacent to the bank. This position was left out of the regression analysis. The analysis demonstrated that there was not a strong relationship between distance from the bank and density (density= $0.962 - 0.311 x$, $R^2 = 0.49$).

At Site 3, no mussels were located past the $x=21$ position during the preliminary survey and the area past this point was not included in the systematic survey. Mussels were absent at this position (Figure 7, Table 19). Within the high density stratum, density was highest in the intermediate positions of $x=2$ and $x=5.5$. Density was lower overall in quadrats placed 0.5 m closer to the bank from $x=2$ at the $x=1.5$ position. As expected, density was lowest within the high density stratum at the $x=7.5$ position, close to the strata boundary region. Density was lower in the low density stratum positions as very few individuals were found past the 200 m upstream mark in this region. Higher numbers were located downstream from this point at $x=12$ and $x=12.5$. Overall, a strong relationship existed between density and distance from the bank at this site (density= $2.130 - 0.108 x$, $R^2=0.75$).

Site 4 was another site where the entire wetted width was not included in the systematic survey. Mussels were absent at positions greater than 16 m from the bank

during the preliminary survey and this area was not included in the survey. Density was lowest in the high density stratum in the position adjacent to the bank ($x=0.5$) but density was very high at this position and at the $x=2$ position in the upper portion of the site around the 150-230 m mark (Figure 8, Table 20). Mussels were very sparse downstream from the 150 m mark at these two positions. A more even pattern could be observed at the other cross-stream positions of the high density stratum with the density dropping off close to the stratum border at the $x=6.5$ position. Density in the low density stratum steadily declined as distance from the bank increased. The relationship between density and distance from the bank was strong at this site (density = $2.60 - 0.180x$, $R^2=0.71$).

Figure 4. Comparison between density estimates (left) and abundance (right) of mussels located on the surface and mussels located on the subsurface separated by size categories. Estimates from Site 1 (A and B), Site 2 (C and D), Site 3 (E and F), and Site 4 (G and H) are displayed. Subsurface Asian clams (*Corbicula fluminea*) are represented by diagonal striped bars, subsurface mussels are represented by solid black bars, and surface mussels are represented by solid grey bars.

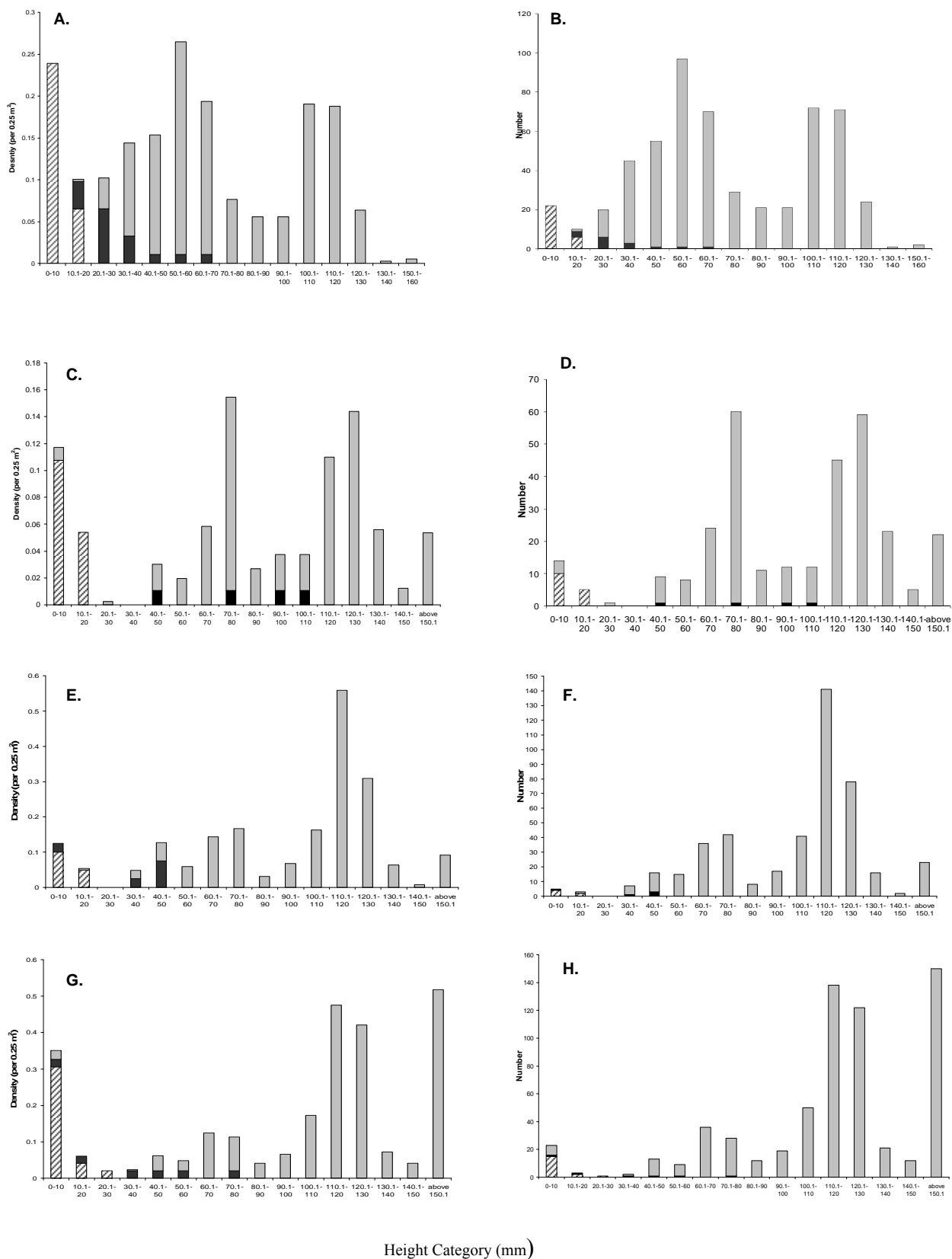


Figure 5. Mussel spatial distribution at Site 1 with total mussel counts in each 0.25 m² quadrat displayed.

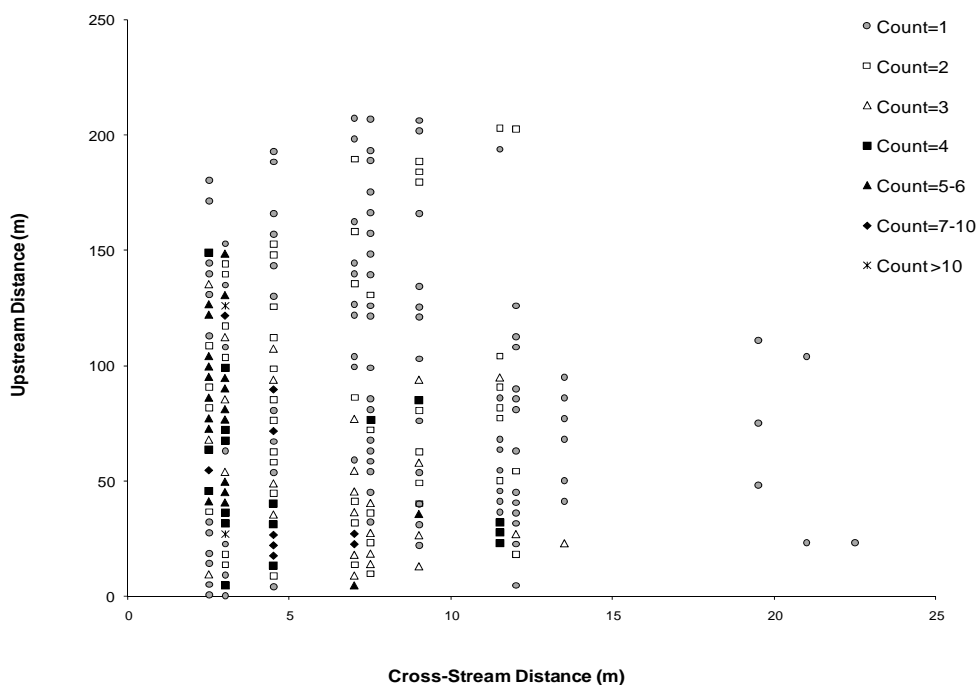


Table 17. Summary statistics for each cross-stream position or distance (m) from left downstream bank at Site 1 (N=number of quadrats, density=mean count of mussels at each cross-stream position, min=minimum number of mussels, max=maximum number of mussels).

Cross-stream Position	N	Density	Standard Deviation	Min	Max
2.5	47	2.09	2.22	0	7
3	47	2.81	2.98	0	14
4.5	46	2.17	2.64	0	10
7	47	1.32	1.77	0	7
7.5	47	0.96	1.02	0	4
9	46	1.04	1.32	0	6
11.5	47	0.75	1.17	0	4
12	47	0.47	0.72	0	3
13.5	23	0.39	0.72	0	3
19.5	23	0.13	0.34	0	1
21	23	0.09	0.29	0	1
22.5	23	0.04	0.21	0	1
28.5	23	0	0	0	0
30	23	0	0	0	0

Figure 6. Mussel spatial distribution at Site 2 with total mussel counts in each 0.25 m² quadrat displayed.

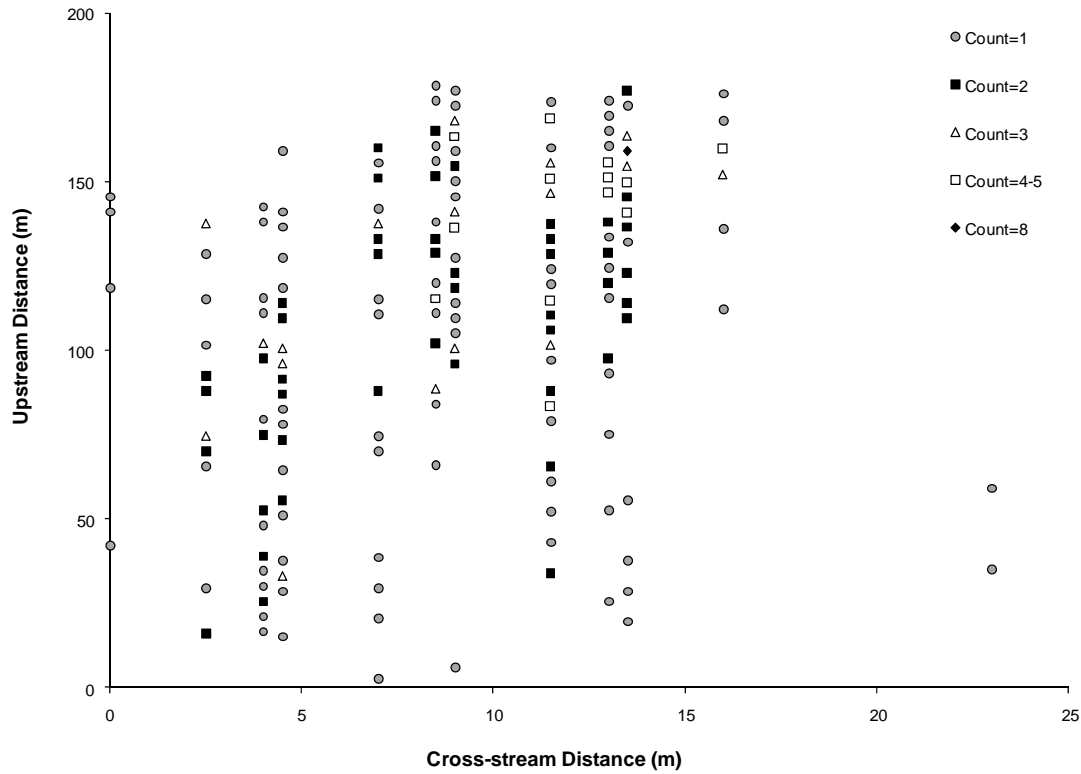


Table 18. Summary statistics for each cross-stream position or distance (m) from left downstream bank at Site 2 (N=number of quadrats, density=mean count of mussels at each cross-stream position, min=minimum number of mussels, max=maximum number of mussels).

Cross-stream Position	N	Density	Standard Deviation	Min	Max
0	41	0.98	0.30	0	1
2.5	41	0.46	0.87	0	3
4	41	0.56	0.81	0	3
4.5	41	0.81	0.95	0	3
7	41	0.56	0.81	0	3
8.5	41	0.66	1.06	0	5
9	41	0.85	1.25	0	5
11.5	41	1.29	1.50	0	5
13	41	0.78	1.22	0	5
13.5	41	1.00	1.67	0	8
16	24	0.50	1.18	0	5
22	23	0	0	0	0
23	23	0.87	0.29	0	1
24	24	0	0	0	0
30	23	0	0	0	0
31	23	0	0	0	0

Figure 7. Mussel spatial distribution at Site 3 with total mussel counts in each 0.25 m² quadrat displayed.

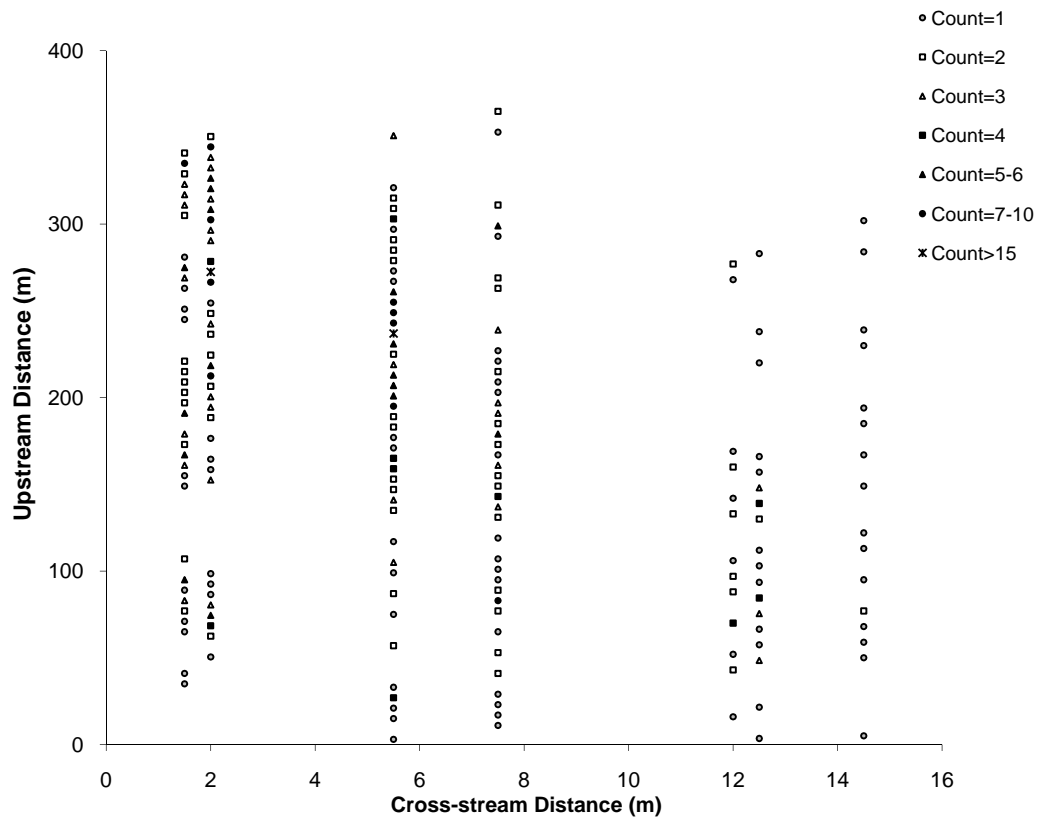


Table 19. Summary statistics for each cross-stream position or distance (m) from left downstream bank at Site 3 (N=number of quadrats, density=mean count of mussels at each cross-stream position, min=minimum number of mussels, max=maximum number of mussels).

Cross-stream Position	N	Density	Standard Deviation	Min	Max
1.5	63	1.33	1.76	0	8
2	63	2.19	3.11	0	18
5.5	63	2.30	2.95	0	16
7.5	63	1.27	1.45	0	7
12	42	0.52	0.92	0	4
12.5	42	0.74	1.27	0	4
14.5	42	0.41	0.54	0	2
21	42	0	0	0	0

Figure 8. Mussel spatial distribution at Site 4 with total mussel counts in each 0.25 m² quadrat displayed.

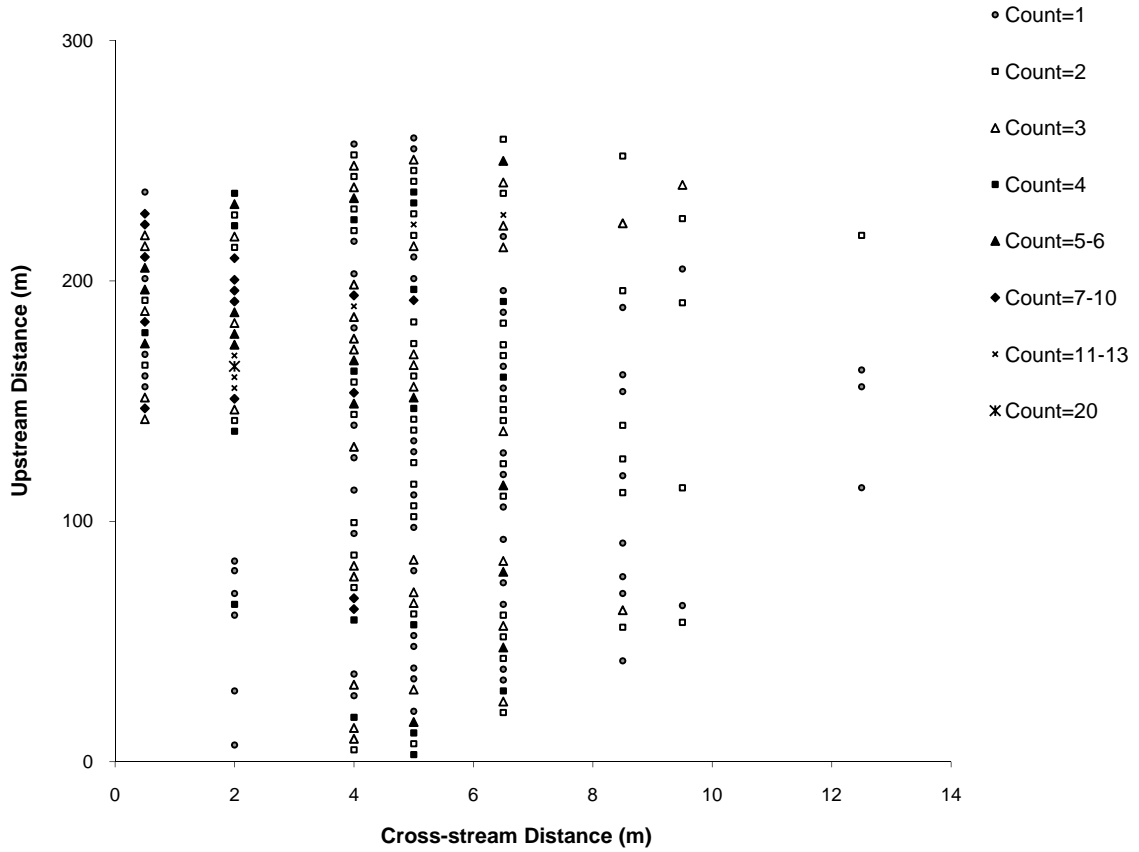


Table 20. Summary statistics for each cross-stream position or distance (m) from left downstream bank at Site 4 (N=number of quadrats, density=mean count of mussels at each cross-stream position, min=minimum number of mussels, max=maximum number of mussels).

Cross-stream Position	N	Density	Standard Deviation	Min	Max
0.5	58	1.50	2.63	0	9
2	58	2.64	4.09	0	20
4	58	2.53	2.75	0	13
5	58	2.21	1.94	0	11
6.5	58	1.81	1.92	0	11
8.5	38	0.68	0.93	0	3
9.5	37	0.35	0.79	0	3
12.5	37	0.14	0.42	0	2
15.5	38	0	0	0	0

Discussion

The four sites selected for extensive sampling were accessible and easily sampled in periods of low water, and all contained a diverse fauna of mussels. The sampling protocol I implemented will allow researchers to monitor changes in these four mussel beds over time. The parameters that can be monitored for change at each site include density, relative abundance, recruitment (Chapter 3), and distribution.

An initial reconnaissance where the mussel bed is clearly delineated and the subsequent two-stage stratified design will allow for an accurate assessment of changes in density and distribution at these sites on the Marais des Cygnes River. The initial reconnaissance was required to measure the extent of the mussel bed by mapping the boundaries and insuring that the sampling included the entire cluster of mussels. These boundaries can be reexamined in future investigations to determine whether the extent of each bed is similar between years.

The stratified design was useful in the Marais des Cygnes River. At each site mussels were heavily clustered along one bank and density decreased from that bank. Rather than limiting the survey to wadeable areas, the initial stage of the stratified design allowed for an approximate delineation of the distribution and cross-stream extent of the cluster. Typically, the 'low density stratum' at each site contained few mussels and was more time consuming and costly to survey. In general the substrate in this area consisted of small and large cobble, boulders, bedrock, or silt (addressed in Chapter 5). Researchers may choose to not resample this area in the future and can systematically resample the 'high density stratum' to analyze temporal changes.

The difficulty that arises from repeating the stratification design in a long-term monitoring study is that the spatial distribution and densities of mussels may change over time (Strayer and Smith 2003). As conditions change the stratification scheme may have to be altered when sampling the entire site. The cross-stream width of the high density stratum will also vary with discharge. These problems can be overcome by sampling during periods where discharge is similar to the discharge during the time frame of this study and possibly repeating the preliminary survey at the sites. A direct comparison of only the high density stratum during similar water levels can also meet long-term research goals and overcome the problems associated with stratification. For example, by limiting the systematic survey at Site 1 to a cross-stream distance of 13 meters (the cross-stream boundary of the high density stratum determined from this study) the densities can be directly compared when discharge is similar. A few meters in the cross-stream direction can be added or subtracted if the sampling occurs at slightly different discharge levels. The question of whether the cross-stream distribution of mussels is changing may be important to researchers. Density can remain fairly constant in the 'high density stratum' over time but the bed may be shrinking as individuals move closer to the bank with deteriorating habitat conditions. The current study allows for this aspect to be addressed in future sampling by providing baseline mean estimates at each cross-stream position. If the original high density stratum area (for example, $x=13$ at Site 1) was systematically resampled in the future and total density was similar, the cross-stream position means could be examined to see if the mean counts in positions furthest from the bank were decreasing and means closest to the bank were increasing. This would

indicate that either individuals have moved closer to the bank or possibly that there were differences in mortality and recruitment along the transects.

The study compared density within areas that were 13 m from the bank (Site 1), 16 m from the bank (Site 2), and approximately 8 meters from the bank (Sites 3 and 4). Density was highest at Sites 3 and 4 but at Site 4 abundance was similar to the abundance at Site 1. The higher density at Site 4 compared to Site 1 indicates the mussels tended to be more crowded in a smaller area at this site. In general within the downstream portion of Site 1, the gravel bar extended further across the stream. Within upstream portions, the gravel and shallow water habitat decreased gradually. At Sites 3 and Sites 4, the decline in mussels and increase in depth or larger substrate was not as gradual. The mussels were more clustered along the bank at these sites. The associations of mussel clusters and habitat conditions are addressed with more detail in Chapter 5. One would expect that as conditions deteriorate on the boundary regions between strata, mussels would move into areas close to the bank with more favorable conditions. Another possibility is there may be differences along the transects in conditions that are favorable to mussel recruitment. Long-term monitoring of the same areas within the sites may detect changes in mussel distribution and densities across the stream.

The systematic sampling protocol with excavation was useful in generating precise estimates of total mussel density and the density of certain species at the sites. The CV or standard error divided by the mean is recommended as an indication of precision (Strayer and Smith 2003, Smith et al. 2001). When using a systematic sampling design and 0.25 m² quadrats, Strayer and Smith (2001) list highly precise estimates as having a CV < 0.20, precise estimates as having a CV < 0.30 and moderately

precise estimates as having a $CV < 0.35$. The total mussel density estimates for the high density strata and entire sites were all highly precise. The most precise estimates were generated for the high density strata. Several of the abundant species had at least moderately precise estimates. The high precision of the estimates will allow researchers to monitor total abundance changes and changes in the mussel community through time.

Total density should not be the only parameter considered in inventories when assessing the quality of a site. Other considerations include species diversity, individual distribution, and recruitment. Site 1 had a lower total density estimate than Site 3 and Site 4 but this site was more diverse according to the Shannon-Weiner diversity index. The distribution of individuals was more evenly spread among the species. At Site 1, the high density stratum was dominated by *A. plicata*, *O. reflexa*, and *Q. pustulosa*. Together these three species comprised approximately 80% of the mussel fauna. Site 1 contained a higher density of *O. reflexa* and *Q. pustulosa* than the other three sites. Site 1 was the only site out of the four sampled that contained *Truncilla truncata* (deertoe) and this species was found in very low numbers. *Megalonaias nervosa* (washboard) was rare at Site 1, but the only young *M. nervosa* recovered during the surveys at all four sites was found stranded at Site 1.

I recovered 17 species of mussels at Sites 3 and 4. The density of mussels was high at these sites. In the high density stratum at Site 3, *A. plicata* comprised over 60% of the individuals and the next most abundant species was *Q. pustulosa* (13%). *A. plicata* and *M. nervosa* were the most dominant species at Site 4. Most of the *A. plicata* and *M. nervosa* collected at these sites were old (probably at least 20 years) and some shell erosion was evident. No evidence of recent washboard recruitment was found at the

sites. Sites 3 and 4 ranked higher than the other sites on the Simpson and Margalef Diversity Indices which are insensitive to evenness. At these sites only one or two species were dominant.

Several rare species were found at Sites 3 and 4. *Actinonaias ligamentina* (mucket) and *Arcidens confragosus* (rock pocketbook) are specified in a Kansas Recovery Plan (Obermeyer, 2002). One live *A. ligamentina* was located in quadrats at Site 3 and Site 4. *A. ligamentina* is a species that is endangered in Kansas and is believed to be extirpated from most of its historic range outside of the upper Osage River system (Obermeyer 2000). Because one *A. ligamentina* was found in the systematic samples, the estimate of the density of this species at each site is very low and imprecise. *Arcidens confragosus* is threatened in Kansas and an individual was found in a quadrat at Site 3. Few live individuals of this species have been collected in Kansas since 1949. Qualitative surveys where catch per unit of time is measured can be used to possibly locate more individuals of the rarer species at these sites but will be biased against the smaller size classes (Strayer and Smith 2003, Dunn 2000).

Site 2 contained fewer mussels than the other sites. The majority of the individuals consisted of *Amblema plicata* and *Q. pustulosa*. The site ranked second among the four sites in evenness. Unlike the other sites, the high density stratum at Site 2 was 16 m wide and included mid-stream riffle areas with cobble and swift moving shallow water and some silty, deep regions near the bank. There was not a strong relationship between density and distance from the bank at this site.

Tactile searches were efficient at detecting the majority of individuals at each site but excavation was necessary to detect *Corbicula fluminea* and juvenile freshwater

mussels. In the preliminary survey where half of the quadrats were excavated, tactile searches without excavation uncovered 95% of the individuals at Sites 2-4, and 87% of individuals at Site 1. I was not able to locate freshwater musselss (that were not attached to other larger individuals) and Asian clams less than 20 mm by tactile searching alone. The results support the findings that surface searches underestimate the density of smaller individuals (Miller and Payne 1988) and that excavation is necessary for observing recruitment (Smith et al. 2000, Miller and Payne 1988, Richardson and Yokley 1996, Vaughn et al.1997). Excavation can be costly in terms of effort and the added disturbance to mussel habitat so I excavated the recommended optimal proportion of quadrats based on a greater than 60% rate of detection by surface searches (Smith et al. 2000). Because I applied the same method of randomly excavating every fourth quadrat to all four sites and habitat within the mussel beds was relatively homogenous, a direct comparison of recruitment could be made between the sites which will be addressed in the next chapter. The size histograms of mussels detected on the surface and subsurface indicate that the majority of freshwater musselss from 10-40 mm in length were found at Site 1. At two sites, the density of *Corbicula fluminea* was significantly greater than the density of freshwater musselss uncovered by excavation. It is reasonable to assume based on size and shell morphology that if the density of *C. fluminea* was similar to the density of juvenile mussels at the sites the probability of uncovering clams by excavation would be similar to the probability of uncovering juvenile (at least 2 years old) mussels of the majority of species but this has not been tested.

The four mussel beds chosen for systematic sampling show variation in several population parameters including density, species richness, species dominance, species

evenness, and area. The distribution of rare species was not even among sites and certain species were noticeably absent or less frequent at particular sites. The diversity and abundance within a mussel bed may be affected by habitat and alterations in the natural flow regime which will be addressed in more detail in Chapter 5. These conditions may play a role in limiting mussel recruitment (historic and present) and survival. The spatial extent of the mussel beds surveyed on the Marais des Cygnes appears to be limited by habitat as the majority of mussels are highly clustered on one side of the channel and the most dense aggregations are present adjacent to the bank. By delineating and mapping the area mussels are occupying at the sites and generating highly precise density estimates, changes in abundance, species richness, and distribution can be monitored through time. In the next chapter, I examine whether recent recruitment is occurring at the four locations.

Chapter 3. Estimating Recent Mussel Recruitment Using Length-at-age Categorical Relationships Based on Internal Annuli

Introduction

Freshwater mussels are long-lived organisms. The life span of most mussel species consists of decades (Bauer and Wachtler 2000) with some populations having mean ages of 50 years or more (Strayer et al. 2004). Because of this longevity, mussel abundance should not be the only factor used for determining the health of a population. A site may support a diverse and dense assemblage but conditions at that site may be unfavorable to mussel reproduction or juvenile survivorship. Low numbers or absence of juveniles has been reported in other studies (Hendelberg 1961, Bauer 1983, Howard and Cuffey 2003).

Most freshwater mussels require parasitism on a host fish during the larval stage of their life cycle. Reproduction is initiated as sperm are released into the water column by males and taken in through the incurrent siphon of females. Fertilization occurs in the interlamellar spaces of the gills. The larva matures in these spaces which act as brood patches and the mature larva or glochidium is ultimately discharged through the excurrent siphon and must attach to the gills or fins of a suitable host fish (Kat 1984). The attached glochidia complete their development and fall from the fish host as juveniles. The juvenile stage is believed to be the most sensitive time in the life cycle of freshwater mussels and mortality of juvenile mussels is high at the time of excystment from their hosts (Hardison and Layzer 2001). There are several factors that could potentially influence the successful establishment of juveniles in an individual mussel bed: low

reproductive adult density, unfavorable discharge patterns or temperature during the spawning season (either during the time of glochidia release or juvenile settlement) (Layzer and Madison 1995, Williams et al. 1993, Payne and Miller 2000), low host fish abundance in the watershed, barriers to host fish dispersal (Neves and Angermeier 1990, Watters 1996), or unfavorable habitat for juvenile settlement (Layzer and Madison 1995, Howard and Cuffey 2003, Morales et al. 2006).

My objective was to determine whether recent mussel recruitment has occurred at the four sampling sites on the Marais des Cygnes River. For this study, recruitment is defined as the addition of individuals of a given age-class into the population. I am interested in the presence and abundance of recent recruits or young individuals at the four sites. To meet this objective I used a collection of mussels that died at Site 1 during a long period of low flow in 2006 from June to November. Several species from this collection were aged in the lab by counting internal shell annuli and age class-length relationships were determined. These relationships were applied to the survey data and allowed for an assessment of the cohort structure at each site. Another objective of the study is to estimate age class specific mortality rates during the low flow period at Site 1 by comparing the quantity of deceased mussels within each age class to the estimated abundances of the age classes at the site prior to the drought.

Materials and Methods

Assessment of Low Flow Period

On 8/2/2006, Site 1 was revisited after the survey was completed. All visible fresh dead shells (shells containing soft-tissue) were collected from the bank and edge of the water. Shells were later identified by species and counted. Site 1 was revisited on four additional dates as water level remained low: 10/14, 10/21, 11/4, and 11/11. On each date all fresh dead shells were collected and removed from the site, identified by species, and counted. In addition this procedure was implemented at Site 3 on 10/21 and Site 4 on 8/18 and 11/12.

I calculated the relative abundance of each species in the mortality collection at each site from total counts of fresh dead shells. Percent mortality during the low flow period was calculated at Sites 1 and 3. Mortality estimates were conservative, limited to the number of shells collected on the bank and edge of the water. The shell count was divided by the abundance estimate and upper and lower limits of the 90% confidence intervals for each species at the sites (abundance and confidence intervals reported in the previous chapter). Percent mortality was not calculated at Site 4 because sampling to determine abundance occurred simultaneously with the collecting of shells on 8/18.

Age Determination

I determined the age of individuals for four species at Site 1, using thin-sectioning techniques, and counting internal annuli. I then determined the relationship between height and age which was applied to field measurements.

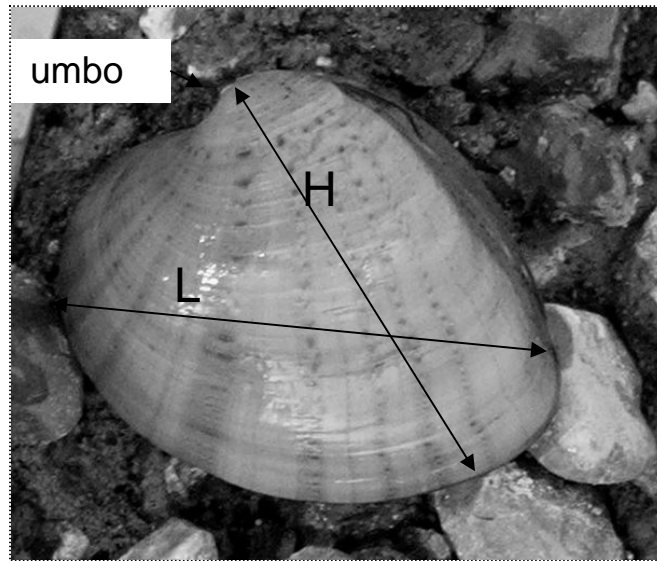
I selected four species for age determination and analysis: pimpleback (*Quadrula pustulosa*, n=84), threehorn wartyback (*Obliquaria reflexa*, n=100), threeridge (*Amblema plicata*, n=32), and butterfly (*Ellipsaria lineolata*, n=42). I used the recently deceased valves (valves containing soft-tissue) collected from Site 1 during the period between August and November, 2006 where the majority of mortalities were observed. Because Site 1 was the only site which contained a large number of valves and a variety of sizes, I used the valves from this site in the study. I assumed that mean annual growth was similar enough among sites for the purpose of generating abundance estimates of multi-year age classes (rather than estimating year-specific recruitment at the four sites for this objective).

Age determinations were accomplished by thin-sectioning techniques (Clark 1980, Neves and Moyer 1988, Jones and Neves 2002, Bruenderman and Neves 1993). This method was demonstrated to be the most consistent and accurate technique for age determinations (versus external growth ring counts, shell ashing, and acetate peels) and it was determined that thin-sections provided the highest degree of resolution for all species, sizes, and ages examined (Neves and Moyer 1988).

The procedure was similar to that described by Clark (1980) and Bruenderman and Neves (1993). Valves were cleaned with soap and water prior to thin-sectioning. The dimensions measured with digital calipers in the field were also measured by the same method on the valves to be sectioned; length (the greatest anterior-posterior distance) and height (the distance from the umbo to the leading edge of the shell) (Figure 1). Both dimensions were measured to the nearest mm. The height measurements were taken along the axis of maximum shell growth which gives the maximum amount of change

between each annulus (MacClintock 1967). The shells were sectioned along this axis to intersect growth rings at right angles (Neves and Moyer, 1988).

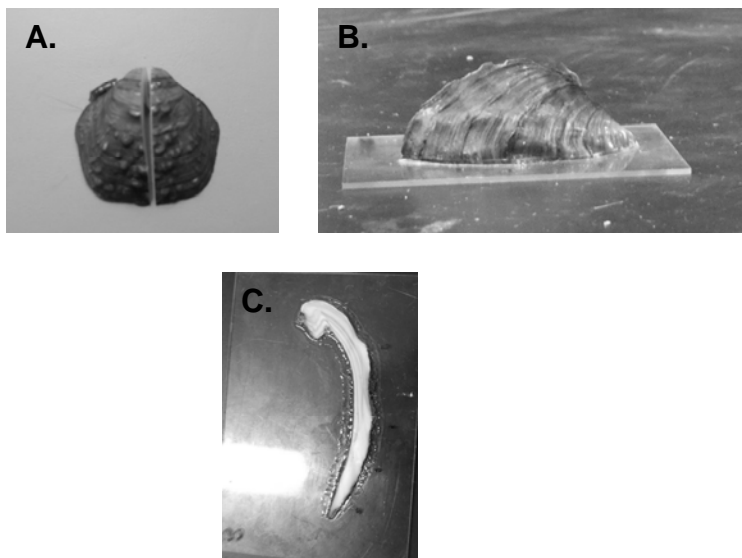
Figure 1. Measurements taken in the field on a male *Ellipsaria lineolata*. The length (L) is the greatest distance from the anterior to the posterior margin of the shell and the height (H) is the distance from the shell umbo to the leading edge of the shell intersecting growth lines at right angles. Shells in the lab were also measured along the two axes and sectioned along the height axis.



A Buehler Isomet 1000 low-speed diamond-tipped saw (Buehler Ltd., Evanston, Illinois) was used to create the sections. An initial cut was made along the axis of maximum growth (height) on one valve (Figure 2A). The sectioned surface and a petrographic slide (27x46 mm or 3x2 inch) were lightly ground with 1000 grit sandpaper. The section was mounted on the slide with Bio-plastic (Ward's Natural Science Establishment, Inc., Rochester, NY) and then placed in an oven set at 70° C for four

hours (Figure 2B). The slide was fit into an appropriate sized chuck and a second cut was made to create a section 300 μm thick (Figure 2C).

Figure 2. Examples shown of three stages in the mussel thin-sectioning process. An initial cut along the axis of maximum growth (height) was made with the Buehler Isomet 1000 low-speed saw on a pimpleback (*Quadrula pustulosa*) shell (A). A threeridge (*Amblyma plicata*) half was mounted to a petrographic slide with Ward's Bioplastic (B). The final cut was made after fitting the slide into a saw chuck (C).



Because a thickness of 280 μm was considered to be optimal for high-resolution thin-sections (Neves and Moyer 1988), the section was lightly ground until the desired thickness was achieved. The sectioned valve and corresponding thin section were examined under a compound microscope and a felt-tip pen was used to mark where the growth line exited the shell surface. True annuli could be traced from the umbo to shell margin on the thin-section and matched with external annuli on the cut valve (Figures 3 and 4). These annuli were counted by two observers to determine the age. Because some shells were collected in early August and in the midst of the summer growing season, one

half year was added to the total count. In young shells, differences in coloration at the edge of the section could be observed in all species and indicated the season of death (Figure 4). For specimens collected in November, I added one full year to the growth ring count.

On younger specimens, I measured the height of the growth lines at previous ages (Bruenderman and Neves 1993). I marked the external annuli on the cut valve which were associated with the internal annuli marked on the slide. I overlaid the cut valve with the uncut valve and measured and recorded the height at previous ages.

The formation of winter annual growth bands has been documented in several studies (Isley 1914, Chamberlain 1931, Negus 1966). Other studies have validated annual winter band formation in riverine species over multiple years (Neves and Moyer 1988, Howard and Cuffey 2006, Haag and Commons-Carson 2008). I conducted no direct annual validation procedure because of the shorter duration of the study but was able to compare different coloration patterns in valves collected at different times of the year. The larger light bands indicated summer growth (Fig. 3B) and a shorter period of growth after the last band before death was observed on young shells collected in August (time of death assumed to occur between June and August 2006, Figure 4A). On the specimens collected in November, a longer period of summer growth was observed between the last annuli and edge of the shell (Figure 4B). The last band was a darker color similar to bands laid down prior to the formation of the winter growth line for previous ages in certain specimens collected in late October or November (Figure 3B).

Age Category Designation

Because of the high variation of size at age with older specimens where it was more frequent that a mussel of a given size could be different ages, I divided the data into categories for the purpose of examining general historic recruitment trends. All aged specimens were placed in both height and age categories. Height categories were determined by examining the data and assigning height increments. Age categories typically consisted of four year intervals. Frequency tables were constructed in SAS which designated the probability of a specimen belonging to a particular age category based on its height category assignment.

I assigned the most probable age category based on height for each individual collected at the four sites. To simplify computations only the data from the high density strata were used. The data were analyzed with the Mussel Estimation Program which generated density, standard error, 90% confidence intervals, and associated abundance estimates.

Figure 3. Thin-sections of *Amblema plicata* (A and B), *Ellipsaria lineolata* (C), and *Obliquaria reflexa* (D). Arrows point to annual growth lines and labels indicate the year the particular band was deposited. The umbo region of a six year old *A. plicata* collected in November of 2006 (A) and the bottom section of the slide (B) are shown. Light regions represent summer growth. The *E. lineolata* and *O. reflexa* shells were each 3 years of age.

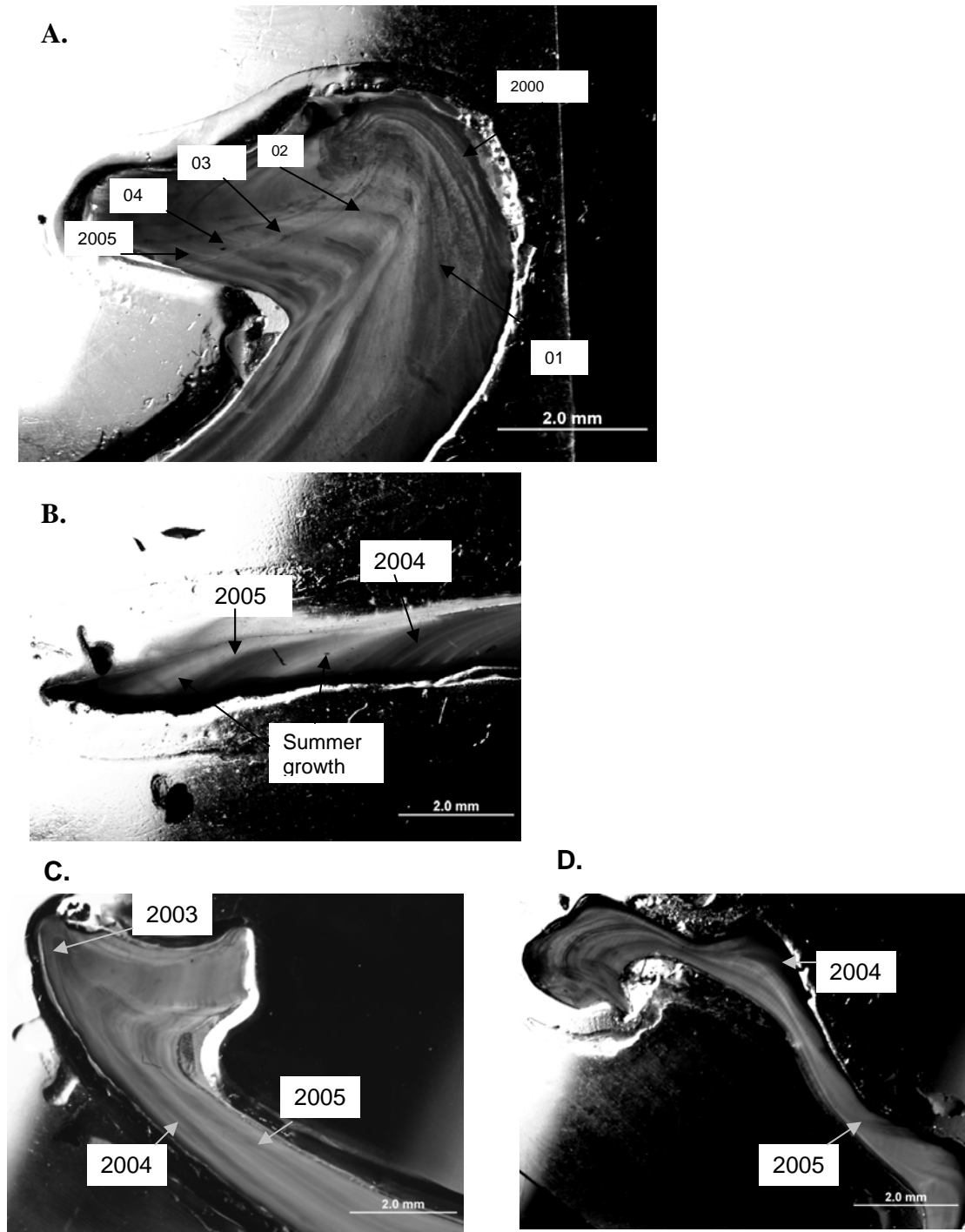
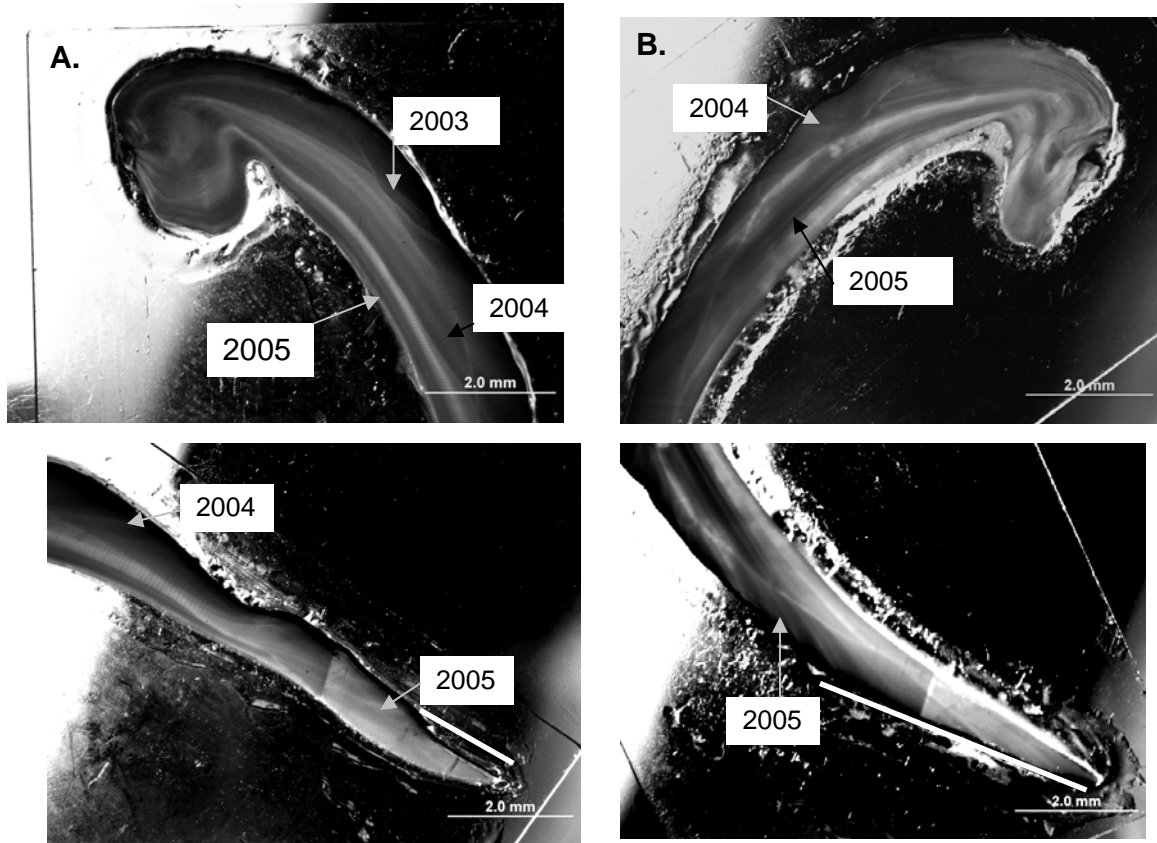


Figure 4. *Amblema plicata* sections of two individuals four (A) and three (B) years of age. Both died during the 2006 drought with the individual depicted in (A) collected in August 2006 and the individual depicted in (B) collected in November 2006. The white bars shown on the edge of the shells in the bottom two photos represent the growth after the last (2005) annuli.



In addition, the probability that an individual of a given height category was categorized into the wrong age category was incorporated into the 90% confidence intervals for the abundance estimates. The total number of individuals assigned to a given age category was multiplied by the probability that they were misclassified and actually belonged to an older or younger category. This effectively decreased the lower limit of the confidence intervals by subtracting individuals that could have potentially been placed in the wrong category based on the pre-determined age category error

probabilities. The upper limit of the interval and error probability for a given age class was used to determine the maximum gain to another age class. These gains were added to increase the upper limit for each confidence interval.

Site Comparisons

The abundance estimates and confidence intervals were used to compare the age distribution of individuals of a species among all four sites. To examine the prevalence of recent recruitment at each site I divided the data into two groups, individuals less than fifteen years of age and individuals fifteen years and older. This served to narrow the confidence intervals and allowed for a direct comparison between sites. In addition, I standardized the abundance estimates of each group by the number of possible 5-year age categories in the group. This allowed for the direct comparison by species and minimized the bias from an over representation of the long-lived species which contained more 5-year age classes. Differences in site abundance estimates were determined by comparing the 90 % confidence intervals.

Site 1: Mortality and Abundance Comparisons

The large number of deceased individuals due to exposure at Site 1 collected from August to November allowed for comparisons between the estimated population and mortalities based on age and size classes. Mortality estimates were conservative, limited to the number of shells collected on the bank. The mortality collection for *O. reflexa* (n=1153) exceeded the number of individuals I could age (n=100). For this species, I

divided un-aged individuals into height categories and used the probability classifications to assign individuals into age categories. I generated confidence intervals for this mortality collection by incorporating the aging error probabilities and potential losses and gains for each age class.

I determined the (conservative) percent mortality due to the low flow period from August to November for each age class where percent mortality was equal to the number of deaths divided by the abundance estimate. Low and high estimates for percent mortality based on the 90% confidence intervals were also generated.

Results

Age Determination

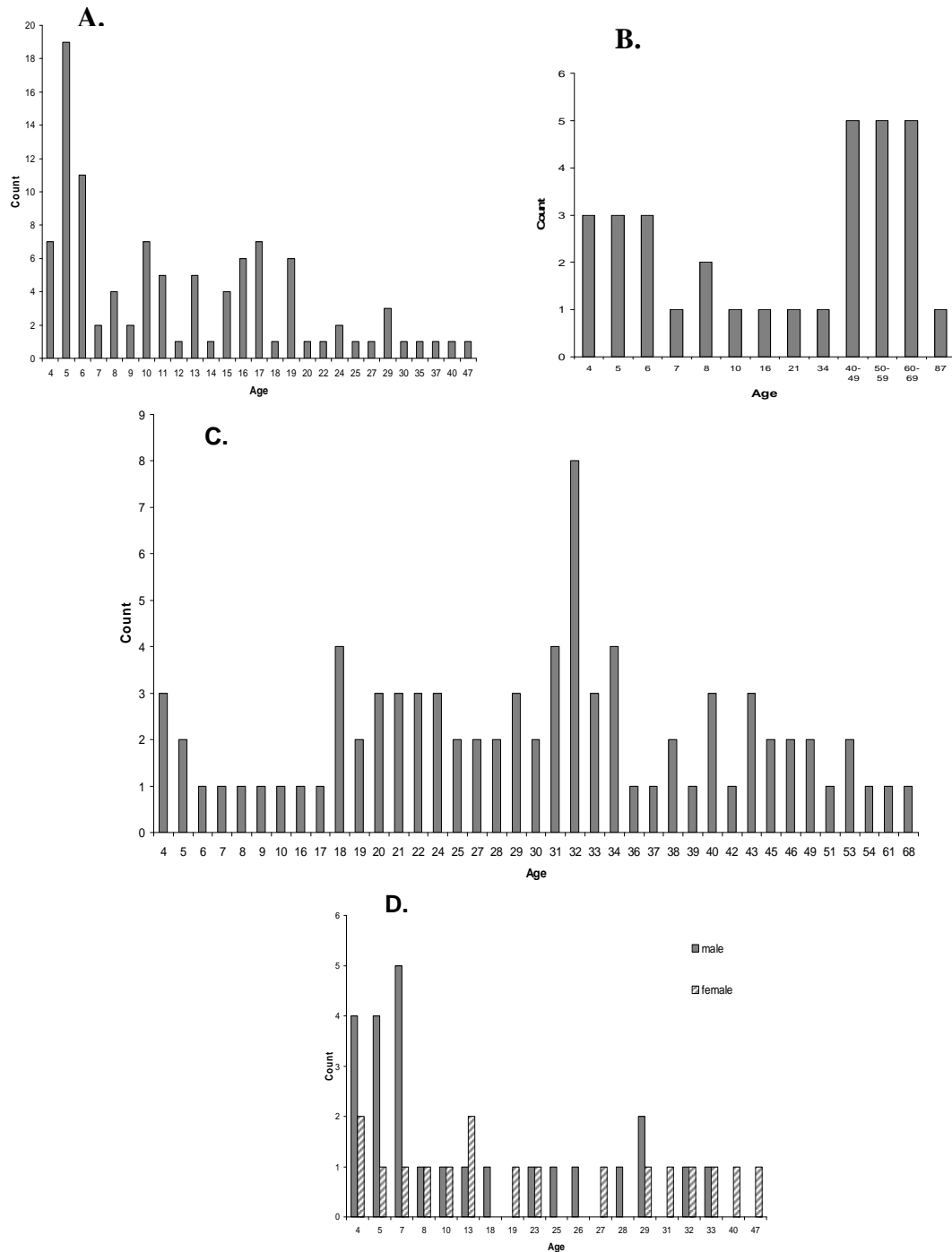
Obliquaria reflexa (n=100) ranged in age from 4 to 47 years old (Figure 5A). The ages indicate the age of the individual in the beginning of 2007 (a four year old individual would have three annuli, summer growth after the last annuli, and an estimated birth year of 2003). Only a random subset of the *O. reflexa* mortality collection was sectioned as 1153 individuals died from the drought in 2006 at Site 1.

It was difficult to age older *Amblema plicata* and a large majority of the individuals I collected from this species were old. Extreme shell weathering in the majority of these specimens led to poor-quality sections where the age could not be conclusively determined. I was able to age 32 individuals including all the smaller shells collected from Site 1 (Figure 5B). The oldest individual aged from this species was 87 years old.

Quadrula pustulosa (n=84) ranged in age from 4 to 68 years old (Figure 5C). I was unable to determine the ages of a few older weathered specimens of this species.

Ellipsaria lineolata was sexually dimorphic and I separated the males (n=25) and females (n=17) in the age distribution (Figure 5D). Five individuals were collected at Site 4. The oldest female was 47 years of age and the oldest male was 33 years of age. Noticeable gaps were evident in the distribution where no individuals were collected from certain years. In addition, I collected a greater percentage of young individuals of *E. lineolata* than the other species with 17 individuals less than or equal to 7 years of age.

Figure 5. Age distribution of thin-sectioned shells where age could be successfully determined. The majority of shells were collected from Site 1 during a low water period from August to November 2006. A: *Obliquaria reflexa* thin-sectioned individuals were randomly selected from 1153 mortalities (n=100). B: The *Amblema plicata* distribution represents only those individuals where age could be determined (n=32). C: The *Quadrula pustulosa* and *Ellipsaria lineolata* distributions were more reflective of the entire mortality collection of these species where only a few older individuals were excluded from aging of *Q. pustulosa* (n=84). D: *Ellipsaria lineolata* could be separated into male (n=25) and female (n=17) distributions.



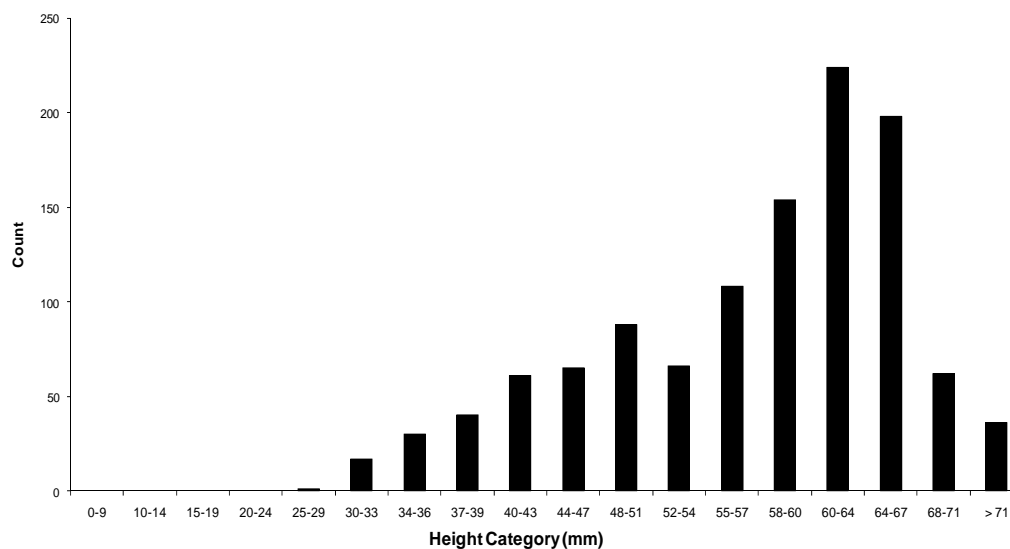
Age and Height Categories Determined from Mortalities

Obliquaria reflexa was divided into 18 height categories (Figure 6). The majority of mortalities were individuals between 58 and 67 mm in height. Individuals fell into 8 age categories with the maximum age of 47 (Table 1). To simplify analysis, I combined the last four age categories into one where all mussels 68 mm and over in length were assumed to be between 20 and 47 years of age.

Table 1. *Obliquaria reflexa* probability classification of age categories based on size ranges. Probabilities determined from actual ages of 100 shells and 590 length-at-age measurements.

Height Category (mm)	Age Category (years)				
	0-5	6-9	10-14	15-19	20-47
0-9	1	0	0	0	0
10-14	1	0	0	0	0
15-19	1	0	0	0	0
20-24	1	0	0	0	0
25-29	1	0	0	0	0
30-33	1	0	0	0	0
34-36	0.979	0.021	0	0	0
37-39	0.882	0.118	0	0	0
40-43	0.559	0.441	0	0	0
44-47	0.233	0.767	0	0	0
48-51	0.024	0.854	0.122	0	0
52-54	0	0.346	0.654	0	0
55-57	0	0.250	0.700	0.050	0
58-60	0	0	0.556	0.444	0
61-64	0	0	0.333	0.556	0.112
65-67	0	0	0	0.583	0.417
68-71	0	0	0	0	1
>71	0	0	0	0	1

Figure 6. Size frequency histogram of *Obliquaria reflexa* shells collected at Site 1. Shells were collected in August-November of 2006 over a period of low flow (N=1153).



Quadrula pustulosa was divided into the same 18 height categories as *O. reflexa* (Figure 7). The size frequency of the mortality collection was dominated by individuals between 60 and 71 mm in height. *Q. pustulosa* was a more long-lived species than *Obliquaria reflexa* with the maximum age of 68 for the specimens I aged. Individuals fell into fourteen age categories originally. I combined the older age categories into three groups: 20-34, 35-44, and 45-70 (Table 2).

Male *Ellipsaria lineolata* could be divided into 10 height categories (Figure 8) because all individuals less than 57 mm in lengths were 5 years old and younger. *E. lineolata* occurred less frequently overall in both the survey and mortality collection. Of the 21 (male) shells the majority were less than 84 mm in height. Male and female height and age categories were assessed separately because males exhibited a higher

Table 2. *Quadrula pustulosa* probability classification of age categories based on size ranges. Probabilities determined from actual ages of 84 specimens and 592 length-at-age measurements.

Height Category (mm)	Age Category (years)						
	0-5	6-9	10-14	15-19	20-34	35-44	45-70
0-9	1	0	0	0	0	0	0
10-14	1	0	0	0	0	0	0
15-19	1	0	0	0	0	0	0
20-24	1	0	0	0	0	0	0
25-29	1	0	0	0	0	0	0
30-33	1	0	0	0	0	0	0
34-36	0.654	0.346	0	0	0	0	0
37-39	0.429	0.571	0	0	0	0	0
40-43	0.118	0.882	0	0	0	0	0
44-47	0	0.952	0.048	0	0	0	0
48-51	0	0.703	0.297	0	0	0	0
52-54	0	0.211	0.684	0	0.105	0	0
55-57	0	0.071	0.714	0.071	0.142	0	0
58-60	0	0	0.250	0.500	0.250	0	0
60-64	0	0	0.214	0.143	0.572	0	0.070
65-67	0	0	0	0.091	0.697	0.151	0.060
68-71	0	0	0	0	0.357	0.429	0.212
>71	0	0	0	0	0	0.143	0.859

Figure 7. Size frequency histogram of *Quadrula pustulosa* shells collected at Site 1. Shells were collected in August-November of 2006 over a period of low flow (n=84).

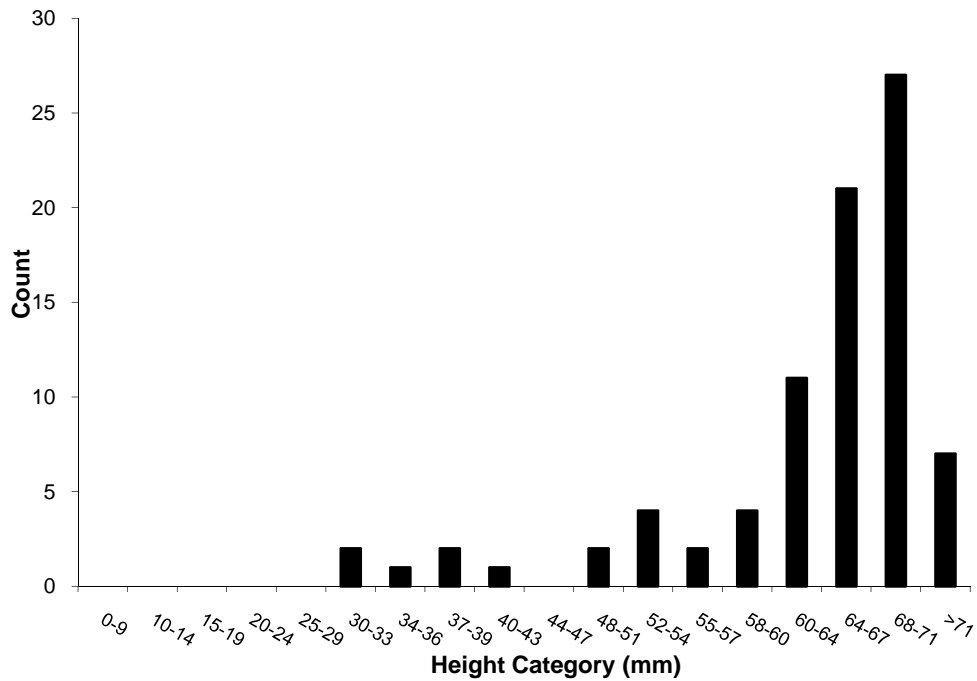


Table 3. *Ellipsaria lineolata* probability classification of age categories based on size ranges. Probabilities determined from actual ages and length-at-age measurements of 25 male specimens (A) and 17 female specimens (B) (number of measurements=132 and 107).

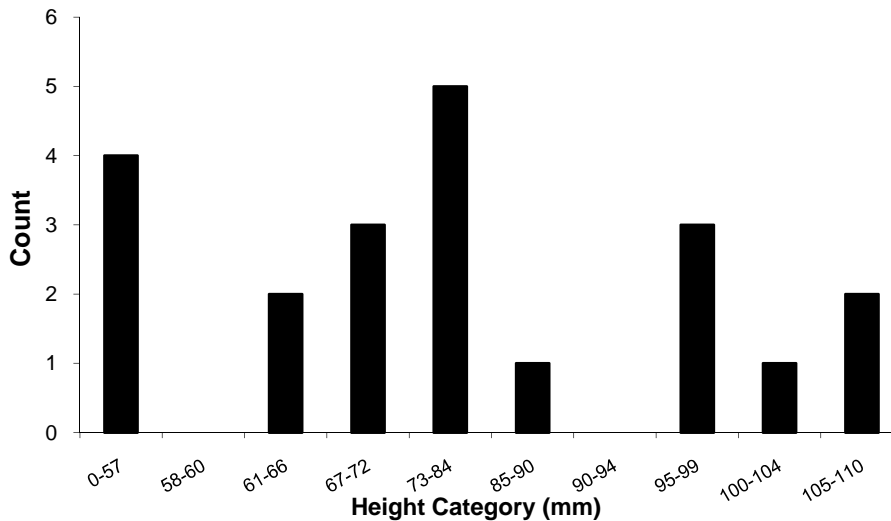
A. Male

Height Category (mm)	Age Category (years)				
	0-5	6-9	10-14	15-19	20-34
0-57	1	0	0	0	0
58-60	0.889	0.111	0	0	0
61-66	0.909	0.091	0	0	0
67-72	0.546	0.455	0	0	0
73-84	0.077	0.923	0	0	0
85-90	0	0	1	0	0
90-94	0	0	0	1	0
95-99	0	0	0	0	1
100-104	0	0	0	0	1
105-110	0	0	0	0	1

B. Female

Height Category (mm)	Age Category (years)				
	0-5	6-9	10-14	15-19	20-47
0-42	1	0	0	0	0
43-48	0.722	0.278	0	0	0
49-52	0.250	0.750	0	0	0
53-59	0.053	0.526	0.421	0	0
60-64	0	0.333	0.667	0	0
65-70	0	0.333	0.667	0	0
71-75	0	0	0	0.250	0.750
76-80	0	0	0	0	1
81-90	0	0	0	0	1

Figure 8. Size frequency histogram for male *Ellipsaria lineolata* shells collected at Site 1. Shells collected in August-November of 2006 over a period of low flow (n=21).



growth rate (Table 3). The oldest male aged was 33 and the oldest female aged was 47. I combined the last groups to create a 20-47 year group which exclusively contained males over 95 mm in height and females over 76 mm in height.

Only 32 *Amblema plicata* were aged because of the low quality of thin-sections. Unlike the other species, I could include several individuals from Site 3 and Site 4 into estimates. Typically, individuals over 15 years old were difficult to accurately age. All individuals less than or equal to 50 mm were 5 years old or younger. I divided the individuals into 11 height categories (Figure 9). The majority of mortalities from Site 1 were individuals that exceeded 100 mm in height. According to my age estimates, these individuals exclusively fell into a large age category of 15 to 85 years (the maximum age I could determine from the aged shells). The other individuals fell into three younger 4- or 5-year age categories (Table 4).

Figure 9. Size frequency histogram for *Amblyma plicata* shells collected at Site 1. Shells collected in August-November of 2006 over a period of low flow (n deaths) = 61.

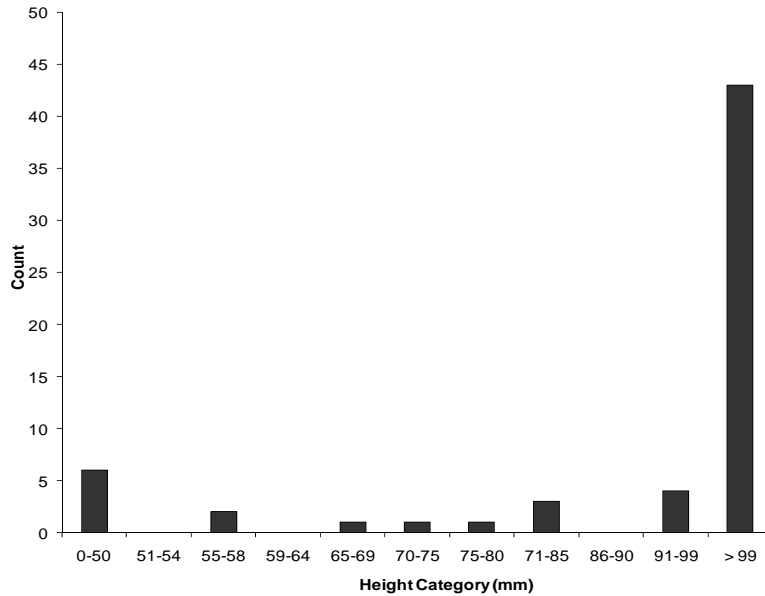


Table 4. *Amblyma plicata* probability classification of age categories based on size ranges. Probabilities determined from actual ages of 32 specimens and 109 length-at-age measurements.

Height Category (mm)	Age Category (years)			
	0-5	6-9	10-15	15-85
0-50	1	0	0	0
51-54	0.667	0.333	0	0
55-58	0.286	0.714	0	0
59-64	0	1	0	0
65-69	0	1	0	0
70-75	0	1	0	0
75-80	0	1	0	0
81-85	0	0	1	0
86-90	0	0	1	0
91-99	0	0	1	0
≥100	0	0	0	1

Site Comparisons

Site 1 contained the greatest abundance of *Obliquaria reflexa* (Table 5). It was the only site where I found individuals less than 36 mm in height. Site 1 contained a higher number of individuals in the 1-5 and 10-14 year categories than the other three sites (Figure 10A, Table 6A).

Site 1 also contained the greatest number of *Quadrula pustulosa* in the survey (Table 7) and was the only site containing individuals less than 40 mm of length. The majority of individuals at the other three sites was greater than or equal to 68 mm, whereas comparatively few in these two height categories were found at Site 1. Site 1 contained a higher number of individuals in the 1-5 year category than the other three sites (Figure 10B, Table 6B). Sites 2 and 3 contained more individuals 45 years of age and older than Sites 1 and 4.

Ellipsaria lineolata was not found at Site 2 and was rare at Site 4 with only 4 individuals collected (Table 8). Site 1 was the only site where *E. lineolata* in the two smallest height categories were found. Two females were found at Site 3 and one female was found at Site 4. All sites were also missing individuals in the 10-19 year age range (Figure 10C, Table 6C). *E. lineolata* at Site 4 were all 20 years or older. No differences between Sites 1 and 3 were found in the abundance estimates.

Table 5. Counts of *Obliquaria reflexa* by size class collected during surveys at the four sites.

Height Category (mm)	Site 1	Site 2	Site 3	Site 4
0-9	0	0	0	0
10-14	0	0	0	0
15-19	0	0	0	0
20-24	0	0	0	0
25-29	2	0	0	0
30-33	5	0	0	0
34-36	4	0	0	0
37-39	4	0	1	1
40-43	6	1	1	1
44-47	4	2	2	0
48-51	10	2	2	0
52-54	13	0	0	2
55-57	19	0	4	1
58-60	10	2	2	2
61-64	13	1	6	2
65-67	5	1	0	1
68-71	1	6	2	2
>71	0	3	1	4
Total	96	18	21	16

I collected the greatest number of *Amblema plicata* at Sites 3 and 4 (Table 9).

Only two individuals at Site 4 were less than 100 mm in height and few were found under this height at the other sites. I found the greatest number of individuals under 100 mm in height at Site 1. Site 3 contained more individuals over 15 years of age (Figure 10D, Table 6D). The abundance estimates at Site 1 were higher for the three younger age classes than the abundance estimates at the other sites but not outside of the 90 % confidence intervals.

Figure 10. Abundance estimates by age category at all four sites (Site 1=grey bars, Site 2=diagonal line bars, Site 3=white bars, Site 4=horizontal line bars). 90% confidence intervals incorporate categorizing errors. Aged species include: A. *Obliquaria reflexa*, B. *Quadrula pustulosa*, C. *Ellipsaria lineolata*, D. *Amblema plicata*.

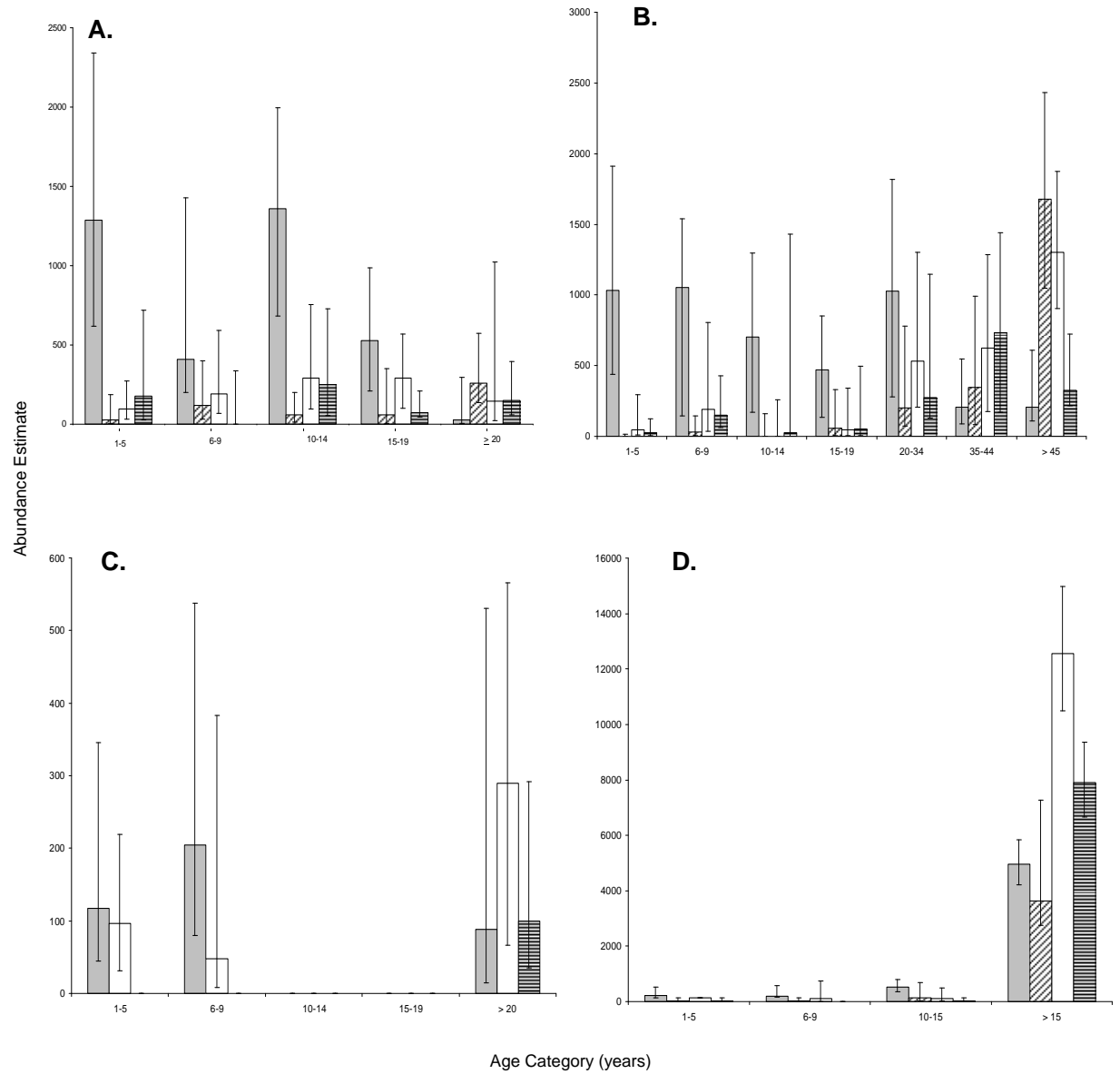


Table 6. Output of density and abundance estimates generated by the Mussel Estimation Program for each aged species at the four sites proportioned into age categories. The probability of miscategorizing an individual or aging error was incorporated into the 90% confidence intervals.

A. *Obliquaria reflexa*

Site	Age Category	Density (per m ²)	SE	CV	90%CI	Abundance Estimate	SE	90% CI	90%CI + Aging error
1	1-5	0.472	0.164	0.348	0.266-0.837	1288	448.78	726-2285	617-2342
	6-9	0.150	0.049	0.326	0.088-0.257	410	133.60	240-701	200-1426
	10-14	0.497	0.078	0.156	0.385-0.643	1357	211.69	1050-1754	684-1994
	15-19	0.193	0.043	0.221	0.134-0.278	527	116.63	366-758	207-987
	> 19	0.011	0.011	0.954	0.002-0.054	29	28.77	6-147	6-296
2	1-5	0.010	0.010	0.960	0.002-0.049	29	28.37	6-145	3-188
	6-9	0.039	0.025	0.650	0.013-0.114	116	75.07	40-336	32-400
	10-14	0.020	0.010	0.491	0.009-0.044	58	28.37	26-130	14-199
	15-19	0.020	0.019	0.983	0.004-0.098	58	56.75	11-291	6-349
	> 19	0.088	0.034	0.393	0.046-0.168	260	102.16	136-496	136-573
3	1-5	0.032	0.016	0.495	0.014-0.072	97	47.75	43-218	31-274
	6-9	0.063	0.031	0.495	0.028-0.143	193	95.5	86-436	70-592
	10-14	0.095	0.039	0.414	0.048-0.188	290	119.85	147-572	96-753
	15-19	0.095	0.027	0.286	0.06-0.152	290	82.71	181-463	100-567
	> 19	0.048	0.055	1.156	0.007-0.319	145	167.31	22-969	22-1021
4	1-5	0.097	0.088	0.910	0.022-0.433	176	160.43	39-788	28-718
	6-9	0	0	0	0	0	0	0	0-336
	10-14	0.137	0.088	0.642	0.048-0.395	250	160.43	87-718	54-726
	15-19	0.041	0	0	0.041-0.041	75	0	75-75	46-210
	> 19	0.083	0.046	0.556	0.033-0.207	151	83.74	60-376	60-397

B. *Quadrula pustulosa*

Site	Age Category	Density (per m ²)	SE	CV	90%CI	Abundance Estimate	SE	90% CI	90%CI + Aging error
1	1-5	0.378	0.127	0.336	0.218-0.657	1033	346.62	595-1794	531-1908
	6-9	0.386	0.035	0.091	0.332-0.448	1054	95.98	907-1224	757-1538
	10-14	0.257	0.043	0.166	0.196-0.338	703	116.63	535-923	375-1296
	15-19	0.172	0.035	0.205	0.122-0.240	468	95.98	334-656	167-852
	20-34	0.375	0.073	0.193	0.273-0.516	1025	198.06	746-1408	483-1816
	35-44	0.075	0.025	0.334	0.043-0.130	205	68.40	118-355	51-546
	45-70	0.075	0.035	0.468	0.035-0.162	205	95.98	95-443	81-609
2	1-5	0	0	0	0	0	0	0	0-17
	6-9	0.010	0.010	0.983	0.002-0.049	29	28.37	6-145	5-145
	10-14	0	0	0	0	0	0	0	0-161
	15-19	0.020	0.019	0.983	0.004-0.098	58	56.75	11-291	5-333
	20-34	0.068	0.030	0.439	0.033-0.141	202	88.79	98-416	71-780
	35-44	0.117	0.040	0.343	0.067-0.206	347	118.87	197-609	85-992
	45-70	0.567	0.109	0.192	0.414-0.777	1678	321.41	1224-2299	1049-2434

table 6 cont.

3	1-5	0.016	0.016	0.990	0.003-0.081	48	47.75	9-246	9-293
	6-9	0.063	0.055	0.867	0.015-0.264	193	167.31	46-803	36-803
	10-14	0	0	0	0	0	0	0	0-257
	15-19	0.016	0.016	0.990	0.003-0.081	48	47.75	9-246	5-342
	20-34	0.175	0.055	0.315	0.104-0.293	531	167.31	316-891	204-1300
	35-44	0.206	0.055	0.267	0.133-0.320	627	167.31	405-973	174-1284
	45-70	0.429	0.055	0.128	0.347-0.529	1303	167.31	1055-1609	904-1874
4	1-5	0.014	0.014	0.980	0.003-0.069	25	24.60	5-126	5-126
	6-9	0.083	0.041	0.490	0.037-0.185	151	73.80	67-337	61-427
	10-14	0.014	0.033	2.384	0-0.696	25	59.84	0-1267	0-1432
	15-19	0.028	0.033	1.192	0.004-0.196	50	59.84	7-357	4-494
	20-34	0.152	0.033	0.217	0.106-0.217	276	59.84	193-394	127-1148
	35-44	0.403	0.150	0.373	0.218-0.744	733	273.50	397-1354	171-1441
	45-70	0.179	0.027	0.151	0.140-0.230	326	49.20	255-418	219-722

C. *Eliipsaria lineolata*

Site	Age Category	Density (per m ²)	SE	CV	90%CI	Abundance Estimate	SE	90% CI	90%CI + Aging error
1	1-5	0.043	0.025	0.584	0.016-0.112	117	68.40	45-306	43-346
	6-9	0.075	0.043	0.569	0.029-0.191	205	116.63	80-523	74-538
	10-14	0	0	0	0	0	0	0	0
	15-19	0	0	0	0	0	0	0	0
	20-47	0.032	0.035	1.093	0.005-0.194	88	95.98	15-530	15-530
3	1-5	0.032	0.016	0.495	0.014-0.072	97	47.75	43-218	31-218
	6-9	0.048	0.027	0.571	0.019-0.122	145	82.71	57-371	8-383
	10-14	0	0	0	0	0	0	0	0
	15-19	0	0	0	0	0	0	0	0
	20-47	0.063	0.042	0.655	0.022-0.186	193	126.34	66-566	66-566
4	1-5	0	0	0	0	0	0	0	na
	6-9	0	0	0	0	0	0	0	na
	10-14	0	0	0	0	0	0	0	na
	15-19	0	0	0	0	0	0	0	na
	20-47	0.055	0.036	0.648	0.019-0.160	100	65.08	35-292	na

D. *Amblema plicata*

Site	Age Category	Density (per m ²)	SE	CV	90%CI	Abundance Estimate	SE	90% CI	90%CI + Aging error
1	1-5	0.086	0.043	0.498	0.038-0.195	234	116.63	103-531	92-531
	6-9	0.075	0.011	0.140	0.060-0.095	205	28.77	163-258	163-575
	10-15	0.193	0.049	0.254	0.127-0.293	527	133.60	347-800	347-800
	16-85	1.816	0.182	0.100	1.540-2.141	4957	496.20	4204-5844	4204-5844
2	1-5	0.010	0.010	0.983	0.002-0.049	29	28.37	6-145	na
	6-9	0.010	0.010	0.983	0.002-0.049	29	28.37	6-145	na
	10-15	0.049	0.047	0.955	0.010-0.235	144	137.87	30-694	na
	16-85	1.229	0.208	0.169	0.930-1.624	3639	616.21	2754-4807	na
3	1-5	0.048	0	0	0.048-0.048	145	0	145-145	na

table 6 cont.

	6-9	0.032	0.039	1.242	0.004-0.245	97	119.85	13-744	na
	10-15	0.032	0.031	0.990	0.006-0.162	97	95.50	19-491	na
	16-85	4.127	0.446	0.108	3.454-4.931	12546	1356.95	10501-14989	na
4	1-5	0.014	0.014	0.980	0.003-0.069	25	24.60	5-126	3-126
	6-9	0	0	0	0	0	0	0	0-2
	10-15	0.014	0.014	0.980	0.003-0.069	25	24.60	5-126	5-126
	16-85	4.345	0.448	0.103	3.666-5.149	7908	816.14	6673-9371	6673-9371

Table 7. Counts of *Quadrula pustulosa* by size class collected during surveys at the four sites.

Height Category (mm)	Site 1	Site 2	Site 3	Site 4
0-9	0	0	1	0
10-14	0	0	0	1
15-19	1	0	0	0
20-24	2	0	0	0
25-29	4	0	0	0
30-33	4	0	0	0
34-36	5	0	0	0
37-39	4	0	0	0
40-43	14	1	2	0
44-47	11	0	0	5
48-51	7	0	2	1
52-54	10	0	0	0
55-57	14	0	0	1
58-60	16	2	1	2
60-64	22	1	6	6
65-67	13	6	5	5
68-71	7	12	13	24
>71.5	7	56	27	13
Total	141	78	57	58

Table 8. Counts of *Ellipsaria lineolata* by size class collected during surveys.
(No individuals were collected at Site 2.)

Male Height (mm)	Female Height (mm)	Site 1 (male)	Site 1 (female)	Site 1 (total)	Site 3 (male)	Site 3 (female)	Site 3 (total)	Site 4 (male)	Site 4 (female)	Site 4 (total)
0-57	0-42	2	0	2	0	0	0	0	0	0
58-60	43-48	1	0	1	0	0	0	0	0	0
61-66	49-52	1	0	1	1	0	1	0	0	0
67-72	53-59	0	0	0	1	0	1	0	0	0
73-84	60-64	6	0	6	1	0	1	0	0	0
85-90	65-70	1	0	1	0	0	0	0	0	0
90-94	71-75	0	0	0	0	1	1	0	0	0
95-99	76-80	2	0	2	2	1	3	1	1	2
100-104	81-90	0	0	0	0	0	0	2	0	2
105-110	na	1	0	1	2	0	2	0	0	0
Total		14	0	14	7	2	9	3	1	4

Table 9. Counts of *Amblyma plicata* by size class collected during surveys at the four sites

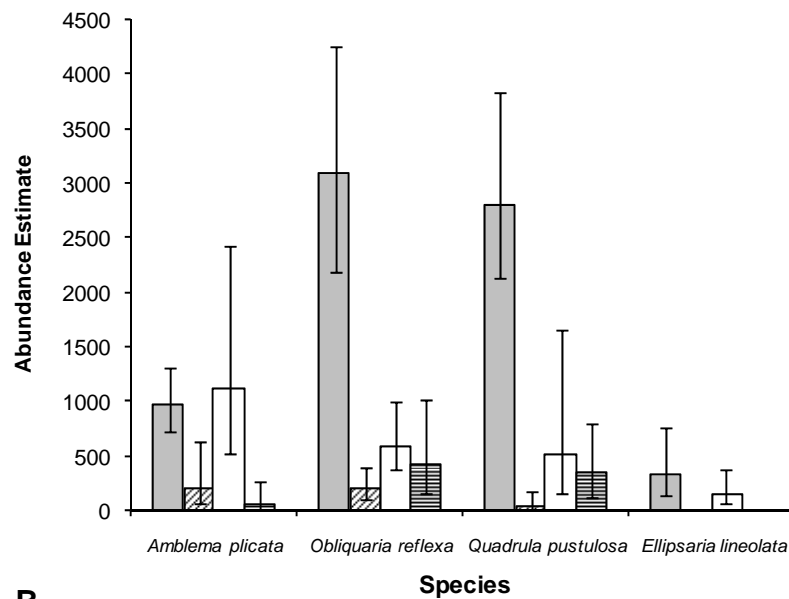
Height Category (mm)	Site 1	Site 2	Site 3	Site 4
0-50	6	1	3	0
51-54	3	0	0	1
55-58	0	0	0	0
59-64	1	0	0	0
65-69	1	0	2	0
70-75	4	0	0	0
75-80	1	1	0	0
81-85	4	1	0	0
86-90	4	0	1	0
91-99	11	4	1	1
≥100	159	126	258	315
Total	194	133	265	317

Site 1 contained higher numbers of *O. reflexa* and *Q. pustulosa* less than 15 years old (Figure 11A) than the other sites. Also, Sites 1 and 3 contained higher numbers of *A. plicata* less than 15 years old than the other two sites. For individuals older than 15, *A. plicata* was more abundant at Sites 3 and 4 (Figure 11B). The abundance of *Q. pustulosa* at Site 4 was slightly less than the estimates at the other three sites in this group.

When the groups were standardized by the number of 5-year age classes, Site 1 exhibited a higher abundance of individuals less than 15 years of age for *O. reflexa* and *Q. pustulosa* (Figure 12A). The abundance estimates for *A. plicata* and *E. lineolata* were not different from each other in the two groups. At Site 2, the abundance estimates for *A. plicata* and *Q. pustulosa* were higher in the older group (Figure 12B). The abundance estimates of the two groups were similar to each other at Site 3 in all species except *A. plicata* where there were more individuals exceeding age 15 (Figure 12C). At Site 4, *A. plicata* exceeding age 15 abundance was higher than the abundance of *A. plicata* under 15 (Figure 12D).

Figure 11. Abundance estimates by species at each site for two age categories: **A.** Individuals younger than 15 years and **B.** Individuals older than 15 years (Site 1=grey bars, Site 2=diagonal stripe bars, Site 3=white bars, Site 4=horizontal line bars).

A.



B.

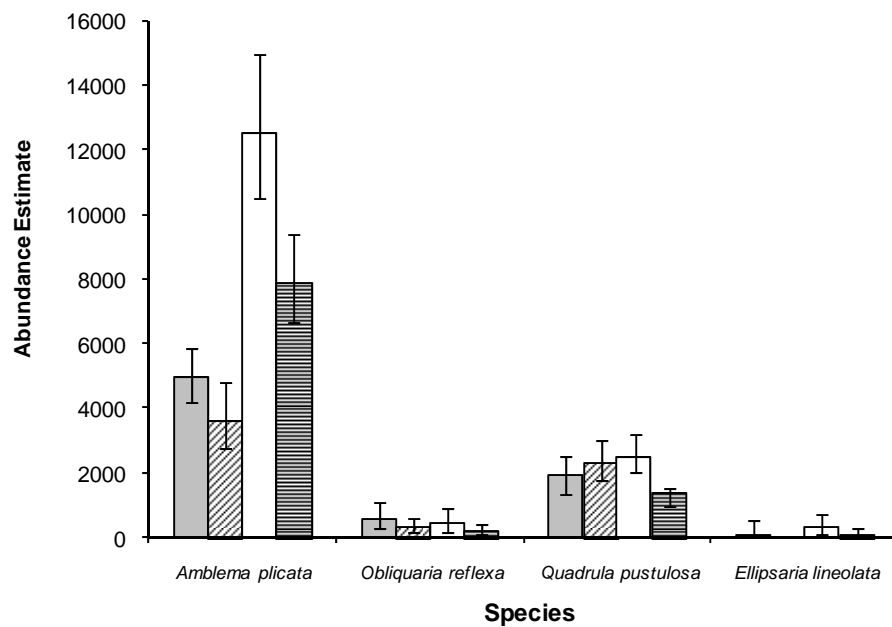
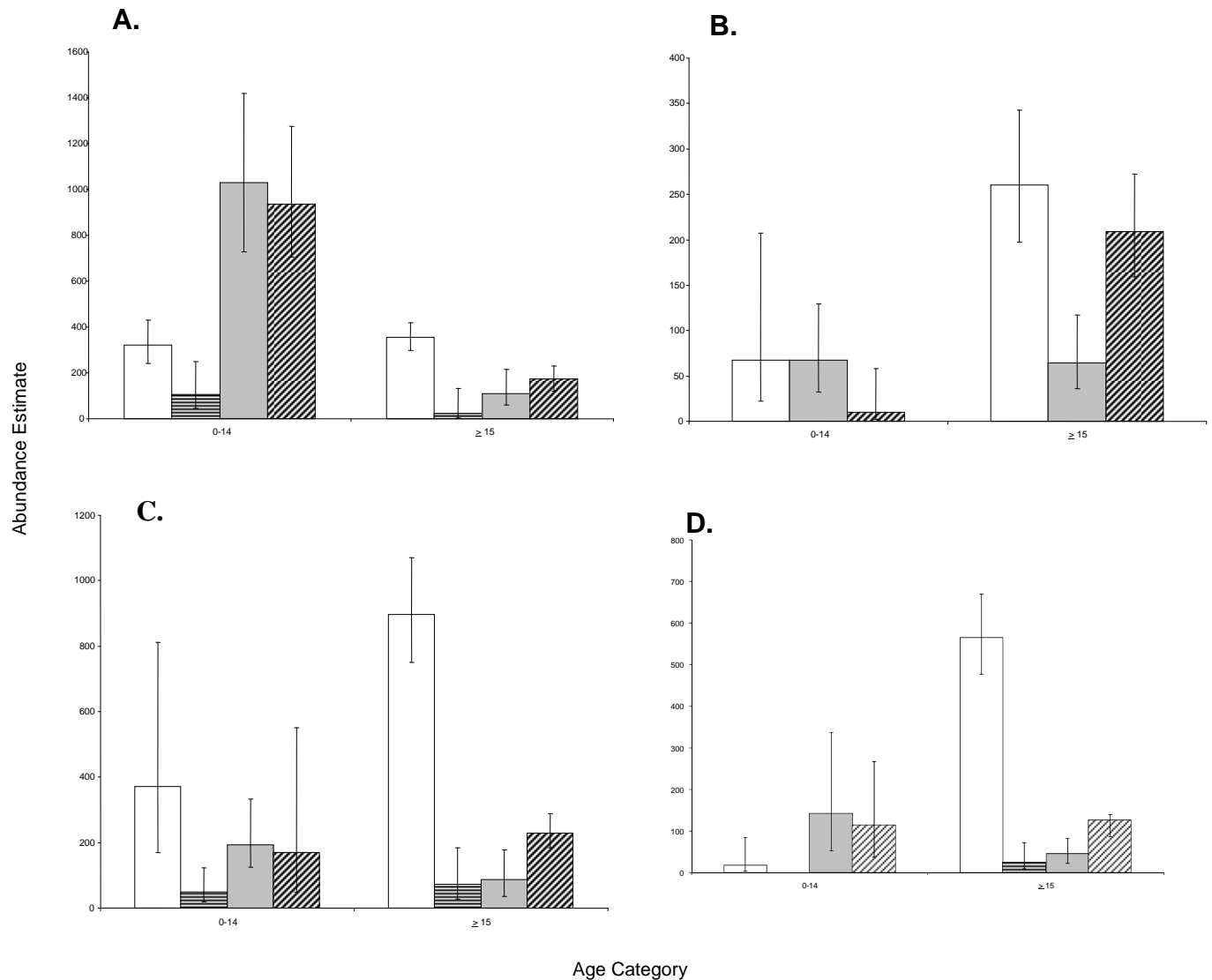


Figure 12. Standardized abundance comparisons between two age groups: individuals less than 15 years of age and individuals 15 years of age and older for each species (*Amblema plicata*=white bars, *Ellipsaria lineolata*= horizontal striped bars, *Obliquaria reflexa*=grey bars, and *Quadrula pustulosa*=diagonal stripe bars). The four sites displayed are Site 1 (A), Site 2 (B), Site 3 (C), and Site 4 (D). Abundance estimates were standardized by estimated number of five-year age categories for each species [Number of categories in each group: all species (<15) n=3, *A. plicata* (≥ 15) n=14, *E. lineolata* (≥ 15) n=4, *Obliquaria reflexa* (≥ 15) n=5, *Q. pustulosa* (≥ 15) n=11].



Assessment of Low Flow Period

From June through November 2006, 94% of days had a flow lower than the median daily discharge on record and 60% of days had a flow lower than the 25th percentile on record (Figure 13, Table 10). A large percentage of fresh dead shells were collected when discharge was above 25 cubic feet per second. I found 1790 stranded mussels at three sites over this time period. The majority of the mortality was observed at Site 1 where 1560 mussels were stranded (Table 11A). *Obliquaria reflexa* comprised 75% of the stranded individuals at Site 1. The species with the highest estimated mortality at Site 1 were *Potamilus ohiensis*, *Obliquaria reflexa*, *Truncilla truncata*, and *Leptodea fragilis* (Table 11B). The 90% confidence interval of *Obliquaria reflexa* mortality was 23%-37%.

Fewer shells containing soft-tissue were collected at Sites 3 and 4 (Table 11, C and D). *Amblema plicata* comprised approximately 51% and 43% of the stranded shells respectively at Sites 3 and 4. Estimated mortality rate at Site 3 was less than 1% of the abundance estimate for each species except *Potamilus ohiensis*.

Figure 13. Figure reproduced from United States Geological Survey Surface-Water Daily Data for the Nation, USGS 06916600 Marais des Cyges River near KS-MO State Line, Kansas (accessed: <http://waterdata.usgs.gov/nwis/dv?> in April 2007). Graph displays daily mean discharge from June 1, 2006 to December 1, 2006 and median daily discharge in the past 49 years from June 1 to December 1. Discharge is measured in cubic feet per second.

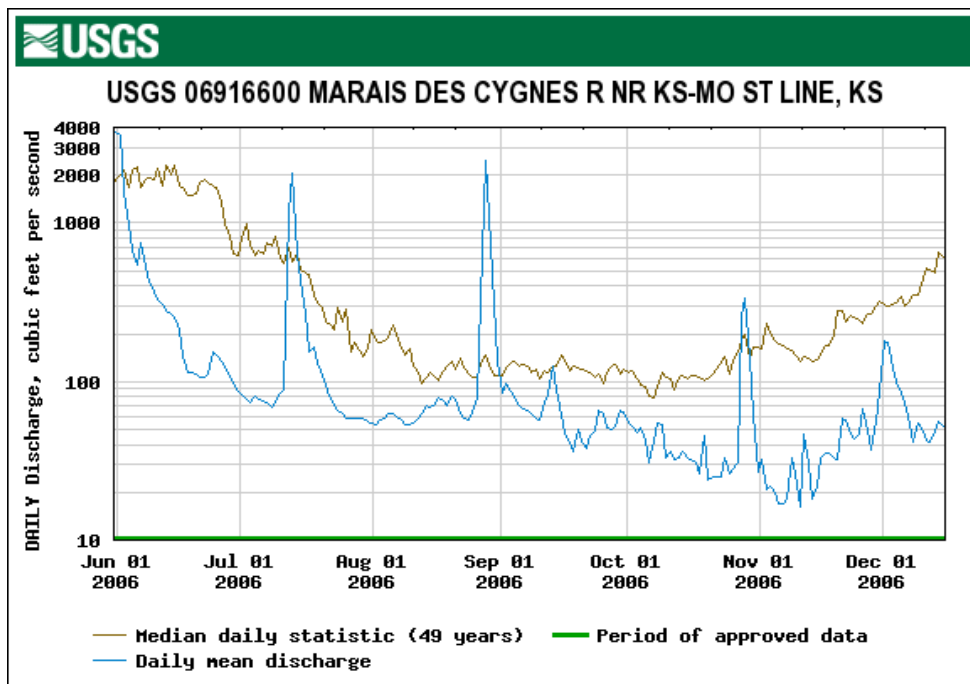


Table 10. Statistics (mean, median, and minimum) of discharge (cubic feet per second) during the collection of shells at Site 1 in 2006. Site 1 was surveyed from 6/20 – 6/29 with no observed mortalities. Fresh-dead shells were collected on 8/2 (n=308), 10/15 (n=934), 10/21 (n=116), 11/5 (n=156), and 11/11 (n=46).

	Time period				
	7/2 - 8/1	8/2 -10/14	10/15 -10/20	10/21 -11/4	11/5 - 11/11
Mean Discharge (cfs)	210.13	114.11	31.83	56.05	21.00
Median Discharge (cfs)	77	59.5	31.5	29	18
Minimum Discharge (cfs)	54	31	24	21	15

Table 11. Shells collected at three sites on the Marais des Cygnes River from 8/2/2006-11/11/2006. Species are listed in order of relative abundance within the total collection of shells. Site 1 (A) was visited on five different dates and numbers represent counts collected on each date. Percent mortality (B) at Site 1 was estimated from total mortalities and the abundance estimates generated from sampling efforts in late June 2006 (see Chapter 2). Site 3 (C) was visited on 10/21 and abundance estimates were generated from sampling efforts in late July 2006. Site 4 (D) was visited on 8/18 and 11/12. Percent mortality was not calculated at Site 4 because sampling to determine Site 4 abundance occurred on 8/18, while the shells were collected. Total abundance estimates include the abundance of species not found in shell collection.

A.

Species	Count					Total	Relative Abundance (%)
	8/2	10/15	10/21	11/5	11/11		
<i>Obliquaria reflexa</i>	225	758	103	64	21	1171	75.06
<i>Quadrula pustulosa</i>	31	34	2	32	6	105	6.73
<i>Amblema plicata</i>	12	25	1	12	11	61	3.91
<i>Leptodea fragilis</i>	4	16	1	11	3	35	2.24
<i>Ellipsaria lineolata</i>	3	22	1	8	1	35	2.24
<i>Tritogonia verrucosa</i>	9	13	0	4	1	27	1.73
<i>Truncilla donaciformis</i>	1	18	4	2	0	25	1.6
<i>Quadrula quadrula</i>	2	16	1	3	0	22	1.41
<i>Truncilla truncata</i>	6	11	1	0	1	19	1.22
<i>Fusconaia flava</i>	3	6	0	8	0	17	1.09
<i>Potamilus alatus</i>	3	11	0	1	1	16	1.03
<i>Potamilus ohioensis</i>	3	3	2	4	0	12	0.77
<i>Pleurobema sintoxia</i>	3	1	0	7	0	11	0.71
<i>Megalanaia nervosa</i>	1	0	0	0	1	2	0.13
<i>Lampsilis teres</i>	2	0	0	0	0	2	0.13
Total	308	934	116	156	46	1560	

B.

Species	Abundance Estimate	Abundance Estimate after Low Water Period	Percent Mortality Estimate	90% Confidence Level of Mortality Estimate
<i>Obliquaria reflexa</i>	3972	2801	29.48	23.3 - 37.3
<i>Quadrula pustulosa</i>	5057	4952	2.08	1.70 - 2.53
<i>Amblema plicata</i>	6338	6277	0.96	0.82 - 1.13
<i>Leptodea fragilis</i>	169	134	20.71	6.24 - 68.63
<i>Ellipsaria lineolata</i>	410	375	8.54	3.35 - 21.74
<i>Tritogonia verrucosa</i>	227	200	11.89	4.86 - 29.03
<i>Truncilla donaciformis</i>	256	231	9.77	4.15 - 22.94
<i>Quadrula quadrula</i>	863	841	2.55	1.71 - 3.79
<i>Truncilla truncata</i>	88	69	21.59	6.01 - 76.00
<i>Fusconaia flava</i>	861	844	1.97	1.15 - 3.39
<i>Potamilus alatus</i>	117	101	13.68	5.23 - 35.56
<i>Potamilus ohioensis</i>	29	17	41.38	8.11 - 100
<i>Pleurobema sintoxia</i>	710	699	1.55	0.87 - 2.77
<i>Megalanaia nervosa</i>	117	115	1.71	0.65 - 4.44
<i>Lampsilis teres</i>	na	na	na	na
Total	19273	17713	8.1	7.10-9.23

C.

Species	10/21 Count	Relative Abundance (%)	Abundance Estimate	Percent Mortality	90% Confidence Level of Mortality Estimate
<i>Amblema plicata</i>	24	51.06	17886	0.13	0.11 - 0.16
<i>Megaloniaias nervosa</i>	6	12.77	1351	0.44	0.32 - 0.61
<i>Quadrula pustulosa</i>	4	8.51	4391	0.09	0.07 - 0.11
<i>Potamilus alatus</i>	3	6.38	639	0.47	0.33 - 0.66
<i>Leptodea fragilis</i>	2	4.26	241	0.83	0.28 - 2.44
<i>Ellipsaria lineolata</i>	2	4.26	941	0.21	0.10 - 0.44
<i>Potamilus ohiensis</i>	2	4.26	48	4.17	0.07 - 100.00
<i>Obliquaria reflexa</i>	1	2.13	2280	0.04	0.04 - 0.05
<i>Tritogonia verrucosa</i>	1	2.13	224	0.45	0.14 - 1.43
<i>Fusconaia flava</i>	1	2.13	1918	0.05	0.04 - 0.06
<i>Pleurobema sintoxia</i>	1	2.13	591	0.17	0.11 - 0.26
Total	47		31360	0.15	0.13 - 0.17

D.

Species	8/18 Count	11/12 Count	Total	Relative Abundance (%)
<i>Amblema plicata</i>	62	15	77	42.08
<i>Megaloniaias nervosa</i>	55	16	71	38.8
<i>Quadrula pustulosa</i>	4	8	12	6.56
<i>Ellipsaria lineolata</i>	5	2	7	3.83
<i>Potamilus alatus</i>	2	2	4	2.19
<i>Fusconaia flava</i>	3	1	4	2.19
<i>Tritogonia verrucosa</i>	2	1	3	1.64
<i>Obliquaria reflexa</i>	1	1	2	1.09
<i>Quadrula quadrula</i>	0	1	1	0.55
<i>Leptodea fragilis</i>	0	1	1	0.55
<i>Pleurobema sintoxia</i>	1	0	1	0.55
Total	135	48	183	

Site 1 Abundance and Mortality Comparisons among Age Classes

Obliquaria reflexa abundance estimates were higher than mortalities for the three younger age categories (less than 15 years old). There was no observable difference between the mortality estimate and abundance estimate for the two older age groups (Figure 14, Table 12). The estimated percent mortality increased with age ranging from 11.57% in the youngest category to 100% in the oldest category. The low estimate for percent mortality within the 20-47 year group was slightly over 1/3 of the Site 1 population.

Quadrula pustulosa abundance estimates were higher than mortalities in each age group (Figure 15, Table 13). Percent mortality was lower overall than *O. reflexa*. A similar trend occurred where percent mortality tended to increase with age past age 14 and was highest for individuals over 20 years of age.

Ellipsaria lineolata abundance estimates were higher than mortalities in three age groups (Figure 16, Table 14). No female or male individuals between the ages of 10 and 19 were found in the survey. The mortality collection contained 16 females and 2 male individuals in the 10 to 19 age range.

Figure 14. Age frequency histogram by category for *Obliquaria reflexa* at Site 1. Abundance estimated from systematic survey data collected in June 2006 (light bars). Mortalities (grey bars) collected in August-November of the same year over a period of low flow (survey n=96, mortalities n=1153). Confidence intervals incorporate error based on the probabilities of a shell of a certain length falling into a particular age class and 90% confidence intervals associated with the abundance estimate.

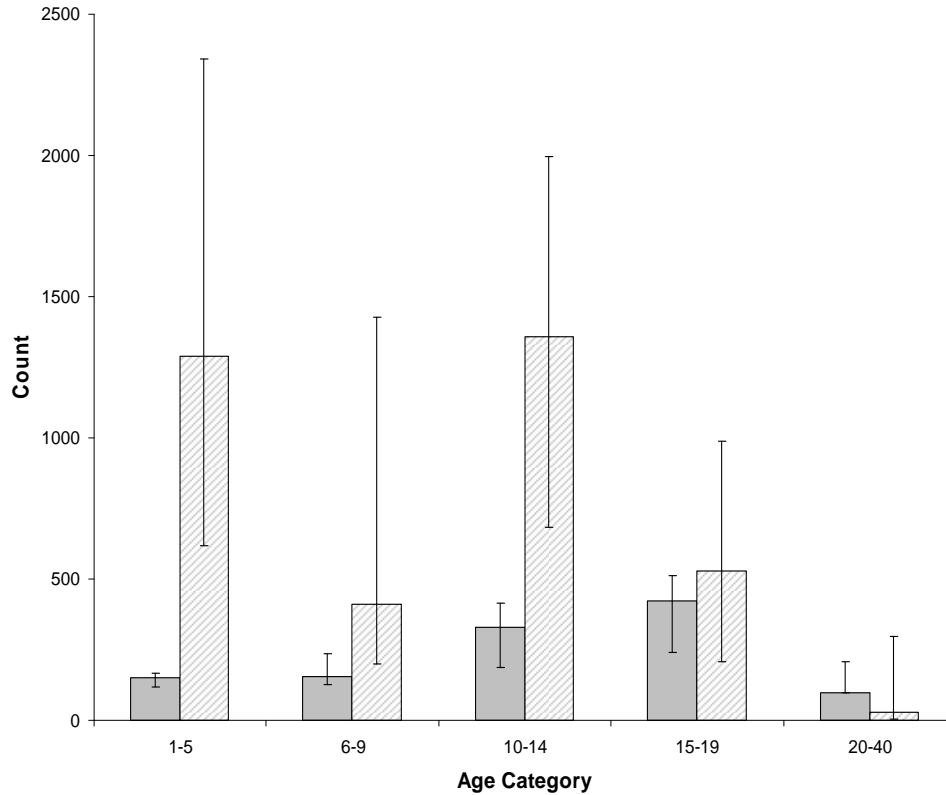


Table 12. *Obliquaria reflexa* age-specific abundance estimates determined from the high density stratum at Site 1 (June 2006). The number of deaths and percent mortality occurred over the low flow period from August-November of the same year. Death confidence intervals incorporated aging error probabilities.

Years	Age Category	Abundance Estimate	90% CI	Deaths	CI (Age error)	% Mortality	% Mortality CI
2001-2006	0-5	1288	617-2342	149	117-166	11.57	5 - 26.9
1997-2000	6-9	410	200-1426	153	125-235	37.32	8.77 - 100
1992-1996	10-14	1357	684-1994	328	187-414	24.17	9.38 - 27.34
1987-1991	15-19	527	207-987	422	239-513	80.08	24.21 - 100
1959-1986	20-47	29	6-296	98	98-206	100	33.11 - 100

Figure 15. Age frequency histogram by age category for *Quadrula pustulosa* at Site 1. Abundance (light bars) estimated from systematic survey data collected in June 2006 (survey n=141). Mortalities (grey bars) were collected in August-November of the same year over a period of low flow (n=84). Confidence intervals incorporate error based on the probabilities of a shell of a certain length falling into a particular age class and 90% Confidence intervals associated with the abundance estimate.

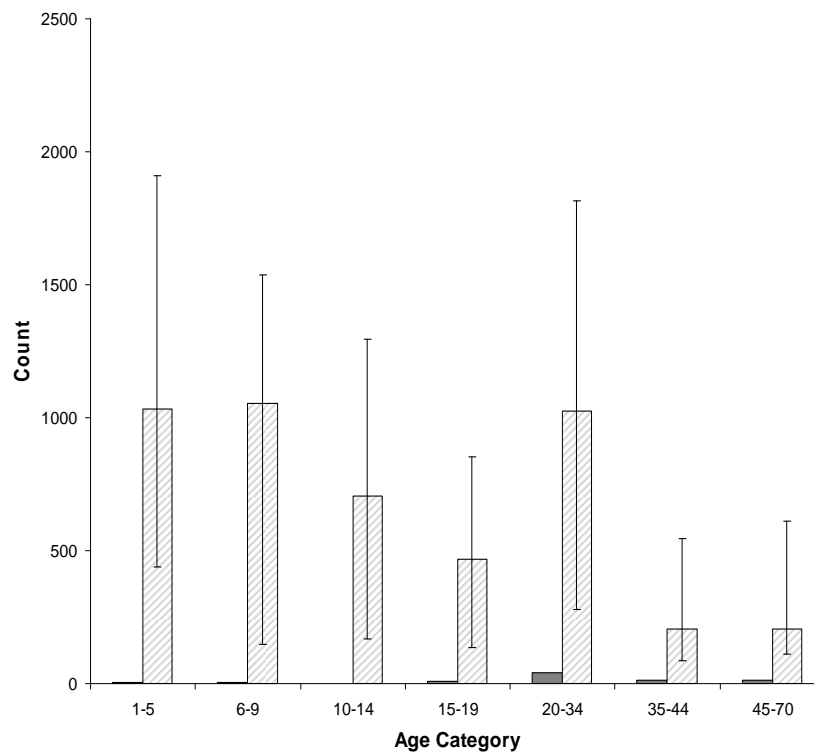


Table 13. *Quadrula pustulosa* age-specific abundance estimates determined from the high density stratum at Site 1 (June 2006). The number of deaths and percent mortality occurred over the low flow period from August-November of the same year.

Years	Age Category	Abundance Estimate	90% CI	Deaths	% Mortality	% Mortality CI
2001-2006	0-5	1033	531-1908	5	0.48	0.26-0.94
1997-2000	6-9	1054	757-1538	4	0.38	0.26-0.53
1992-1996	10-14	703	375-1296	1	0.14	0.08-0.27
1987-1991	15-19	468	167-852	8	1.71	0.94-4.79
1972-1986	20-34	1025	483-1816	42	4.1	2.31-8.70
1962-1971	35-44	205	51-546	12	5.85	2.20-23.53
1936-1961	45-70	205	81-609	12	5.85	1.97-14.81

Figure 16. Age frequency histogram by category for male *Ellipsaria lineolata* at Site 1. Abundance (light bars) were estimated from systematic survey data collected in June 2006 (n=14). Mortalities (grey bars) were collected in August-November of the same year over a period of low flow (n=21). Confidence intervals incorporate error based on the probabilities of a shell of a certain length falling into a particular age class and the 90% confidence intervals associated with the abundance estimate.

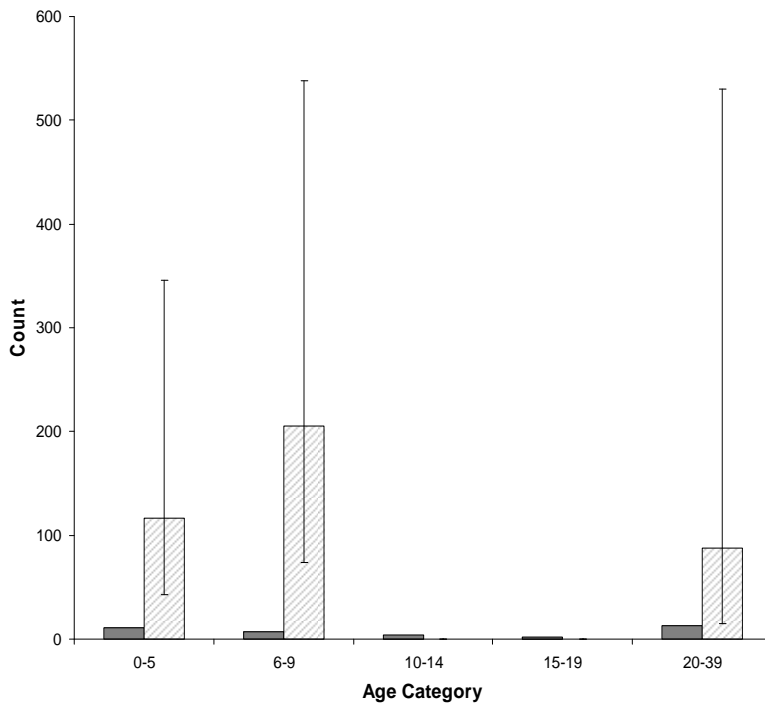


Table 14. *Ellipsaria lineolata* age-specific abundance estimates determined from the high density stratum in Site 1 (June 2006). The number of deaths and percent mortality occurred over the low flow period from (August-November) of the same year.

Years	Age Category	Abundance Estimate	90% CI	Deaths (female)	Deaths (male)	% Mortality	% Mortality CI
2001-2006	0-5	117	43-346	3	8	9.4	3.18-25.58
1997-2000	6-9	205	74-538	1	6	3.41	1.30-9.46
1992-1996	10-14	0	0	3	1	0	0
1987-1991	15-19	0	0	1	1	0	0
1967-1986	20-39	88	15-530	8	5	14.77	2.45-86.67

Figure 17. Age frequency histogram by category for *Amblema plicata* at Site 1. Abundance (diagonal stripe bars) was estimated from systematic survey data collected in June 2006 (survey n=194). Mortalities (solid bars) were collected in August-November of the same year over a period of low flow (n=61). Confidence intervals incorporate error based on the probabilities of a shell of a certain length falling into a particular age class and the 90% confidence intervals associated with the abundance estimate.

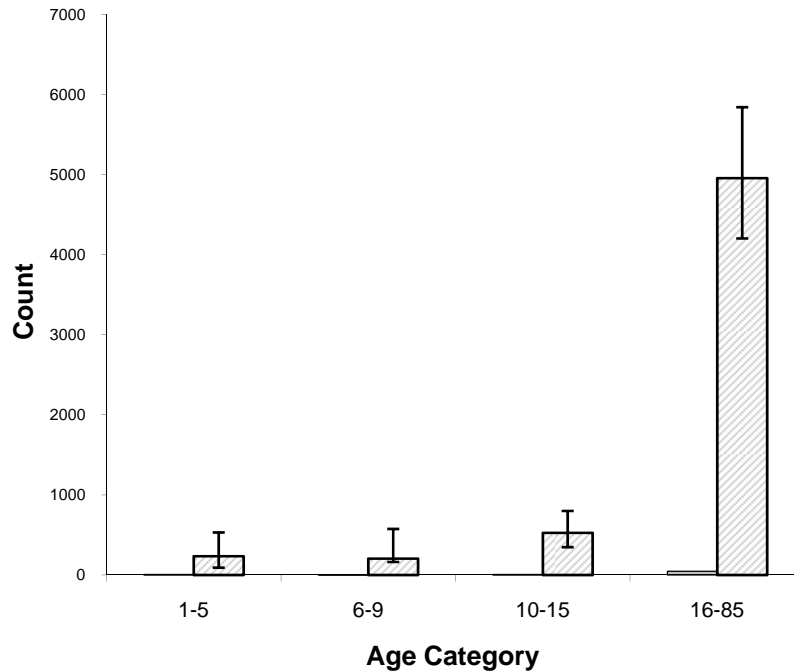


Table 15. *Amblema plicata* age specific abundance estimates determined from the high density stratum at Site 1 (June 2006). The number of deaths and percent mortality occurred from August-November of the same year.

Years	Age Category	Abundance Estimate	90% CI	Deaths	% Mortality	% Mortality CI
2001-2006	1-5	234	92-531	5	2.14	0.94-5.43
1997-2000	6-9	205	163-575	4	1.95	0.7-2.45
1991-1996	10-15	527	347-800	6	1.14	0.75-1.73
1921-1990	16-85	4957	4204-5844	46	0.93	0.79-1.09

Percent mortality was estimated to be highest in the youngest (0-5 year) and oldest (20-39) age category. I did not consider individuals between 10 and 19 year of age because of low samples size.

Amblyma plicata abundance estimates were higher than mortalities in the four age categories at Site 1 (Figure 17, Table 15). The abundance estimate for individuals over 16 was significantly higher than the three younger age categories. Unlike the other species, percent mortality was estimated to be highest in the youngest age category (1 to 5 years) and decreased with age. *Amblyma plicata* percent mortality overall was comparatively low.

Discussion

This study demonstrates that recruitment has occurred recently on the Marais des Cygnes River in all four mussel species that were sampled. The majority of recent recruits were found at Site 1. Site 1 contained more *O. reflexa* and *Q. pustulosa* under 15 years of age than individuals 15 years and older. Additionally, Site 1 contained more individuals less than 15 years of age of these two species than the other sites. There were no differences between sites in the 15 years of age and older category of these two species. The age class analysis revealed that Site 1 also had a greater estimated number of individuals in the three youngest cohorts than the other sites. The total abundance estimates for *O. reflexa* and *Q. pustulosa* were significantly greater at Site 1. The discrepancy in abundance of these two species between sites can be explained by the higher numbers of younger (< 15 years old) individuals at Site 1. These results indicate

that there are differences in recruitment success between mussel beds and that the abundances of *O. reflexa* and *Q. pustulosa* at Sites 2-4 may decline with mortality.

There are several possible scenarios that could account for the higher recruitment success at Site 1. Both species are tachytictic and are reported to be gravid from June to August (Howard 1914, Utterback 1915). Several host fish species are listed for *Q. pustulosa* (Appendix: Table 1, Fuller 1974). The host fish is unknown for *O. reflexa* and it has been suggested that parasitism may not be required (Utterback 1915, Oesch 1984). Because of the proximity of the sites where a host fish with glochidia attached could conceivably migrate between sites, the most likely scenarios would involve the part of the life cycle where juveniles drop from the gills of the host fish and settle into the substrate. There are no tributaries connecting to the stretch of the river where the four sites are located and discharge should be similar at all sites. At Site 1, the mussel bed may be better sheltered from high flows and shear stress during flood events and more suitable for juvenile settlement and survivorship than the other sites. Increased shear stress in the boundary layer in certain locations during high flows is believed to impact juvenile survivorship by displacing juveniles downstream or scouring the substrate where juveniles may be located (Layzer and Madison 1995, Hardison and Layzer 2001, Howard and Cuffey 2006). The habitat and availability of food at Site 1 may also be more favorable to the fish host species and a disproportionate number of infected fish may be aggregating at the site.

The high density stratum abundance estimates of *A. plicata* (Chapter 1) indicated that *A. plicata* was more abundant at Site 3 than the other sites. The total abundance estimate at Site 4 was higher than the abundance at Site 1 but not outside of the

confidence intervals. Site 2 had the lowest estimate of *A. plicata* abundance. The abundance estimates of *A. plicata* less than or equal to 15 years of age were higher at Sites 1 and 3 than the other sites but there was no difference between estimates at the two sites. Abundance estimates of *A. plicata* greater than 15 years of age were significantly higher at Sites 3 and 4. The aging data indicate that *A. plicata* is a more long-lived species than the other aged species but it was difficult to create high quality thin sections and conclusively age older specimens. The oldest individual aged was 87 years old but it is probable that the maximum age for *A. plicata* is higher. When estimates were standardized according to the estimated number of 5-year intervals in each age category and assuming a maximum age of 85, the abundance of *A. plicata* greater than or equal to 15 years of age was higher than the abundance of *A. plicata* less than 15 years of age at Sites 2-4. There was no difference between the two groups at Site 1.

A. plicata recruitment is low at Site 4. The high abundance of *A. plicata* at this site was estimated to be comprised of mainly older individuals at least 15 years of age. As these individuals die, *A. plicata* abundance will decline at this site without the recruitment of juveniles into the population. *A. plicata* recruitment at Site 2 was lower than Sites 1 and 3 when considering the presence of individuals less than 10 years old. The number of *A. plicata* equal to or less than 15 years of age at Site 1 was lower than the estimates of *Q. pustulosa* and *O. reflexa* in this age category. If *A. plicata* are longer-lived than the other species, strong recruitment may be less critical to the population. Implicit in that assumption is that older females continue to contribute offspring and members of long-lived species are fecund for most of their lives. There are few studies that examine reproductive senescence in freshwater mussels but one study of length and

age-related fecundity of six species including *A. plicata*, *Q. pustulosa*, and *O. reflexa* suggests that although the rate of increase in fecundity declines with age, older individuals continue to produce a large quantity of offspring (Haag and Staton 2003). At Site 1 the lower *A. plicata* abundance estimates for the 1-5, 6-9, and 10-14 age categories than the other species may be sufficient to sustain a healthy population of *A. plicata* at the site.

E. lineolata abundance estimates were similar between Sites 1 and 3. The abundance estimate at Site 4 was lower than the other sites but within the margin of error. There was no evidence of *E. lineolata* recruitment at Site 4 and all individuals sampled were estimated to be 20 years of age or older. Evidence of recent recruitment was found at Sites 1 and 3 with individuals present in the two youngest age categories at both sites (1-5 years and 6-9 years). At both sites there was no difference between abundance of individuals less than 15 years of age and the abundance estimate of individuals 15 years of age and older. *E. lineolata* is a rare species on the Marais des Cygnes River. There is evidence of recruitment at the mussel beds in Sites 1 and 3 which are close in proximity. *E. lineolata* is bradyctictic and is gravid from August to June or July of the following year (Ortmann 1919, Oesch 1984). The presence of recruitment at two of the sites suggests that these sites may attract higher concentrations of infected host fish or have more favorable conditions for juvenile settlement and survivorship than the other sites.

Age-specific mortality from low water

Of four species examined, *O. reflexa* experienced the highest rate of mortality from the low water period (n=1153). The abundance of *O. reflexa* was estimated to be reduced by approximately 32% during this period (90% confidence interval: 25%-40%) at Site 1. The largest number of *O. reflexa* fresh-dead shells was found at Site 1 and comparatively few shells were found at the other sites. Percent mortality tended to be higher in older individuals of this species but the long period of low water affected all age classes.

Similarly, *Q. pustulosa* percent mortality was highest in individuals > 15 years old during the low flow period. The greatest number of *A. plicata* mortalities occurred in the 16-85 age class (n=46) but there were no differences in percent mortality among age classes. There were also no differences in *E. lineolata* mortalities among the age classes. The number of *E. lineolata* fresh-dead shells was 35 and represented 9% of the estimated *E. lineolata* abundance at Site 1 (90% confidence interval: 4%-23%).

The estimates of mortality caused by the low flow period are conservative and limited to visible fresh dead shells either on the bank or in the shallow water adjacent to the bank. The low flow period may have caused additional physiological stress to individuals at Site 1 associated with decreased respiration and growth. Initially, decreases in depth and water velocity reduce levels of oxygen and food in the water column. Prolonged exposure to drought conditions cause mussels to face increasing water temperature, hypoxia, and eventually exposure to air or stranding (Golladay et al 2004). Several studies have indicated that there are various species-specific adaptations

that have evolved which allow mussels to survive in drought conditions (Holland 1991, White 1979), and that species vary in tolerance to drought stress (Golladay et al 2004).

The mortality data at Site 1 indicate that *O. reflexa* was particularly susceptible to stress from the low water. There also were differences in drought tolerance among age classes in *O. reflexa* and *Q. pustulosa* with the older age classes experiencing higher mortality. The findings emphasize the need for the maintenance of adequate flow and continued monitoring of the mussel beds on the Marais des Cygnes River. Site 1 supports a diverse assemblage of species and age classes. However, a trade-off may be occurring at this site where shallow water habitat conditions provide refugia from high water events which enhance host fish densities and/or juvenile survivorship but render the mussel bed more susceptible to higher temperatures and dewatering during long periods of low flow. Impacts of long periods of low flow on Site 1 are particularly important because it supports a higher abundance of recent recruits in the species I examined than the other sites. To insure that the mussel population on the refuge section of the Marais des Cynges River is viable in the long-term, mussel beds that support recruitment should be closely monitored and the conditions that may have lead to higher levels of recruitment at these locations should be investigated.

Chapter 4. A Comparison of Recent Recruitment Events and Annual Growth Patterns with Annual Discharge and Temperature

Introduction

A goal of this study was to determine if historical recruitment trends are related to annual discharge fluctuations. The majority of freshwater mussel species have a unique life cycle that involves parasitism on a host fish (Coker et al. 1921). The cycle begins when sperm is released by males and becomes entrained in the water column. The sperm must reach the incurrent siphon of the female mussel. The sperm is passed through the incurrent siphon and retained in the gills where fertilization occurs. The fertilized egg is retained in brood pouches located in the gills. Potential bottlenecks at this stage include a low number of reproductive adults or uneven sex ratio. Mussels which are highly clustered may have an advantage with finding a mussel of the opposite sex in close vicinity (Strayer et al. 2004). Flow extremes may present a problem as the velocity should be adequate to allow the flow to entrain the sperm but if it is too high, there is a potential for the entrained sperm to not reach nearby female mussels (Payne and Miller 2000).

Mature glochidia are released into the water column by most mussels and must come into contact with a host fish. They can be released singly or in groups depending on the species. Glochidia which do not contact a suitable host fish within a few days will die or if they attach to an improper host species may be sloughed off. In addition researchers have reported that certain host fish previously infected with glochidia appear to develop an immunity to re-infection (Reuling 1919, Arey 1924, Bauer and Vogel 1987). The number of glochidia produced by a single female may vary from 75,000 to 3

million (Coker 1921 et al., Kat 1984) and survival during this time is extremely low (Young and Williams 1984).

The next stage occurs when the newly formed juvenile mussel excysts off the host fish and drops to the bottom of a lake or stream. The juvenile must fall into suitable habitat and there are several bottlenecks associated with this stage.

A juvenile will not survive if it falls into an area such as a floodplain that may become dry during low flows. Juveniles may become displaced if high velocities impede them from burrowing into the substrate or attaching to sediment with a byssal thread (Holland-Bartels 1990) and mortality may be higher in locations that would be subject to high shear stresses acting on the substrate (Yeager et al 1994, Layzer and Madison 1995).

Discharge patterns may influence recruitment in several ways. Lower velocity may facilitate fertilization as sperm is released by males into the water column and drawn in by the incurrent siphon of nearby females (Yokely 1972, Payne and Miller 2000). High velocity events may have a positive or negative influence on the behavior of host fish depending on the species. High discharge in the spring may attract spawning aggregations of certain host fish species while causing emigration and juvenile mortality of others (Howard and Cuffey 2006, Payne and Miller 2000). Researchers have suggested that low velocity is favorable during settlement when the newly metamorphosed juvenile drops from the host fish into the water column (Holland-Bartels 1990, Payne and Miller 2000, Howard and Cuffey 2006). Payne and Miller (2000) identified a complex spring hydrograph pattern that only occurred in two years from 1977 through 1996 in the lower Ohio River. Demographically complete sampling of *Fusconaia ebena* from 1983 through 1998 revealed two successful recruitment years, the

same years that reflected the complex discharge pattern. Specifically, brief periods of below average discharge in early April and May when it is believed fertilization occurs were followed by rapid rises in discharge which may have attracted spawning aggregations of the host fish *Alosa chrysochloris*. Rapid declines in discharge occurred in late June favoring juvenile settlement.

Howard and Cuffey (2006) observed that during periods of low flow the pearl mussel (*Margaritifera margaritifera*) recruited more successfully. Hardison and Layzer (2001) observed that unnaturally high discharge during spring and early summer could result in higher shear stresses exerted on mussel beds at the time of juvenile exystment in lampsiline species in Kentucky. Early season breeders had lower recruitment success than amblemine species that bred later in the year when flows were typically lower on average.

Although demographically complete sampling over a long time period is not possible in the study, successful recruitment years can still be identified by examining the current age-structure of the population (Howard and Cuffey 2006). A complication in using the current demographic make-up to assess recruitment is accounting for the influence of mortality on cohort structure. Researchers have found that mortality is higher in older individuals (>20 years) of the pearl mussel *M. margaritifera* (Bauer 1983, Howard and Cuffey 2006). In younger mussels, nearly all mortality occurs in the early life stages when the individual is a glochidium larva or when the metamorphosed juvenile excysts off the fish host (Payne and Miller 2000, Hardison and Layzer, 2001). For the purpose of this study, I assumed mortality rate was not drastically influenced by age for the first 10 to 15 years of life (with the exception of age-0 mussels). By applying this

assumption to our sample from the population, I determined whether annual recruitment was constant or if certain year classes in the last fifteen years were disproportionately represented or missing in the population. I then examined the effect of temperature and discharge during the breeding season on the recruitment success of several species. I also examined annual growth variation for the four aged species to determine whether growth was correlated with discharge and temperature.

Materials and Methods

Frequencies for each annual spawning event were calculated two ways: 1) Age frequencies were directly determined from the mortality collection at Site 1 and 2) Age frequencies were determined by application of an accurate size-at-age model to sizes (heights) measured in the field from the survey at Site 1. A chi-square analysis was performed to examine if counts were proportioned evenly among years or if there were significant differences in annual recruitment. Growth and age frequencies were examined for correlations with both temperature and discharge during the spawning season for the four species.

Growth Models

Height and age data for each individual and all back-calculated height and age data were plotted using TableCurve 2D v 5.01 (Systat Software Inc.). The program generated the best-fitting model and appropriate parameter estimates based on the data (*Obliquaria reflexa* N=596, *Quadrula pustulosa* N=569, male *Ellipsaria lineolata*

N=130, female *Ellipsaria lineolata* female N=104, *Amblyma plicata* N=98). Because of the large sample size, outliers could be removed for the *Obliquaria reflexa* and *Quadrula pustulosa* models and the models were refit to include only data less than three standard deviations from the best-fit estimate. Outliers were not removed from the *Amblyma plicata* and *Ellipsaria lineolata* models due to smaller sample sizes.

The accuracy and error rate of each model were determined by applying the inverse of the model to the initial height measurements and generating the predicted age. Residuals were calculated by subtracting the predicted age from the actual age. Typically, the models were accurate through the first five years and accuracy decreased with age. I applied the models to heights measured in the field at Site 1. Because of the inaccuracy of the models when applied to older individuals, only the younger ages were considered (generally less than 15 years of age). The probability of an incorrect predicted age was directly incorporated into age frequency estimates to account for error. To accomplish this, individuals were assigned to ages based on model estimates. The probability that individuals actually belonged to a different year-class than what the model dictated was used to reclassify a proportion of the individuals into the other year-class. Finally, I determined the proportion of individuals over the range of years examined that was born in each year. This variable was used in further analysis.

The mortality collection was analyzed over the range of ages selected. The proportion of individuals born in each year was determined over the specified range. For *Obliquaria reflexa*, the entire mortality collection of individuals less than 15 years of age could not be aged directly. The shells were measured and the *Obliquaria reflexa* growth model was applied to the data to assign age and error probabilities. Both survey and

mortality recruitment values were examined for equal proportions among years by a chi-square test for *O. reflexa*, *Q. pustulosa*, and *A. plicata*. I did not analyze *E. lineolata* recruitment based on the survey collection because of the relatively low sample size of butterfly under 10 years of age in this collection (N=9) where only a few years were represented and the lower accuracy of the growth model. The mortality collection contained a larger sample size of individuals under ten years of age (N=21) and was used in further analysis.

Determination of Annual Growth Variation

To determine differences in growth between years, I considered each aged specimen separately. Typically younger individuals contained very clear internal annuli that could be traced from the umbo region of the shell to the exterior periostracum. Growth was higher in early years and could be determined by measuring the distance between annuli on the shell margin. For all growth measurements of a given species, mean growth and standard error were calculated for each one year growth interval. Generally more observations were gathered for the high growth during early years and fewer observations were gathered for later years where growth decreased significantly.

Each individual growth measurement, the year when the growth occurred, and the one year growth interval were examined to determine an annual growth parameter. I calculated the difference between each individual growth measurement and the mean growth over the specific one year interval. For each year, the average of the differences was determined and this value represented the growth parameter.

Analysis of the Relationship between Annual Growth and Recruitment with Temperature and Discharge

For each species a dataset was created which contained the year, recruitment frequency parameters (from mortality collection and survey collection when appropriate), the growth parameter, monthly mean discharge, and monthly mean air temperature. Monthly mean ambient air temperatures for each year were downloaded from NOAA, National Climatic Data Center, Asheville NC. Monthly mean discharges were downloaded from surface-water monthly statistics (USGS 06916600 Marais des Cygnes River near KS-MO state line). The USGS gage is located within the study reach. Because there are no major tributaries present along the reach from the gauging station to Site 1, the discharge values were assumed to accurately reflect on-site discharge.

Literature estimates pertaining to the possible breeding season for each species were examined. *Obliquaria reflexa*, *Quadrula pustulosa*, and *Amblema plicata* are considered tachytictic. The breeding season of *A. plicata* is reported to occur from June to August (Baker 1928, Frierson 1914, Ortmann 1912, Watters 1994). *Q. pustulosa* and *O. reflexa* were also reported to breed during these months (Howard 1914, Utterback 1915-1916). I averaged monthly discharge and temperature over this interval, July and August, and May through October. In addition, mean monthly discharges for each month throughout the year and mean annual discharge were also considered in the correlation analysis.

Ellipsaria lineolata, a bradytictic species is reported to be gravid from August to June or July of the following year (Ortmann 1919). The average monthly discharges and temperatures over July and August, June through August, May through October, and

August of the previous year were considered in addition to mean discharge and temperature for each month throughout the year.

The data were analyzed using SAS 9.1.3 Software (SAS Institute Inc., Cary, NC, USA). A chi-square test was performed on each species to test for equally proportioned recruitment among recent years. Counts derived from age frequencies generated from the survey and mortality collections were both used in the chi-square analysis for all species except *E. lineolata* which had a low sample size in the survey. Only the counts directly determined from the mortality collection were used to examine annual differences in *E. lineolata* recruitment. If there were significant differences in recruitment among recent years, the age frequency data were examined for correlations with discharge and temperature.

The discharge and temperature values were log transformed and a Pearson correlation matrix was constructed. I examined the results for significant correlations between growth, recruitment (when significant annual differences were apparent), discharge, and temperature. Next, variables with the strongest significant correlations were used in simple linear regression models to examine the relationship between year class strength (age frequency) and discharge or temperature.

If mean monthly discharge during the breeding season was significantly correlated with recruitment frequency, I created a new variable to further quantify the relationship. I calculated the median monthly discharge for each month based on daily discharge data from 45 years (1960 to 2005) as reported by the gaging station. The number of days during the breeding season (months with significantly correlated mean monthly discharges) with discharge less than the median discharge on record for each

specific month was determined. The relationship between recruitment frequency and the new variable (number of days with discharge below forty-five year median monthly discharge) was tested by simple linear regression. This new variable was created to assess the relationship between the duration of lower than average flows throughout the breeding season and recruitment. The magnitude of mean discharge may be disproportionately affected by extremely low flows when flow is actually highly variable. The new variable represented constant lower than average flow conditions throughout the breeding season.

Results

Obliquaria reflexa

Growth Model

The best-fitting model for *Obliquaria reflexa* was $\text{height}^{0.5} = 9.557 + -7.242/\text{age}^{0.5}$ (Figure 1, Table 1). The model fit the data well ($R^2 = 0.96$). The majority of data points were below 20 years of age. The model correctly predicted age 89.71% of the time when the predicted age was between 1 and 15 but only 12% of the time for ages greater than 15 (Table 1). Error rates were highest for ages 8, 10, and 11-15 of the 1-15 year old individuals but all estimates within this group correctly predicted age within one year.

Table 1. Table of Regression Coefficients (A) and ANOVA table (B) for the *Obliquaria reflexa* growth model ($\text{height}^{0.5} = a + b/\text{age}^{0.5}$). $R^2=0.96$, Adjusted $R^2=0.96$, and Fit SE=3.32

A.					
Parameter	Coefficient	SE	<i>t</i>	<i>P</i>	
a	9.557	0.068	140.112	<.0001	
b	-7.242	0.150	-48.413	<.0001	

B.					
Source	SS	df	MS	F	<i>P</i>
model	157511.700	1	157511.700	14288.202	<.0001
residual	6559.220	595	11.0		
total	164070.920	596			

Table 2. *Obliquaria reflexa* inverse model accuracy for determining age based on length of 596 observations. Includes the predicted age or range of ages and probabilities that the predicted value is correct (residual=0) or accurate within one year (residual= +/- 1).

Predicted Age	Ratio (Correct/Total)	Percent Correct	Percent Correct within 1 Year
1-15	497/554	89.71	100.00
16-51	5/42	12.00	47.00
1-4	234/234	100.00	100.00
5-6	91/108	84.26	100.00
7	18/18	100.00	100.00
8	22/23	95.65	100.00
9	8/8	100.00	100.00
10	9/16	56.25	100.00

Recruitment Frequencies

A large number of *Obliquaria reflexa* mortalities occurred and I directly determined the ages in the lab for 68 individuals between the ages of 3 and 15 (Table 3, Figure 2). For the remaining 490 individuals, I obtained height measurements and applied the growth model and error rates. The model and error rates were also applied to the 69 individuals I measured in the survey and the survey frequencies for individuals born in each year from 1992 to 2003 were calculated. The survey collection demonstrated a somewhat bimodal distribution with a high frequency of individuals born in 2000 and 1997 and a very low frequency of individuals born in 1996. The mortality

collection demonstrated a more even distribution of individuals born across years. The chi-squared nonparametric test on both the survey and mortality count data indicated that the null hypothesis of an even distribution of individuals among years over this time period could not be rejected (survey $X^2=13.88$, $df=11$, $P=0.24$; mortality $X^2=15.03$, $df=11$, $P=0.18$).

Figure. 1. *Obliquaria reflexa* growth model ($\text{height}^{0.5}=9.577 - 7.242 \text{ b/age}^{0.5}$). Graph indicates best fitting linear model of height vs. age with 90 % confidence intervals (black lines) and prediction intervals (grey lines). Ages determined from internal annuli and back-calculated heights at previous ages ($n=596$). Individual data points displayed are within 1 SD (black), 2 SD (dark grey), and 3 SD (light grey) of the model ($R^2=0.96$).

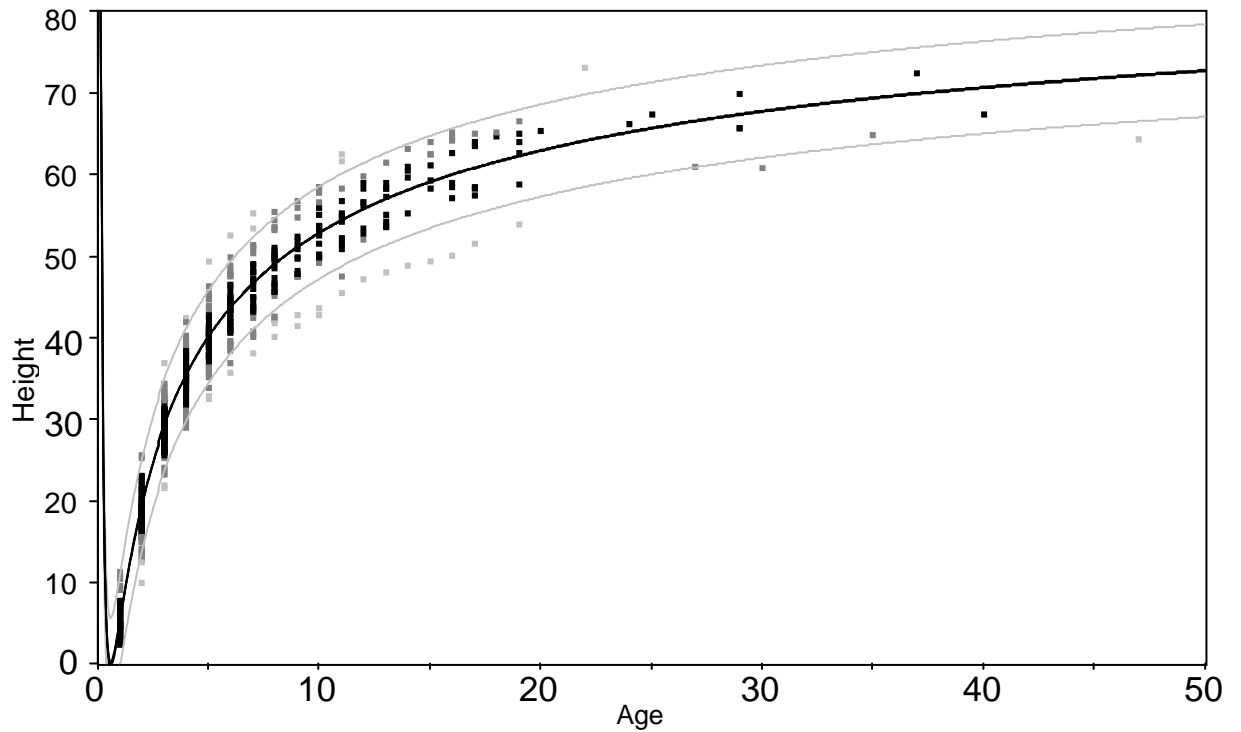
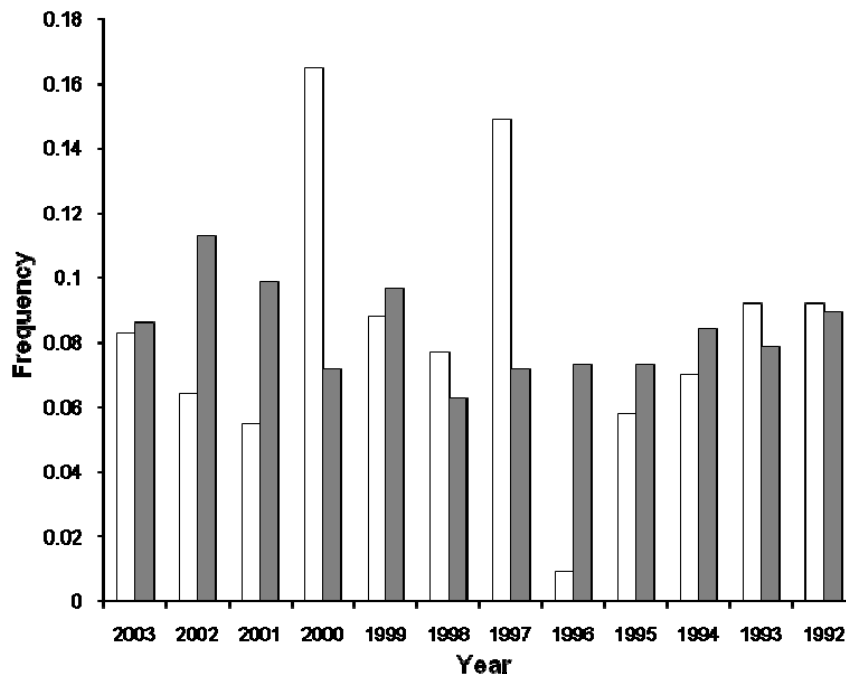


Table 3. Counts and frequency distribution of *Obliquaria reflexa* by year of birth as represented in the survey and mortality collections. For the survey collection, year of birth was estimated using model parameters and heights measured in the field (N=69). Error probabilities were incorporated into survey frequency values. Mortality frequencies were estimated by considering shells directly aged (N=68) and model estimates generated from measurements of fresh-dead shells (N=490) of the 2006 mortality collection. Only individuals born from 1992 to 2003 were considered.

Year of birth	Survey Count	Survey Frequency	Mortalities (Aged by Model)	Mortalities (Aged Directly)	Total Mortalities	Mortality Frequency
2003	6	0.083	41	7	48	0.086
2002	7	0.064	44	19	63	0.113
2001	6	0.055	44	11	55	0.099
2000	4	0.165	38	2	40	0.072
1999	1	0.088	50	4	54	0.097
1998	9	0.077	33	2	35	0.063
1997	5	0.149	33	7	40	0.072
1996	8	0.009	36	5	41	0.074
1995	6	0.058	40	1	41	0.074
1994	11	0.070	42	5	47	0.084
1993	4	0.092	43	1	44	0.079
1992	2	0.092	46	4	50	0.090
Total	69		490	68	558	

Figure. 2. Age frequency distribution of *Obliquaria reflexa* from 1992 to 2003 determined from model estimates of the survey collection (white bars) and a combination of model estimates and lab aged specimens from the mortality collection (grey bars).



Annual Growth

Growth was highest between ages 1 and 2 and decreased exponentially in subsequent years (Figure 3). Growth was similar in years after the interval between years 7 and 8. Growth continued to decrease after year 12 but the difference between annuli became more difficult to discern and measure on the outside of the shell margin after this year.

Individuals experienced the highest growth on average in 2000 (Table 4). The second highest growth occurred in 2002. Individuals grew the least in 2005 and 1999. Years prior to 1998 contained two or fewer observations and were not considered.

Figure 3. Mean early annual growth in height for *Obliquaria reflexa* with standard error for specific yearly intervals. Growth determined individually for each aged specimen by measuring distances between internal annuli.

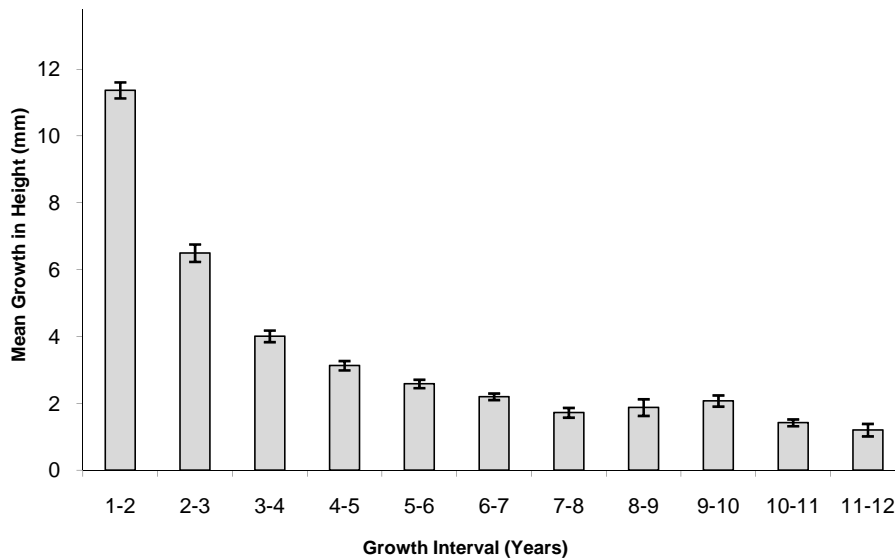


Table 4. Growth parameter for *Obliquaria reflexa* for each year from 1998-2005. Determined by averaging the difference between individual growth and the mean for a specific age interval for each year. Positive values indicate net above average growth in a given year while negative values indicate below average growth. Only years with more than two observations considered.

Year	Growth Parameter
1998	0.117
1999	-0.251
2000	2.272
2001	0.365
2002	0.742
2003	0.184
2004	0.027
2005	-1.129

Analysis of the Relationship between Annual Growth and Recruitment with Temperature and Discharge

Annual recruitment differences were not examined at this stage of the analysis for *Obliquaria reflexa* because the chi-square test indicated that I could not reject the null hypothesis of individuals proportioned evenly across spawning years. Several variables were significantly correlated with growth at the $P < 0.05$ level (Table 5). The strongest correlation was between growth and mean May-September discharge ($r = -0.924$, $P = 0.001$). Discharge over the late summer monthly interval of July thru September was also strongly correlated with growth ($r = -0.818$, $P = 0.013$). Growth was negatively correlated with mean monthly discharge in July, August, and September ($P < 0.05$). *Obliquaria reflexa* growth was positively correlated with August air temperature ($r = 0.733$, $P < 0.05$).

Table 5. Pearson correlation coefficients representing significant correlations ($P < 0.05$) between the growth parameter for *Obliquaria reflexa* and parameters relating to mean monthly discharge and mean monthly air temperature from 1998-2005 (N=8). Temperature and discharge were log transformed [Q = discharge (cfs), T = temperature (°F)].

Parameter	r	$P > \{r\}$
Q july	-0.733	0.039
Q aug	-0.746	0.034
Q sept	-0.741	0.035
Q (may-sept)	-0.924	0.001
Q (july-aug)	-0.811	0.015
Q (july-sept)	-0.818	0.013
T aug	0.733	0.038

Quadrula pustulosa

Growth Model

The best-fitting model relating size to age of *Quadrula pustulosa* was

$\text{height}^{0.5} = 9.582 - 7.922/\text{age}^{0.5}$ (Figure 4, Table 6). The model fit the data well

($R^2 = 0.96$). The majority of data points were below 20 years of age. The model was less accurate than the *Obliquaria reflexa* model. The model correctly predicted age 60.72% of the time when the predicted age was between 1 and 10, 13.33% correct for ages 11-15, and only about 4% correct for ages greater than 15 (Table 7). Model accuracy decreased with age for individuals between 1 and 10 years of age but the majority of predictions were correct within one year.

Figure 4. *Quadrula pustulosa* growth model ($\text{height}^{0.5}=9.582 - 7.923/\text{age}^{0.5}$). Graph indicates best fitting linear model of height vs. age with 90 % confidence intervals (black lines) and prediction intervals (grey lines). Ages determined from internal annuli and back-calculated heights at previous ages (n=569). Individual data points displayed are within 1 SD (black), 2 SD (dark grey), and 3 SD (light grey) of the model ($R^2=0.96$).

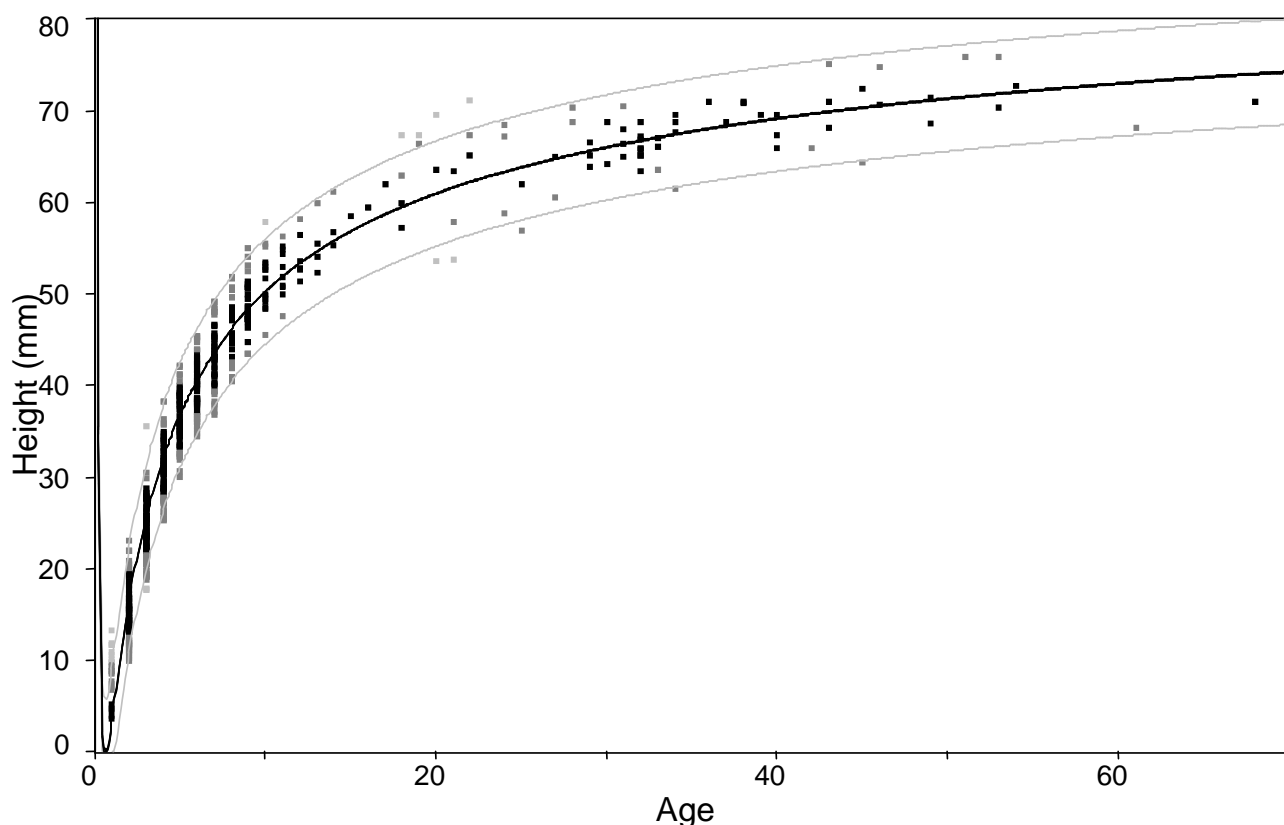


Table 6. Table of Regression Coefficients (A) and ANOVA table (B) for the *Quadrula pustulosa* growth model ($\text{height}^{0.5}=a + b/\text{age}^{0.5}$). $R^2=0.96$, Adjusted $R^2=0.96$, and Fit SE=3.210

A.

Parameter	Coefficient	SE	<i>t</i>	<i>P</i>
a	9.582	0.060	160.713	<.0001
b	-7.922	0.143	-55.260	<.0001

B.

Source	SS	df	MS	F	<i>P</i>
model	147239.110	1	147239.110	14287.925	<.0001
residual	5853.321	568	10.305		
total	153092.440	569			

Table 7. *Quadrula pustulosa* inverse model accuracy for determining age based on length of 551 observations. Includes the predicted age or range of ages and probabilities that the predicted value is correct (residual=0) or accurate within one year (residual= +/- 1).

Predicted Age	Ratio (Correct/Total)	Percent Correct	Percent Correct within 1 Year
0-10	269/443	60.72	93.45
11-15	4/30	13.33	26.67
16-84	3/78	3.85	10.26
1	11/11	100	100
2	70/75	93.33	100
3	62/85	72.94	100
4	48/63	76.19	100
5	28/61	45.90	95.08
6	16/43	37.21	95.35
7	14/34	41.18	88.24
8	12/32	37.50	78.13
9	5/21	23.81	61.90
10	3/18	16.67	72.22

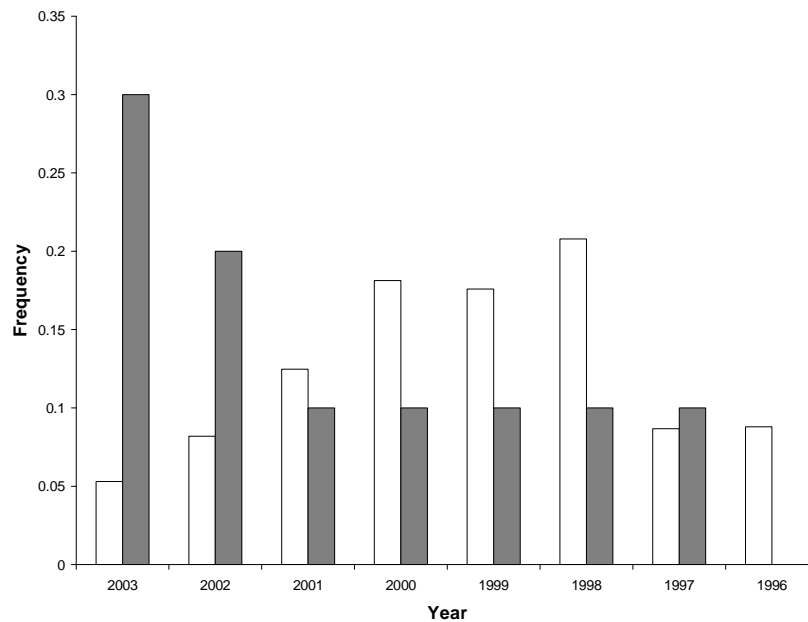
Recruitment Frequencies

At Site 1, 56 *Q. pustulosa* were collected in the survey between 4 and 11 years of age (Table 8). Only ten individuals from this age range were represented in the mortality collection and all were aged in the lab. The frequency of individuals among years in the survey collection had a slightly higher magnitude in 1998-2000 (Figure 5). The mortality collection was skewed to the right, with more individuals representing 2002 and 2003. Although sample size was low, each year contained at least one individual except 1996. The chi-square test on both the survey and mortality data indicated that the null hypothesis of an even distribution of individuals among years could not be rejected (survey $X^2 = 9.947$, $df = 7$, $P = 0.192$; mortality $X^2 = 4.4$, $df = 7$, $P_{\text{exact}} = 0.853$). An exact test was used on the mortality data to account for the low sample size.

Table 8. Counts and frequency distribution of *Quadrula pustulosa* by year of birth as represented in the survey and mortality collections. For the survey collection, year of birth was estimated using model parameters and heights measured in the field (N=56). Error probabilities were incorporated into survey frequency values. Mortality frequencies were estimated by considering shells directly aged (N=10) from the 2006 mortality collection. Only individuals born from 1996 to 2003 were considered.

Year	Survey Count	Survey Frequency	Mortalities (Aged Directly)	Mortality Frequency
2003	4	0.053	3	0.3
2002	8	0.082	2	0.2
2001	6	0.125	1	0.1
2000	13	0.181	1	0.1
1999	7	0.176	1	0.1
1998	4	0.208	1	0.1
1997	4	0.087	1	0.1
1996	10	0.088	0	0
Total	56		10	

Figure 5. Age frequency distribution of *Quadrula pustulosa* from 1996 to 2003 determined from model estimates of the survey collection (white bars) and aged specimens of the mortality collection (grey bars).



Annual Growth

Growth was highest between ages 1 and 2 and decreased exponentially in subsequent years (Figure 6). Growth was similar in years after the interval between years 7 and 8. As with *Obliquaria reflexa*, growth continued to decrease less significantly after year 11 but the difference between annuli became more difficult to discern and measure on the outside of the shell margin after this year.

Individuals experienced the highest growth on average in 2001 (Table 9). The second highest growth occurred in 2002. *Quadrula pustulosa* grew the least in 2005. Only years containing more than two observations were considered.

Analysis of the Relationship between Annual Growth and Recruitment with Temperature and Discharge

Annual recruitment differences were not examined at this stage of the analysis for *Quadrula pustulosa* because the chi-square test indicated that we could not reject the null hypothesis of individuals proportioned evenly across spawning years. The *Quadrula pustulosa* growth parameter was not significantly correlated with mean monthly discharge or temperature at the $P < 0.05$ significance level.

Figure 6. Mean early annual growth in height for *Quadrula pustulosa* with standard error for specific yearly intervals. Growth determined individually for each aged specimen by measuring distances between internal annuli.

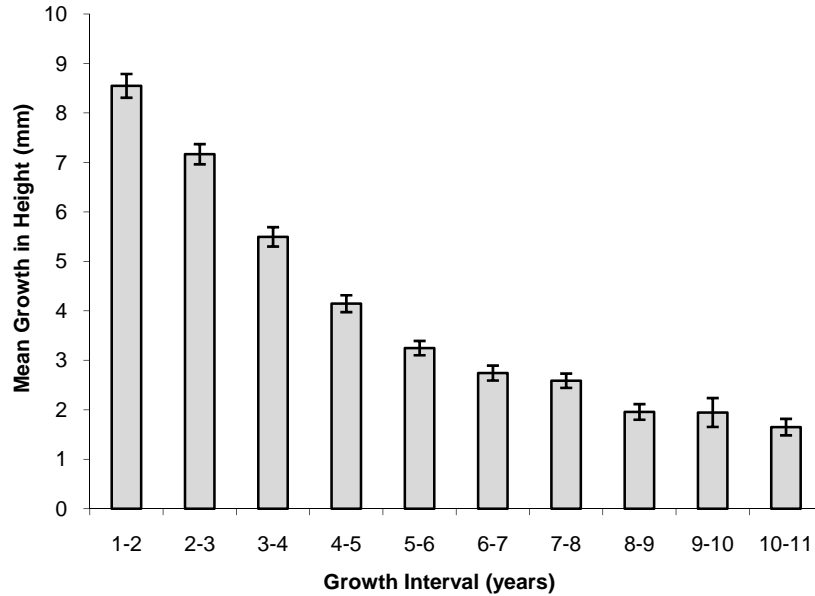


Table 9. Growth parameter for *Quadrula pustulosa* for each year from 1991-2005. Determined by averaging the difference between individual growth and the mean for a specific age interval for each year. Positive values indicate net above average growth in a given year while negative values indicate below average growth. Only years with more than two observations considered.

Year	Growth Parameter
1991	0.2852
1992	-0.0608
1993	0.2872
1994	0.9972
1995	-0.0368
1996	.
1997	.
1998	.
1999	.
2000	0.2816
2001	1.7212
2002	1.2212
2003	0.62
2004	0.3327
2005	-0.5155

Ellipsaria lineolata

Growth Model

Ellipsaria lineolata is sexually dimorphic and the sexes exhibited differences in growth rate. The best-fitting model for male *E. lineolata* was $\text{height}^{0.5} = 11.662 - 8.219/\text{age}^{0.5}$ (Figure 7A, Tables 10A and 10B). The model fit the data well ($R^2 = 0.94$). The majority of data points were below 10 years of age. The model correctly predicted age from height 53.28% of the time when the predicted age was between 1 and 10 but only 20% of the time for ages greater than 10 (Table 11A). The model was less accurate than the other models. Predictions for age 3 individuals were correct only 52.38% of the time and the percent correct decreased with subsequent ages.

Table 10. Tables of Regression Coefficients (A, C) and ANOVA tables (B, D) for *Ellipsaria lineolata* growth models ($\text{height}^{0.5} = a + b/\text{age}^{0.5}$). Male model (A and B) fit statistics: $R^2 = 0.94$, Adjusted $R^2 = 0.94$, and Fit SE = 5.54. Female *E. lineolata* model (C and D) fit statistics: $R^2 = 0.95$, Adjusted $R^2 = 0.95$, Fit SE = 3.81.

A.

Parameter	Coefficient	SE	<i>t</i>	<i>P</i>
a	11.662	0.198	58.900	<.0001
b	-8.219	0.392	-20.967	<.0001

B.

Source	SS	df	MS	F	<i>P</i>
model	65589.178	1	65589.178	2133.807	<.0001
residual	3965.215	129	30.738		
total	69554.393	130			

C.

Parameter	Coefficient	SE	<i>t</i>	<i>P</i>
a	9.609	0.151	63.449	<.0001
b	-6.182	0.333	-18.577	<.0001

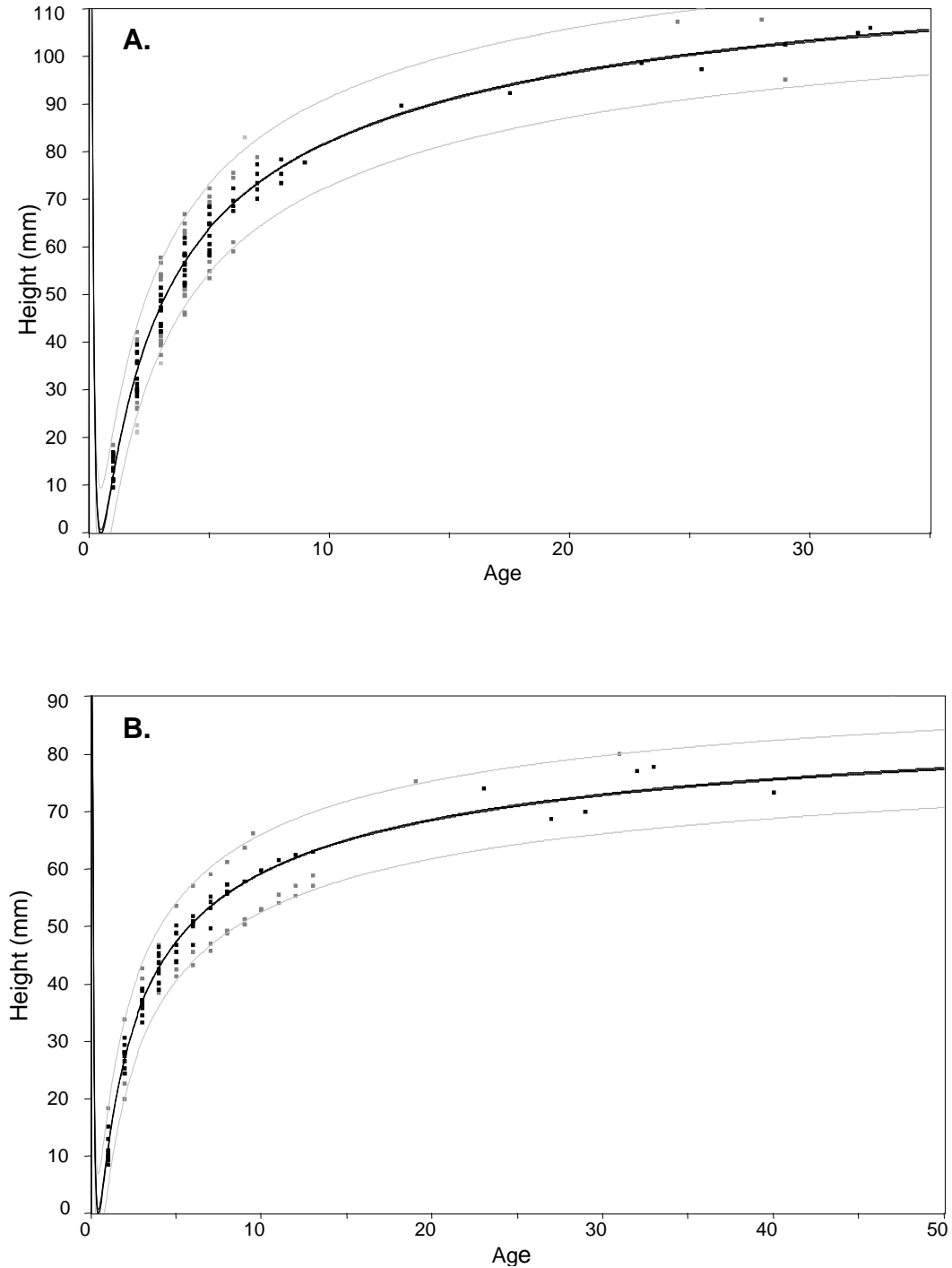
D.

Source	SS	df	MS	F	<i>P</i>
model	26501.802	1	26501.802	1826.54	<.0001
residual	1494.460	103	14.509		
total	27996.262	104			

Table 11. *Ellipsaria lineolata* inverse model accuracy for determining age based on length of 238 observations. Male (A, n=132) and female (B, n=106) models assessed separately. Includes the predicted age or range of ages and probabilities that the predicted value is correct (residual=0) or accurate within one year (residual= +/- 1).

Predicted Age	Ratio (Correct/Total)	Percent Correct	Percent Correct (Within 1 Year)
A. male			
1-42	67/132	50.76	90.15
1-10	65/122	53.28	95.08
11-42	2/10	20	40.00
1	15/18	83.33	100.00
2	20/28	71.43	100.00
3	11/21	52.38	100.00
4	8/20	40	100.00
5	5/11	45.45	100.00
6	3/10	30	100.00
7	2/6	33.33	83.33
8	1/5	20	80.00
9	0/2	0	50.00
10	0/1	0	0
B. female			
1-56	46/106	43.4	71.70
1-10	46/91	50.55	80.22
11-56	0/15	0	20.00
1	10/11	90.91	100.00
2	11/11	100	100.00
3	11/15	73.33	100.00
4	6/13	46.15	92.31
5	2/10	20	80.00
6	3/11	27.27	63.64
7	2/6	33.33	50.00
8	1/6	16.67	66.67
9	0/5	0	40.00
10	0/3	0	33.33

Figure 7. Male (A) and female (B) *Ellipsaria lineolata* growth models. Graph indicates best fitting linear model of height vs. age with 90 % confidence intervals (black lines) and prediction intervals (grey lines). Ages determined from internal annuli and back-calculated heights at previous ages (male n=104, female n=130). Individual data points displayed are within 1 SD (black), 2 SD (dark grey), and 3 SD (light grey) of the models (male model: $\text{height}^{0.5} = 11.662 - 8.219/\text{age}^{0.5}$, $R^2=0.94$, female model: $\text{height}^{0.5} = 9.610 - 6.182/\text{age}^{0.5}$, $R^2=0.95$).



The best-fitting model for female *E. lineolata* was $\text{height}^{0.5} = 9.610 - 6.182/\text{age}^{0.5}$ (Figure 7B, Table 10C and 10D). The model fit the data ($R^2=0.95$). Female *E. lineolata* grew at a slower rate than male *E. lineolata*, reaching a maximum height below 80 in aged specimens. The model correctly predicted age from height 50.55% of the time for ages 1-10 (Table 11B).

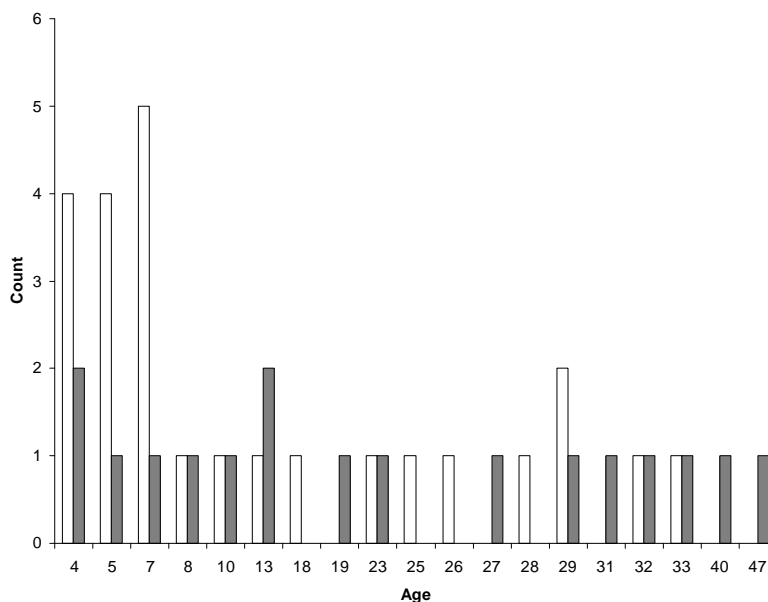
Recruitment Frequencies

The survey collection contained 9 male *E. lineolata* less than 11 years of age. Because of the low sample size and inaccuracy of the growth model for individuals under 11 years, I determined recruitment frequencies based on the mortality collection. The mortality collection consisted of 42 individuals (Figure 8). The distribution was skewed to the right with the majority of individuals being distributed in the 4, 5, and 7 year classes. Six year old mussels born in 2001 were noticeably absent from the collection. The resulting frequencies from 2003 through 1994 (ages 4-13) showed an uneven distribution where individuals were absent from four years and relatively abundant in three years 2003, 2002, and 2000 (Table 12). The unevenness of the distribution could be contrasted with the other species aged by the mortality collections. *Quadrula pustulosa* and *Amblema plicata* had lower sample sizes but individuals were more evenly distributed. The exact chi-square test indicated that the null hypothesis of an equal proportion of individuals among years could be rejected ($X^2 = 23.5$, $df = 9$, $P_{\text{exact}} = 0.0063$).

Table 12. Counts and frequency distribution of male and female *Ellipsaria lineolata* by year of birth as represented in the mortality collection. Mortality frequencies were estimated by considering shells directly aged (N=24) from the 2006 mortality collection. Only individuals born from 1994 to 2003 considered.

Year	Age	Male Count	Female Count	Total	Frequency
2003	4	4	2	6	0.250
2002	5	4	1	5	0.208
2001	6	0	0	0	0
2000	7	5	1	6	0.250
1999	8	1	1	2	0.083
1998	9	0	0	0	0
1997	10	1	1	2	0.083
1996	11	0	0	0	0
1995	12	0	0	0	0
1994	13	1	2	3	0.125
Total		16	8	24	

Fig. 8. Counts of male (white bars) and female (grey bars) *Ellipsaria lineolata* by age determined from shells aged in the lab from the 2006 mortality collection (male n=25, female n=17).



Annual Growth

Growth was highest between ages 1 and 2 and decreased exponentially in subsequent years (Figure 9). Males grew significantly more than females during all intervals except between the ages of 6 and 7 where mean growth was similar. The difference in growth between the sexes was greatest during the 3 to 4 year interval. Growth continued to decrease less significantly after year 7 but the difference between annuli became more difficult to discern and measure on the outside of the shell margin after this year.

Individuals experienced the highest growth on average in 2001, 2000 and 1998 (Table 13). *E. lineolata* grew the least in 2005 and 2002. Only years containing more than two observations were considered.

Figure 9. Mean early annual growth in height for male (white bars) and female *E. lineolata* (gray bars) with standard error for specific yearly intervals. Growth determined individually for each aged specimen by measuring distances between internal annuli.

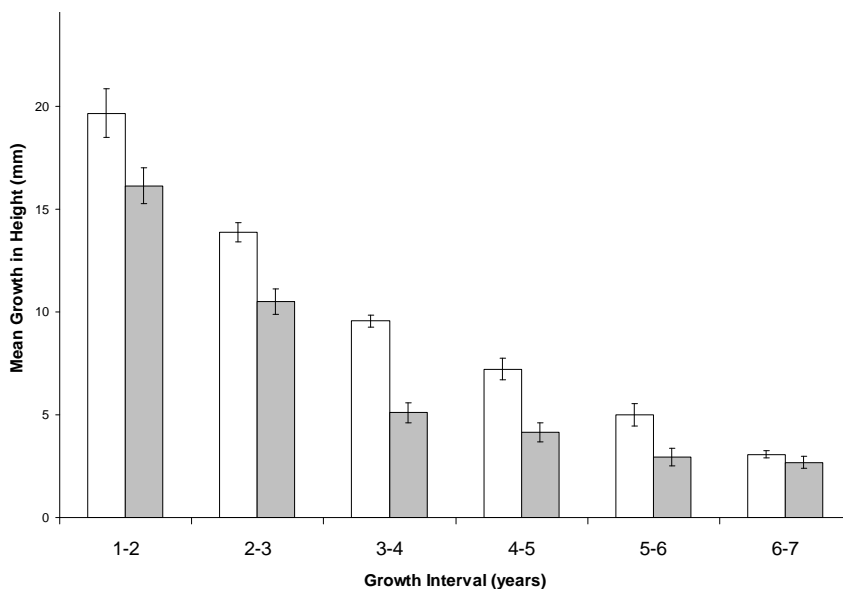


Table 13. Growth parameter for male *Ellipsaria. lineolata* for each year from 1998-2005. Determined by averaging the difference between individual growth and the mean for a specific age interval for each year. Positive values indicate net above average growth in a given year while negative values indicate below average growth. Only years with more than two observations considered.

Year	Growth Parameter
2005	-0.6222
2004	0.2796
2003	0.4594
2002	-0.4292
2001	1.7922
2000	1.0058
1999	0.3708
1998	1.3283

Analysis of the Relationship between Annual Growth and Recruitment with Temperature and Discharge

The chi-square test indicated that the null hypothesis of an even distribution of *E. lineolata* individuals among years could be rejected. I tested the relationship between both annual recruitment (or age frequency) and the annual *E. lineolata* growth parameter with mean monthly temperature and discharge for each year. Because *E. lineolata* is a bradytictic species, I also examined mean monthly temperature and discharge for the previous spawning season. The strongest correlation was between the recruitment frequency and mean June through August discharge during the spawning year ($r = -0.925$,

P= 0.0001; Table 14). Several monthly mean discharge parameters were also significantly negatively correlated with age frequency including mean monthly discharge in July, August, October, and the July-August interval. June mean discharge was also negatively correlated with recruitment but the relationship was not significant at the $P < 0.05$ level. *E. lineolata* growth was positively correlated with one parameter, May air temperature.

In order to examine the duration of lower flows over the June-August interval and examine how these flows compare to historical flows, I created a new parameter: the number of days from June-August where the discharge was less than or equal to the median monthly discharge from 1960-2005 (Table 15). A strong positive correlation existed between this parameter and recruitment frequency ($r = 0.919$, $P = 0.0002$, Table 14).

Regression analysis displayed a strong negative linear relationship between recruitment frequency and mean June-August discharge ($y=0.875-0.116 x$; $R^2= 0.855$, Figure 10A). The years with high recruitment: 2000, 2003, and 2002 had lower average discharge values during the summer months. A second regression displayed a strong positive linear relationship between *E. lineolata* recruitment frequency and the number of days in June-August where the discharge was less than or equal to the median monthly discharge ($y= -0.112+.0044 x$; $R^2= 0.844$, Figure 10B). The three years exhibiting the highest recruitment had over 70 days from June through August with discharge at or below the median discharge level. Some variation in this parameter existed among years exhibiting no recruitment but all had fewer than 45 days less than the median discharge level during the three month period.

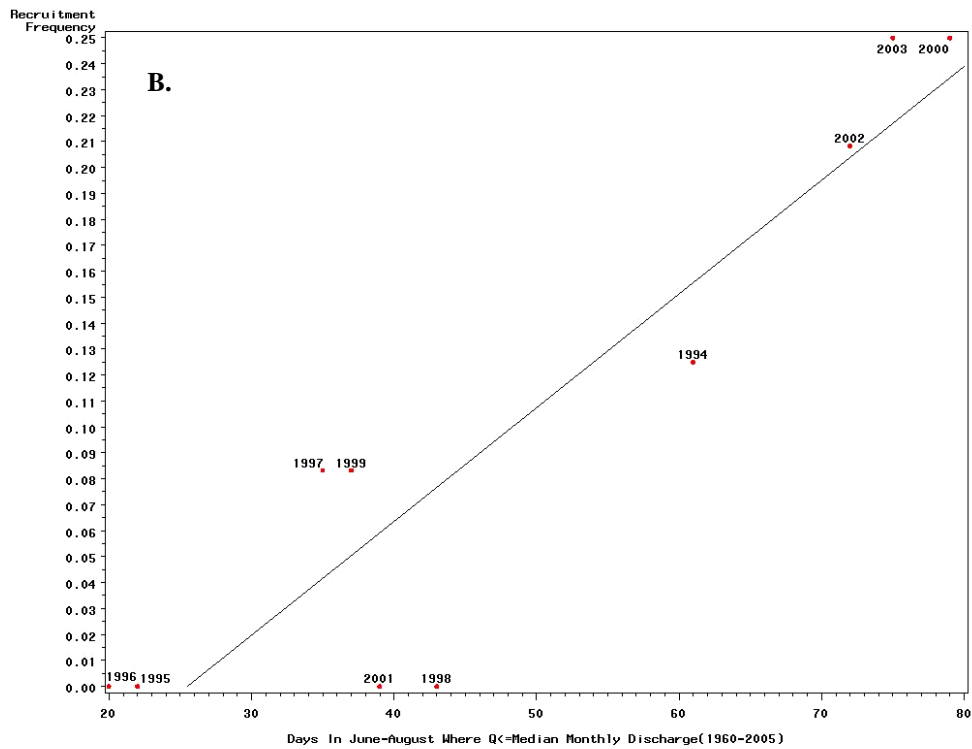
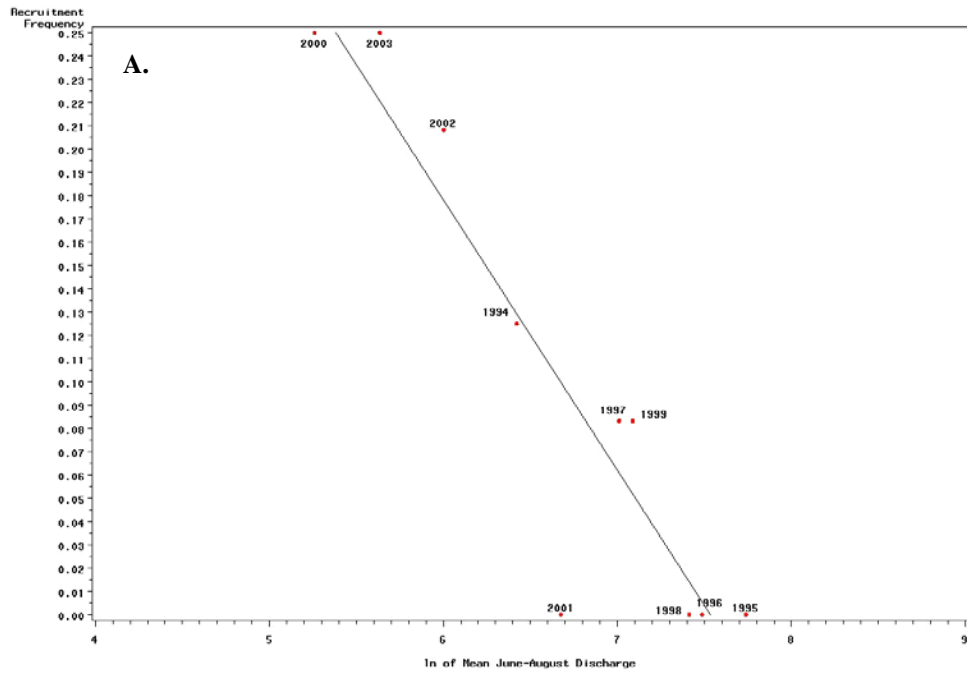
Table 14. Pearson correlation coefficients indicating significant correlations ($P < 0.05$) between *E. lineolata* annual growth and recruitment with several parameters relating to mean monthly discharge and air temperature for each year from 1994-2003 (N=10). Variables relating to discharge and temperature were log transformed [Q=discharge (cfs), T=temperature (°F)].

Flow/Temperature Parameter	Biological Parameter	r	$P > r $
Q july	age frequency	-0.789	0.0067
Q aug	age frequency	-0.793	0.0062
Q oct	age frequency	-0.696	0.0253
Q (june-aug)	age frequency	-0.925	0.0001
Q (july-aug)	age frequency	-0.870	0.0110
No. days $Q \leq Q_{50}$ (june-aug)	age frequency	0.919	0.0002
T (may)	growth	0.778	0.0236

Table 15. Median monthly discharge (cfs) on the Marais des Cygnes River near KS-MO state line (USGS 06916600) for June-August from 1960-2005.

Month	Q_{50} (1960-2005)
June	1785
July	467
August	132

Figure 10. Simple linear regression of the relationship between annual *Ellipsaria lineolata* recruitment frequency from 1995 to 2003 and the mean June-August discharge (cfs) (A) and the number of days at or below the median discharge level from 1960-2005 in the months of June, July, and August (B).



Amblema plicata

Growth Model

Amblema plicata was the most difficult species to age. Specimens from Site 1 were typically in good condition, but the majority of specimens came from Sites 3 and 4 and were of poorer quality. Most shells greater than approximately 90 mm in length were not included in the model due to a high degree of shell erosion and morphological variability at Sites 3 and 4. A lower sample size of aged specimens resulted in less variability. The best-fitting model was $\text{height}^{-1} = 0.0079 + .1265/\text{age}^{-1}$ (Figure 11, Table 16). The model fit the data well ($R^2=0.97$). The model after age 10 was generated by only one individual and its previous height-at-ages. I aged 18 *A. plicata* that were greater than 100 mm in length at the three sites (Figure 12). These individuals exhibited high variation and no relationship between height and age. The oldest was 87 years old. The model correctly predicted age 76.95% of the time when the predicted age was between 1 and 10 (Table 17).

Table 16. Table of Regression Coefficients (A) and ANOVA table (B) for the *Amblema plicata* growth model ($\text{height}^{-1}=a + b/\text{age}^{1.5}$). $R^2=0.97$, Adjusted $R^2=0.97$, and Fit SE=3.87

A.				
Parameter	Coefficient	SE	<i>t</i>	<i>P</i>
a	0.0079	0.0001	56.2616	<.0001
b	0.1265	0.0028	45.5495	<.0001

B.					
Source	SS	df	MS	F	<i>P</i>
model	56364.801	1	56364.801	3757.33	<.0001
residual	1455.123	97	15.001		
total	57819.924	98			

Figure 11. *Amblema plicata* growth model ($\text{height}^{-1} = 0.0079 + 0.1265/\text{age}^{-1}$). Graph indicates the best fitting linear model of height vs. age with 90 % confidence intervals (black lines) and prediction intervals (grey lines). Ages determined from internal annuli and back-calculated heights at previous ages (n=98). Individual data points displayed are within 1 SD (black), 2 SD (dark grey), and 3 SD (light grey) of the model ($R^2=0.97$).

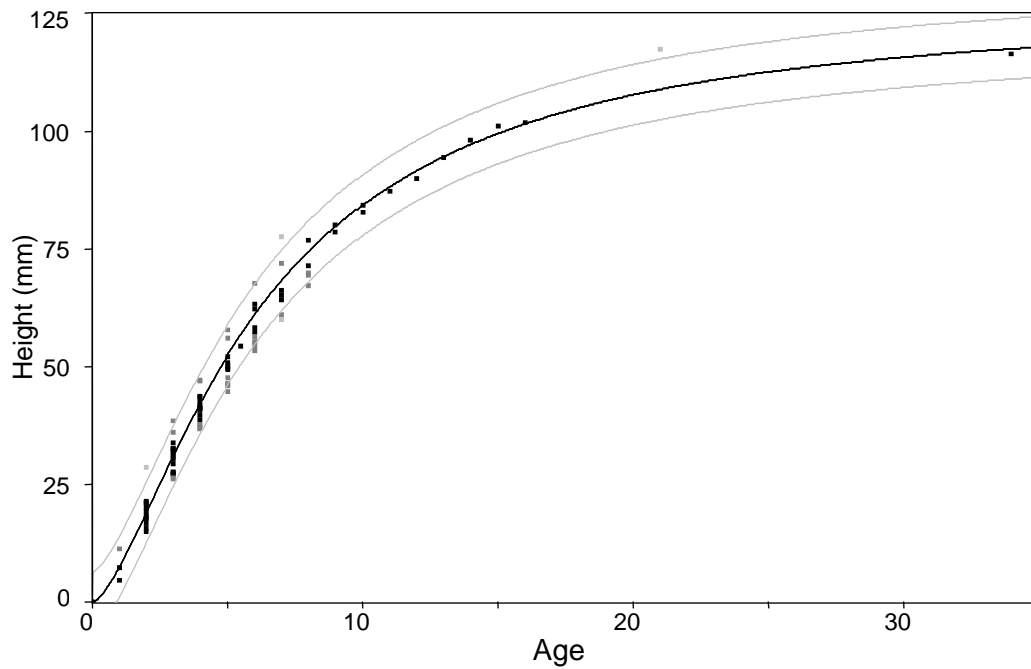


Figure 12. *Amblema plicata* height vs. age for 18 aged specimens exceeding 100 mm in length and 16 years of age from three sites: Site 1=grey circles, Site 3=white triangles, and Site 4=black diamonds.

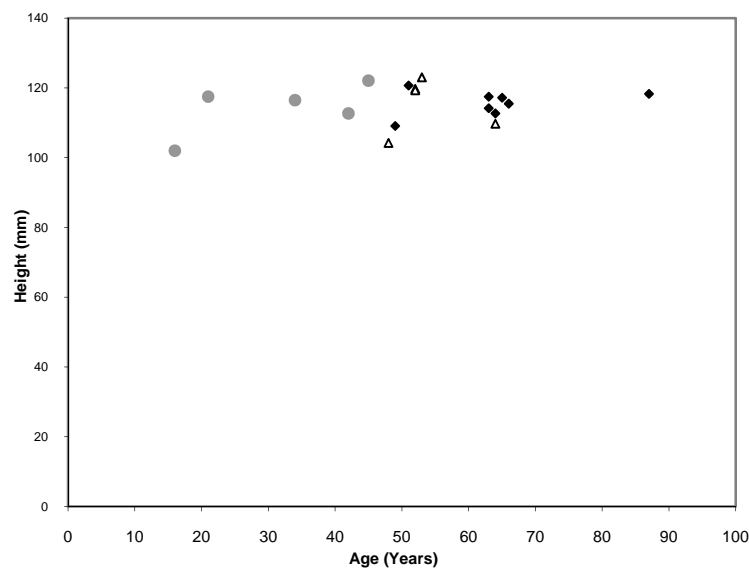


Table 17. *Amblema plicata* inverse model accuracy for determining age based on length of 91 observations. Includes the predicted age or range of ages and probabilities that the predicted value is correct (residual=0) or accurate within one year (residual= +/- 1).

Age	Ratio (Correct/Total)	Percent Correct	Percent Correct (Within 1 Year)
1-10	70/91	76.95	98.90
1	3/3	100.00	100.00
2	14/14	100.00	100.00
3	16/17	94.12	100.00
4	16/21	76.19	100.00
5	9/13	69.23	100.00
6	4/8	50.00	100.00
7	2/6	33.33	100.00
8	2/3	66.67	100.00
9	2/3	66.67	66.67
10	2/2	100.00	100.00

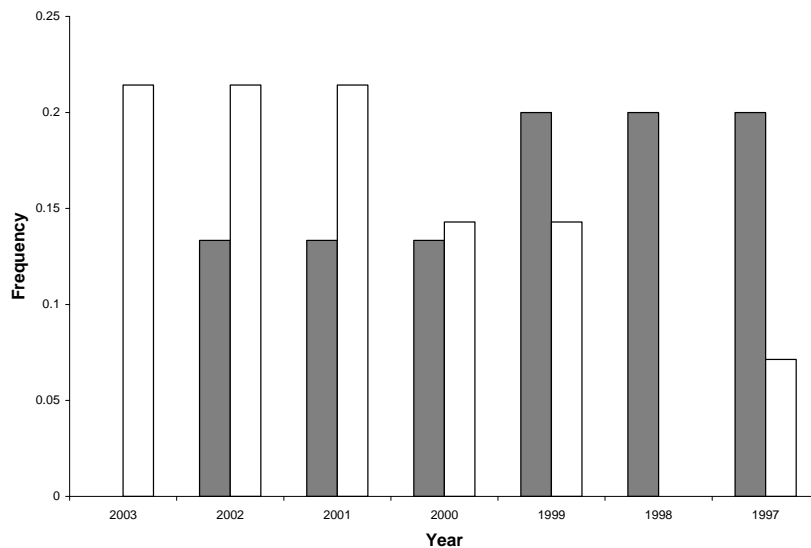
Recruitment Frequencies

At Site 1, 15 *Amblema plicata* were collected in the survey between 5 and 10 years of age (Table 18). In the mortality collection from Site 1, 14 *A. plicata* were collected between 4 and 10 years of age. The recruitment frequency distribution of the survey collection was similar among years with an absence of individuals from 2003 (Figure 13). The chi-square test indicated that the null hypothesis, an equal proportion of individuals across years, could not be rejected ($X^2=3.199$, $df = 6$, $P=0.7834$). The recruitment frequency distribution of the mortality collection was skewed to the right with slightly higher frequencies in the three more recent years and a lack of individuals in 1998. The chi-square test indicated that the null hypothesis of equal proportions could not be rejected ($X^2=4.6$, $P_{\text{exact}}=0.6768$).

Table 18. Counts and frequency distribution of *Amblyma plicata* by year of birth as represented in the survey and mortality collections. For the survey collection, year of birth was estimated using model parameters and heights measured in the field (N=15). Error probabilities were incorporated into survey frequency values. Mortality frequencies were estimated by considering shells directly aged (N=14). Only individuals born from 1997 to 2003 considered.

Year	Survey Count	Frequency	Mortalities (aged directly)	Frequency
2003	0	0	3	0.2143
2002	2	0.133	3	0.2143
2001	2	0.133	3	0.2143
2000	2	0.133	2	0.1429
1999	3	0.200	2	0.1429
1998	3	0.200	0	0
1997	3	0.200	1	0.0714
Totals	15		14	

Figure 13. Age frequency distribution of *Amblyma plicata* from 1997 to 2003 determined from model estimates of the survey collection (white bars) and aged specimens of the mortality collection (grey bars)



Annual Growth

Amblema plicata growth was highest between ages 1 and 2 where it averaged close to 12 mm in one year and decreased gradually in subsequent years (Figure 14). Growth continued to decrease after year 10.

Individuals experienced the higher growth on average in 2002, 2003, and 2001 (Table 19). *A. plicata* grew the least in 2000. In 2004 and 2005, growth was also lower than average. Only years containing more than two observations were considered.

Analysis of the relationship between annual growth and recruitment with temperature and discharge

Because the chi-square test indicated equal proportions among years in both the survey and mortality collections, *A. plicata* recruitment proportions were not considered in the correlation analysis. A negative correlation between growth and average ambient air temperature during the month of October was the only significant Pearson correlation at the $P < 0.05$ level ($r = -0.817$, $P = 0.0473$). An examination of the relationship revealed the error variance was not constant across all levels of temperature and that the relationship was highly dependent on one year, 2002. Other years tended to deviate from the model. During 2002, a relatively low average October temperature coincided with the highest growth value.

Figure 14. Mean early annual growth in height of *Amblema plicata* with standard error for specific yearly intervals. Growth determined individually for each aged specimen by measuring distances between internal annuli.

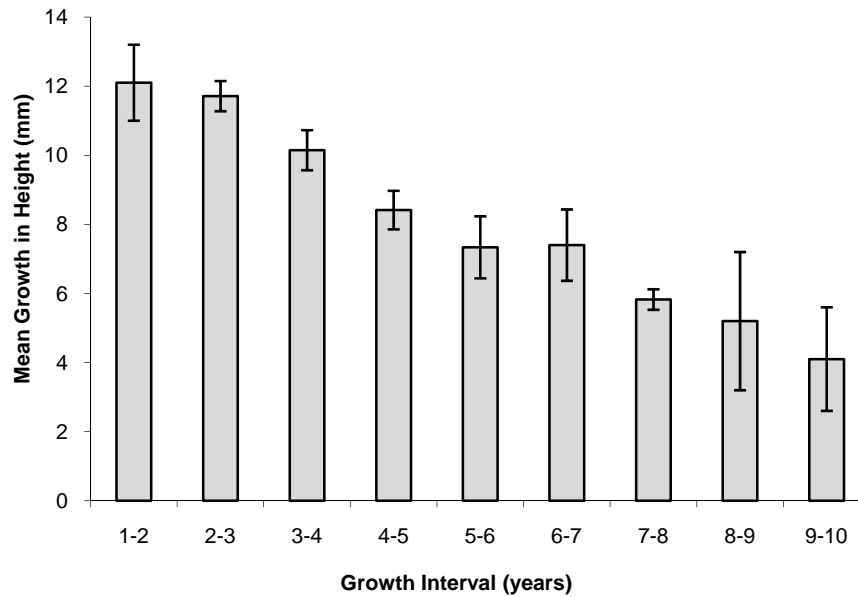


Table 19. *Amblema plicata* growth parameters for each year from 2000-2005, determined by averaging the difference between individual growth and the mean for a specific age interval for each year. Positive values indicate net above average growth in a given year while negative values indicate below average growth. Only years with more than two observations considered.

Year	Growth Parameter
2000	-1.1235
2001	0.6197
2002	1.7086
2003	1.0672
2004	-0.2422
2005	-0.3151

Discussion

Assessment of growth models

The relationship between age and height was best described by simple linear equations where the variables were transformed. *Obliquaria reflexa*, *Quadrula pustulosa*, and *Ellipsaria lineolata* incorporated square-root transformations of the variables and the *Amblema plicata* model incorporated inverse transformations. I tested other standard non-linear models to the data originally including the von Bertalanffy (1938), Gompertz, and monomolecular growth models. The simple linear models with transformations were selected because they were more accurate at predicting the correct age for individuals < 15 years of age for all species examined. In general there were comparatively few data points for individuals > 15 years of age and the models were inaccurate for individuals in this age group. Model inaccuracies are expected particularly for older individuals because mussel growth declines with age and varies annually.

The *Obliquaria reflexa* model was the most accurate and could predict the correct age 90% of the time and within 1 year 100% of the time through age 15. The *Quadrula pustulosa* model correctly predicted age from length about 60% of the time in mussels < 10 years old. The *Q. pustulosa* and *A. plicata* models had high predictive power (within 1 year, at least 90% of the time) through age 7. This level of accuracy is useful for examining general recruitment trends. Another approach is to age younger mussels of some certain species directly in the field. External growth rings can be pronounced and could be used for aging instead of the model for younger individuals if exact ages are desirable. Rogers et al (2001) found that variability between counting external rings and internal rings was small up until age 8 in the tan riffleshell (*Epioblasma florentina*

walkeri). However, the external features of *O. reflexa* and darker periostracum of *A. plicata* caused the growth rings to be less pronounced even in very young individuals and the models were more applicable. Also several individuals exhibited false growth lines which could only be distinguished from annual growth lines on the thin sections. In order to assess the differences in annual recruitment from the field sample by using the models, the probability of an incorrect prediction had to be directly incorporated in age estimates.

Mean growth in height was high between ages 1 and 2 in *O. reflexa* (~11 mm) and decreased significantly in the next year interval (~ 6 mm). Growth remained fairly constant (~2 mm/year) from year 6 to year 12. *Quadrula pustulosa* average growth was lower between ages 1 and 2 (~ 8.5 mm) and steadily declined. *Q. pustulosa* growth remained constant at approximately 2 mm from age 8 to age 11. The model reflected a slightly higher rate of growth. Size and age are positively related to fecundity in these two species (Haag and Staton 2003). Haag and Staton researched the age at reproductive maturity of *Q. pustulosa* (and seven other species) in the Sipsy River AL, Buttahatchee River, MS and Little Tallahatchie River, MS. While a low percentage of *Q. pustulosa* was sexually mature at age 3, the percentage increased gradually and 100% maturity was reached by age 7. After age 6 in the mussels collected on the Marais des Cygnes, growth rate remained fairly constant at 2.5 mm/year and gradually decreased to 2 mm per year by age 10. A gradual decline in growth rate may be reflective of the shift in resource allocation from growth to reproduction. There are several benefits associated with early high growth. Achieving a large size after juvenile settlement during the first year may be critical to surviving through the first winter (Hanlon and Neves 2006). In riverine

systems, larger juveniles may also experience less displacement or damage during periods of high flows or sediment movement.

A. plicata exhibited the highest growth rate of the four species and the decline in growth over time was more gradual. Mean growth decreased from about 12 mm/year from ages 1-3 to a mean growth of 4 mm during years 9-10. The *A. plicata* growth model correctly predicted age from length about 77% of the time but this model was based off of a lower sample size. The few older (>20 years of age) individuals that I was able to age conclusively showed high variation in height at age. Shell erosion was present on the majority of older three ridge individuals in both the field and mortality collections and this resulted in an underestimation of age based on length using a simple linear model.

Ellipsaria lineolata is sexually dimorphic and separate models were constructed for each sex. Males exhibited a higher growth rate than females. Males grew significantly more on average for every annual interval until age 6. The difference was most pronounced in the 3-4 age interval. Males grew close to 20 mm on average and females grew about 15 mm between ages 1 and 2. During the 3-4 age interval, female *E. lineolata* grew about 5 mm and growth gradually decreased to about 2.5 mm/year during the 6-7 age interval. The larger growth differential between male and females beginning at age 3 may coincide with the onset of reproduction in this species. Higher growth in males of certain species has been documented in previous studies (Rogers et al. 2001, Hanlon and Levine 2004, Eads et al. 2006). Bradytictic females that brood glochidia over the winter may allocate less resources into somatic growth and more into brooding glochidia (Rogers et al. 2001, Eads et al. 2006). Additionally, it has been suggested that

females may not be able to feed properly when their gills are modified to brood glochidia (Bauer 1988, Hastie et al. 2000).

The relationship between annual growth variation, discharge, and temperature

Growth varied annually and was below average for all species in 2005.

Freshwater mussel growth may vary with temperature, water turbulence, and food availability in a given time frame (Bailey and Green 1988, Bauer and Wachtler 2000).

The data indicate that *O. reflexa* growth was negatively correlated with mean discharge in May-September and positively correlated with August mean ambient air temperature.

Air temperature is highly correlated with water temperature measurements at the site based on historical water quality data. *E. lineolata* growth was positively correlated with May mean temperature. I did not find a relationship between discharge or temperature and growth in the other species. A possible explanation for the lower growth in certain species during months of high discharge is that these individuals may be burrowing deeper into the sediment when flows are high. Vertical movements during high flows could impede surface filter feeding if mussels remain burrowed for long periods of time during summer months (Pusch et al. 2001) or invoke an additional energetic cost.

Schwalb and Pusch (2007) studied vertical and horizontal movements of three species of mussels in a lowland river in Germany from May to October and found that discharge was the dominant factor in influencing the vertical movements of mussels but day length and temperature were contributing factors.

Examination of year class strength in relation to annual discharge and temperature variation

The difficulty with investigating year class strength in freshwater mussel populations in a short-term study is that younger individuals may be rare in the population and difficult to age in certain species without using thin sectioning techniques. Researchers may not wish to sacrifice the young mussels of a declining population collected in a random sample for aging analysis. Examining a collection of fresh dead shells for recruitment differences is useful if the event that caused the mortalities had a similar random effect on all year classes. The results in Chapter 3 indicate that the low water period had less of an impact on younger individuals of all four species. The sample size of the mortality collection may be too low to accurately reflect year class strength in the population in some cases and conducting random sampling and applying the growth models to determine ages from shell size may be preferable. I used both approaches to assess whether there are any implications of strong and weak year classes in the mussel assemblage at Site 1.

In the last ten to fifteen years, there is evidence of *O. reflexa*, *Q. pustulosa*, and *A. plicata* recruitment for every year at Site 1. The estimated recruitment frequencies were not similar between field samples and mortality samples, but these samples generally had a low size. The chi-square tests indicated that the null hypothesis of an even distribution in counts among the year classes could not be ruled out when considering either the field or mortality samples for all three species. The even distribution of individuals among year classes in these three species may be a result of a high probability of successful

contact between the glochidia and a suitable host fish during summer months despite discharge or temperature fluctuations.

All three species are tachytictic. The breeding season of *A. plicata* is reported to occur from June to August (Baker 1928, Frierson 1914, Ortmann 1912, Watters 1994). *Q. pustulosa* and *O. reflexa* were also reported to breed during these months (Howard 1914, Utterback 1915-1916). *A. plicata* and *Q. pustulosa* glochidia have been found to infect a variety of fish hosts, many of which are found in the West Osage River Basin (Appendix: Table 1) while the host fish for *O. reflexa* is unknown (Oesch 1984, Parmalee and Bogan 1998). Host fish density in the vicinity of the mussel bed may not be a limiting factor for recruitment success in certain years when the glochidia can parasitize on the gills of a number of species.

In contrast to the three tachytictic species, the mortality collection of *Ellipsaria lineolata* revealed several strong year classes from 1994 to 2003. There was no evidence of *E. lineolata* recruitment in certain years and the proportions of individuals were not distributed evenly among years. *E. lineolata* females of this species are reported to be gravid in August through June or July of the following year (Ortmann 1919). There was no relationship between mean monthly discharge or temperature throughout the year when fertilization occurs and recruitment frequency in the following year. There was a strong negative relationship between *E. lineolata* recruitment frequency and mean monthly discharge in June-August. There also was a strong positive relationship between the number of days throughout this period at or below the median monthly discharge on record and recruitment frequency. The median discharges in June, July, and August over the past 47 years were 1785 cfs, 467, cfs, and 132 cfs respectively. The years with high

recruitment had more than 60 days ($> 65\%$) in these three months under the median monthly discharges while the years with little or no evidence of recruitment had less than 45 days ($< 50\%$) under the median monthly discharges. The rarity of *E. lineolata* in the assemblage may be reflective of episodic recruitment that is closely linked to long bouts of lower than average discharge from June through August. This period coincides with the reported time frame when *E. lineolata* females release glochidia, the host fish become infected, and juveniles excyst from the gills of the fish and drop into the substrate. The three reported host fish for *E. lineolata* glochidia are green sunfish (*Lepomis cyanellus*), sauger (*Stizostedion canadense*), and freshwater drum (*Aplodinotus grunniens*) (Fuller 1978). The freshwater drum and green sunfish are found in the Kansas portion of the West Osage River basin (Dent et al. 2007) but this study did not verify these fish as suitable hosts for *E. lineolata* in the Marais des Cygnes River. Host infection may involve predation by the molluscivorous *A. grunniens* on gravid females (Coker et al. 1921). The timing of freshwater drum spawning is reported as May through June (Wrenn 1969, Fremling 1980) and coincides with the reported time frame when female *E. lineolata* are believed to release glochidia. Freshwater drum form spawning aggregations triggered by the communication of male drum (Fremling 1980). If these aggregations are formed over a mussel bed containing gravid female butterfly mussels, then there could be a high rate of infestation. Gravid *E. lineolata* mussels have been reported to respond to shadows and touch by flipping the mantle margin back and exposing the marsupia which could aid in releasing glochidia (M. Davis, Minnesota Department of Natural Resources in Barnhart et al. 2008).

A long duration of lower discharge from June to August could facilitate *E. lineolata* recruitment in several possible ways. The low discharge may increase water clarity and cause molluscivorous host fish to aggregate over mussel beds in their search of food. In turbid rivers impacted by sedimentation, periods of low flow may result in increased water clarity or less turbidity and suspended load (Leopold and Maddock 1953, Roos and Pieterse 1994, Gordon et al. 2004) which serves to decrease host fish reaction distance to visual cues (Barret et al. 1992). Rutherford et al. (1995) suggest growth of freshwater drum was enhanced during conditions of low turbidity because of greater macroinvertebrate production and foraging efficiency of freshwater drum in these conditions (1995). When visibility is increased, the host fish could be more inclined to prey upon gravid females when they observe the exposed marsupia.

Low discharge creating depositional conditions during the time of juvenile settlement is believed to positively influence annual recruitment success in certain species of mussels (Payne and Miller 2000, Howard and Cuffey 2006). Additionally, higher flows after settlement can displace juveniles downstream (Howard and Cuffey, 2006). The discharge-stage relationship is another factor to consider and glochidia that are excysted during high flow conditions may have a higher probability of falling in the floodplain or areas that are adjacent to the main channel that will become dry late in the summer. Because the other species experienced successful recruitment in all the years under consideration at Site 1, the specific conditions at this site could be providing refuge for the newly metamorphosed juveniles from high flows. However, discharge in late July and August is typically much lower than spring and early summer discharge on the Marais des Cygnes River. *E. lineolata* are reported to release glochidia in June or July

(Ortmann 1919) and the timing of juvenile excystment from the host fish may be earlier than the other species and more dependent on lower than average flows throughout the entire summer. These findings support the suggestion by Hardison and Lazyer (2001), that the recruitment of amblemine (the three tachytictic species) species is less affected by unnaturally high discharge during spring and early summer because they tend to breed later in the year when flows are typically lower on average. Prolonged high discharge in spring and early summer could result in higher shear stresses exerted on mussel beds at the time of excystment in lampsiline species (Hardison and Lazer 2001). *E. lineolata*, a threatened species in Kansas is rare on the Marais des Cygnes and according to the recruitment frequency data presented in this study, that rarity can be partially explained by episodic recruitment linked to intermittent years of below average discharge for the majority of days from June to August.

More research is needed on *E. lineolata* and its host fish in the Marais des Cygnes River before conclusively identifying the reasons for the associations between recruitment success and low summer discharge. Studies should verify the timing of early life cycle events in the Marais des Cygnes River, verify the host fish, and examine the feeding ecology, movements, and the habitat preferences of the host fish in the river. The relative success of annual recruitment in the three tachytictic species at Site 1 compared to the other three sites as described in Chapter 2 should also be investigated. The habitat at Site 1 may support higher densities of host fish during the summer months or it could provide a refuge for age-0 mussels from shear stress during high flow events. The current conditions at Site 1 could be similar to the conditions at the other sites over 15 years ago that supported recruitment of these species and have since deteriorated. Habitat

variability and mussel habitat preferences at the four sites are described in more detail in the following chapter.

Chapter 5. The Relation of Habitat Conditions to Mussel Density in the Marais des Cygnes River

Introduction

In the preceding chapters, the quality of four locations along the Marais des Cygnes River was assessed as it relates to overall mussel density, species richness, and recruitment. Variation in these parameters was observed both within a particular site in the cross-stream direction and among the four locations. Within a site, mussels were aggregated and high and low density strata were assigned. Mussels were absent from the majority of quadrats within the low density stratum. In general, a particular mussel bed contained in the 'high density stratum' was located in the area adjacent to the bank and had a limited extent both across the stream and in the upstream/downstream direction.

There may be several factors that limit a mussel bed to a confined area of the stream. Mussels can move both vertically and horizontally and may be showing a preference to a particular habitat type thereby not extending their range at a site (Vannote and Minshall 1982, Strayer 1993, Vaughn 1997, Morales et al. 2006). Studies that have attempted to predict mussel densities from microhabitat variables have had limited success (Holland-Bartels 1990, Strayer and Ralley 1993), but a few have shown mussel preference to specific habitat conditions (Salmon and Green 1983, Layzer and Madison 1995, Howard and Cuffey 2003). The range may also be limited to the preferred habitat of host fish or the high mortality of newly metamorphosed juveniles in certain areas (Watters 1992, Strayer 1999, Vaughn and Taylor 2000, Morales et al. 2006).

This objective describes the habitat conditions at each site by examining depth and substrate differences and relating these variables to mussel density. Researchers

have suggested that the presence of mussel beds and particularly beds that support juveniles in an area may indicate substrate stability during high flows (Vannote and Minshall 1982, Di Maio and Corkum 1995, Strayer and Ralley 1993). The question of whether conditions limit the distribution pattern of mussels on the Marais des Cygnes River can be explored by comparing the habitat where mussels are found within the mussel bed and where mussels are absent in a particular location. The findings reported in the previous chapters indicate that there are differences among sites in the level of species richness and mussel recruitment. This chapter will compare the habitat variables between mussel beds and address how those differences may relate to abundance and recruitment.

Materials and Methods

Field Study

The preliminary survey was repeated during low flow conditions in August through September 2007 at each site. Each site was divided into twenty cross-stream transects. Along each transect at 3-m intervals from a random start, one 0.25 m² quadrat was sampled for mussels and the depth was measured in the center of the quadrat. Quadrats were surveyed for mussels by using the same tactile procedure used during the previous year without excavation. I conducted Wolman pebble counts (Wolman 1954) along five transects at each site. I divided each transect into the high and low density strata and also divided each stratum into two adjacent sections. A designated count of pebbles was assigned to each section and particles were selected at random until the

count was reached. The total count for each transect was 100 particles. Particles were measured to the nearest mm along the longest axis. A variety of habitat variables were derived from these counts (Table 1). The median particle size (**d50**), 84th percentile size (**d84**), 16th percentile size (**d16**), the average particle size, and standard deviation of the particle size were calculated in each section. I also determined the percent of particles comprising each substrate category (Table 2).

Table 1. List of microhabitat variables measured at the four sites. The median particle diameter, 84th percentile size, and 16th percentile size are represented by d50, d84, and d16 respectively.

Variable	Unit
depth	m
d50	mm
d84	mm
d16	mm
average particle size	mm
stdev particle size	mm
percent silt	%
percent sand	%
percent medium gravel	%
percent coarse gravel	%
percent very coarse gravel	%
percent total gravel	%
percent small cobble	%
percent large cobble	%
percent boulder	%
percent bedrock	%

Table 2. Adapted from Wentworth classes and applied grade scales for particles sizes (from Gordon et al. 2004, Brakensiek et al. 1979). Variable abbreviations are used to refer to particle size classes in this study.

Class	Variable Abbreviation	Size Range (mm)
silt	psilt	< 0.0625
fine sand	pfs	0.0625-1
sand	psand	1-2
medium gravel	pmg	2-16
coarse gravel	pcg	16-32
very coarse gravel	pvcg	32-64
medium and coarse gravel	pgravel	2-32
gravel (all)	pall	2-64
small cobble	psc	64-128
large cobble	plc	128-256
boulder	pbou	256-2048
bedrock	pbck	>2048

Interpolation was used to assign depth values to x positions along the entire transect for each of the 20 transects. The x,y pairings surveyed in 2006 were matched with the closest x,y pairing along the transects sampled for depth and substrate. In addition two transects were sampled at 25 and 50 meters above and below each site to compare conditions outside of the site to conditions within the site.

Mussel distributional patterns were compared between years to examine whether changes occurred. The distributional patterns and general position of mussels along transects were similar from 2006 to 2007.

Data Analysis of Site Habitat Comparisons

I used SAS 9.2 and SAS Studio 3.1 to analyze the mussel count and habitat data. A combination of descriptive, graphical, and statistical analyses was used to examine the habitat and density patterns at each site and to assess whether conditions were similar at the four sites and between the high and low density strata within a site.

Graphical representations of each site were generated using SAS Studio 3.1. I generated separate contour plots of depth, percent medium and coarse gravel, and mussel density from quadrat data. The plots represented the variable of interest as a response variable of cross-stream (x-axis) and upstream distance (y-axis). The generated contours are level curves of the response function. These plots were useful in identifying general density and habitat spatial patterns at each site.

Scatter plots were also created to qualitatively examine the relationship between depth and the number of mussels per quadrat at each site. The relationship between percent gravel (medium plus coarse) and the number of mussels per quadrat at each site was also described.

Two sample non-parametric tests were used to examine differences in location and distribution between all four sites for the variables depth and percent gravel. For these tests I only used the data from the high density strata at the sites. The Wilcoxon rank sum test was used to examine similarities in location and the Kolmogorov-Smirnov test was used to examine similarities in distribution. I also tested whether there were differences in location between the high and low density strata at all four sites in each substrate category.

Discriminant Analysis of Mussel Density and Habitat Patterns

Categorical analysis was used because of an overabundance of zero counts per quadrat at the sites. I divided the quadrats into four categories based on the count of individuals present in a quadrat: mussels absent, one mussel, two mussels, and three or more mussels. The data could then be assessed to determine if the density categories could be discriminated based on the habitat conditions in the area of each quadrat. Habitat that supported three or more mussels in a quadrat was considered optimal mussel habitat. Quadrats with a high count were often found near the center of the mussel bed. Occasionally quadrats that were in locations distant from the densest part of the mussel bed would contain one or two mussels and have similar conditions to adjacent quadrats where mussels were absent. By grouping the quadrats by count, the habitat differences in the densest part of the mussel bed (three or more mussels in a quadrat) could be compared to regions lacking mussels.

I used canonical discriminant analysis to build discrimination models that could summarize the variation between classes based on the habitat variables. Canonical Discriminant Analysis is a dimension reduction technique that analyzes between-class variation by generating canonical variables from linear combinations of a set of predictor quantitative variables. I used this technique to generate linear combinations of the habitat variables that could best predict the classification variable (density groups). I was interested in the predictive power of the models and the role individual habitat variables

played in the models (determined from the sign and magnitude of the pooled-within-class canonical structure coefficients).

The Step-wise Discriminant (STEPDISC) procedure was used to select a subset of the quantitative variables that could discriminate between the four density groups. Stepwise selection removes variables that do not contribute to the discriminatory power of the model as measured by Wilks' lambda. Variables are selected if they meet the criterion to stay in the model. Because different conditions at each site were associated with density patterns, separate discriminant models were constructed at each site for greater accuracy in predicting whether a particular quadrat contained mussels. The mean, standard deviation, 90 % confidence interval, maximum value, and minimum value were computed for all variables selected by the STEPDISC procedure. Univariate one-way ANOVA's were conducted to determine if group means were equal for each variable. The variables selected by the STEPDISC procedure were then used in canonical discriminant (CANDISC) analysis to build discrimination models that could summarize the variation between classes based on the habitat variables.

The prior probability of group membership was assumed to be equal. The CANDISC procedure generated multivariate statistics, a Mahalanobis distance matrix, and the canonical structure with correlations between the canonical variables and the original variables. In addition raw coefficients and the between canonical structure coefficients were examined to assess the relative importance of each quantitative variable in the components. I plotted the observations with corresponding group membership on the components that were significantly correlated with group membership to assess whether the majority of observations could be grouped along the axes. The observations

were then classified by the resubstitution method to determine if their group membership could be predicted based on canonical component scores. The classification error rate for each density group and the error rate associated with misclassifying a quadrat that contained mussels into the absent group were generated. In addition the procedure generated a predicted group membership variable for each observation based on its canonical scores. The misclassification of quadrats where mussels were present into the 'absent' category corresponds to the percent of quadrats sampled in unfavorable habitat where mussels were found. Conversely, the misclassification of quadrats where mussels were absent into the 'present' category corresponds to the percent of quadrats in favorable habitat that did not contain mussels.

Results

General Description of Site Density Patterns and Microhabitat

The majority of mussels were located in the shallow portion of Site 1 adjacent to the left downstream bank (Figure 1). Higher percentages of gravel were found in the shallow areas closest to the bank and the downstream portion of the site near the island. Depth increased with distance from the bank and the mussel density decreased. The portion of the stream adjacent to the opposite bank was shallow, contained a low percentage of gravel particles, and mussels were absent.

Site 2 had the lowest mussel density of the four sites (Figure 2). The site was generally shallow with large particle sizes including many boulders. The majority of mussels were

Figure 1. Contour plots of the variables: depth (A), percent medium and coarse gravel (B), and mussel density (C) measured at Site 1. The x-axis represents cross-stream distance (m) from the left downstream bank and the y-axis represents upstream distance (m) from the downstream site boundary. Contour levels are displayed on each plot. Depth (A) was measured in m at regular intervals along 20 transects, percent gravel (B) represents the percent of particles within the 2 to 32 mm range collected during the pebble count along 5 transects and mussel density (C) is the number of mussels collected in 0.25 m² quadrats during the systematic survey. Blank spaces represent missing data.

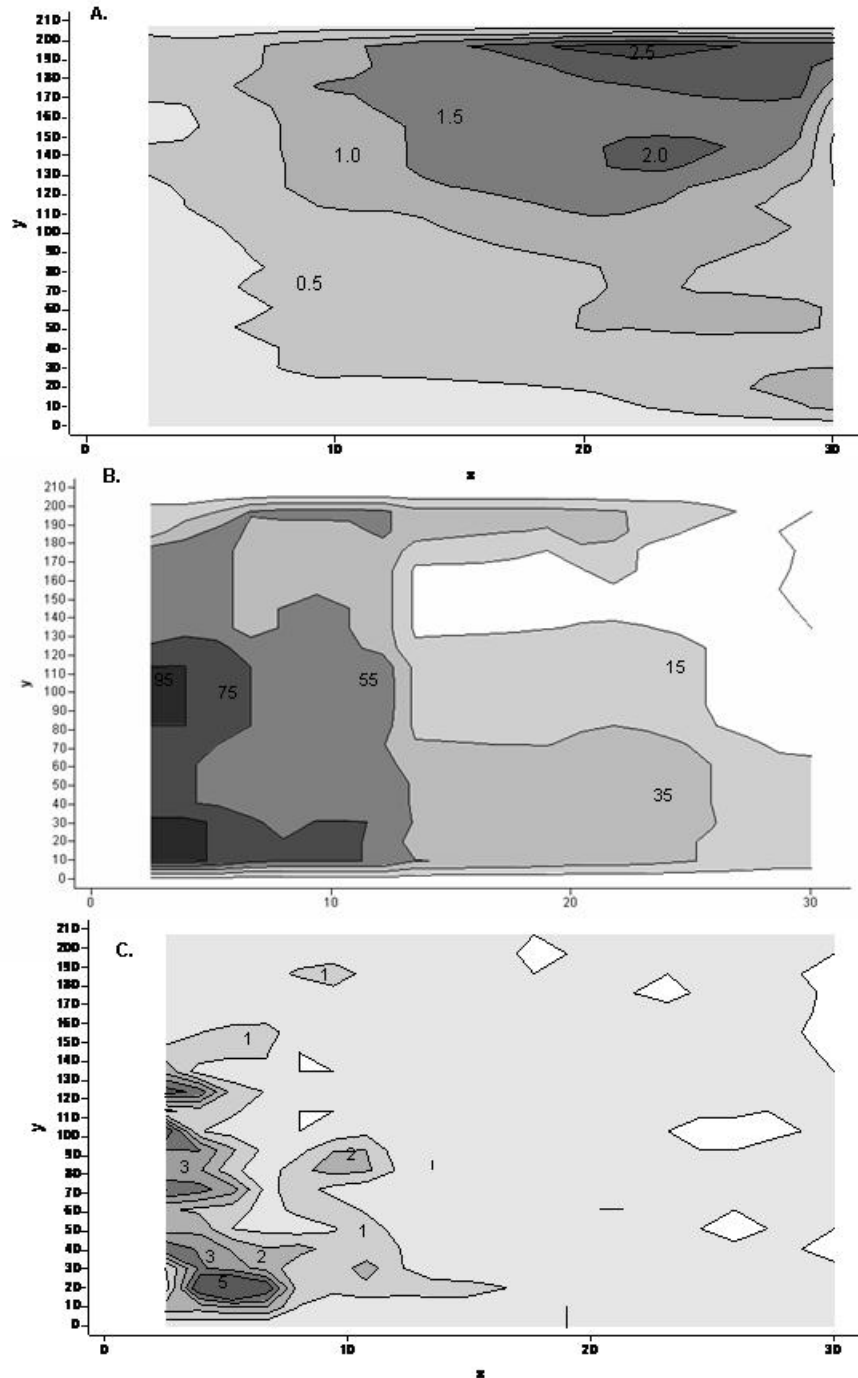


Figure 2. Contour plots of the variables: depth (A), percent medium and coarse gravel (B), and mussel density (C) measured at Site 2. The x-axis represents cross-stream distance (m) from the left downstream bank and the y-axis represents upstream distance (m) from the downstream site boundary. Contour levels are displayed on each plot. Depth (A) was measured in m at regular intervals along 20 transects, percent gravel (B) represents the percent of particles within the 2 to 32 mm range collected during the pebble count along 5 transects and mussel density (C) is the number of mussels collected in 0.25 m² quadrats during the systematic survey. Blank spaces represent missing data.

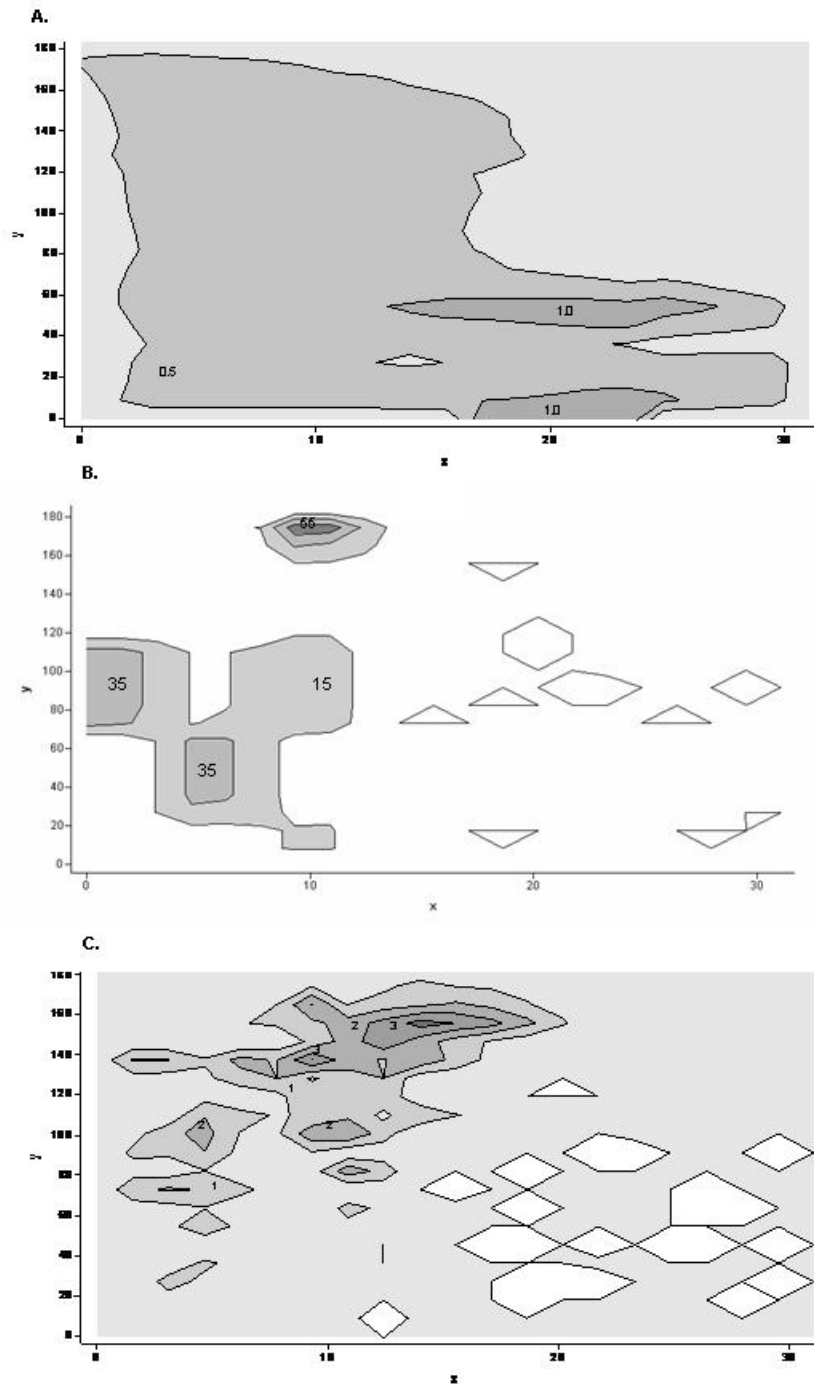


Figure 3. Contour plots of the variables: depth (A), percent medium and coarse gravel (B), and mussel density (C) measured at Site 3. The x-axis represents cross-stream distance (m) from the left downstream bank and the y-axis represents upstream distance (m) from the downstream site boundary. Contour levels are displayed on each plot. Depth (A) was measured in m at regular intervals along 20 transects, percent gravel (B) represents the percent of particles within the 2 to 32 mm range collected during the pebble count along 5 transects and mussel density (C) is the number of mussels collected in 0.25 m² quadrats during the systematic survey. Blank spaces represent missing data.

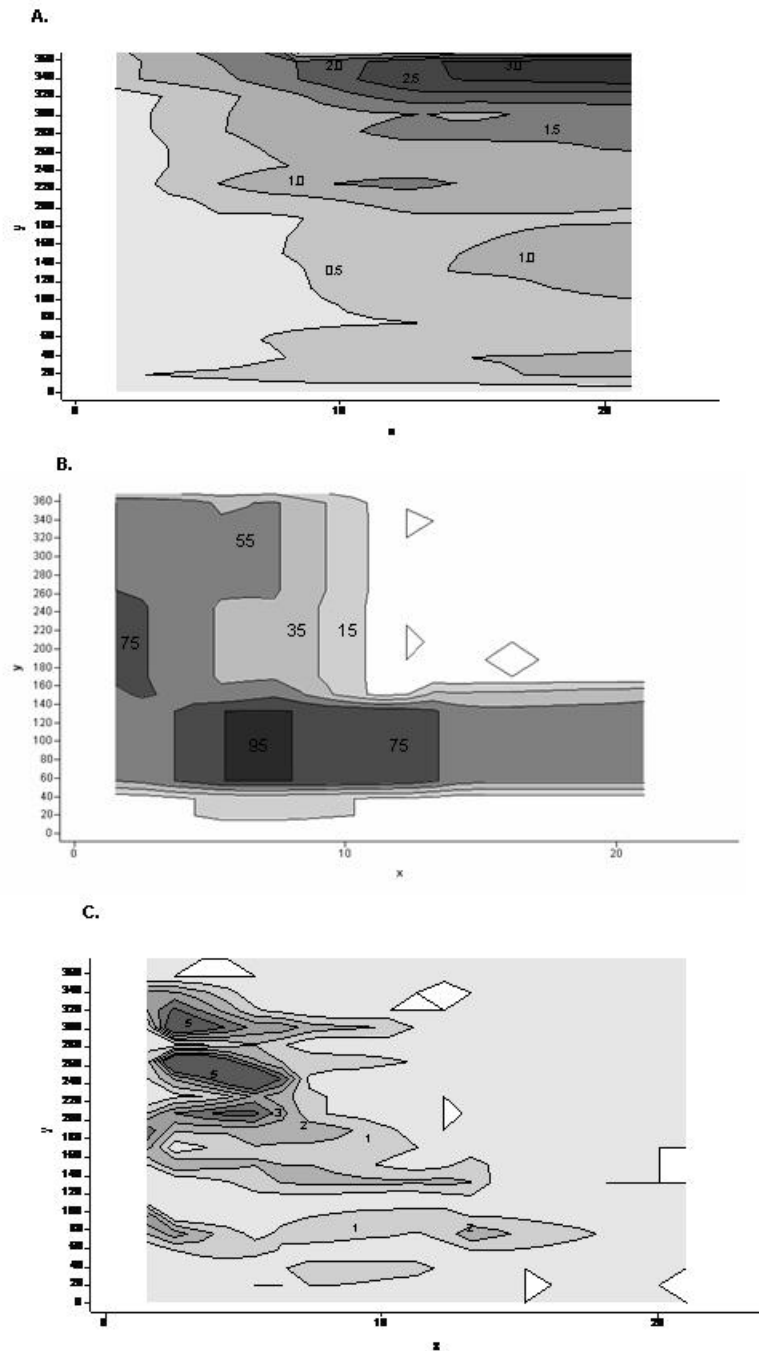
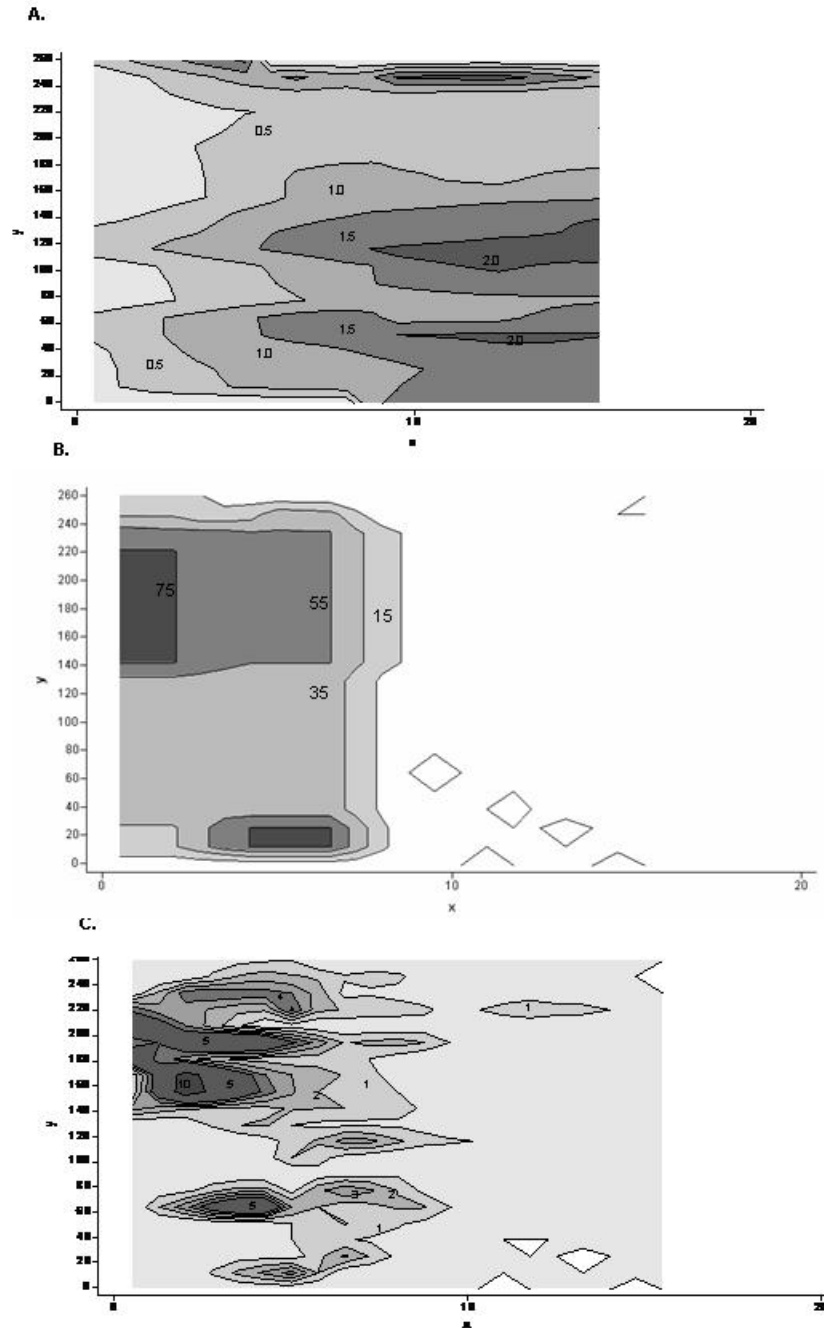


Figure 4. Contour plots of the variables: depth (A), percent medium and coarse gravel (B), and mussel density (C) measured at Site 4. The x-axis represents cross-stream distance (m) from the left downstream bank and the y-axis represents upstream distance (m) from the downstream site boundary. Contour levels are displayed on each plot. Depth (A) was measured in m at regular intervals along 20 transects, percent gravel (B) represents the percent of particles within the 2 to 32 mm range collected during the pebble count along 5 transects and mussel density (C) is the number of mussels collected in 0.25 m² quadrats during the systematic survey. Blank spaces represent missing data.



found within the upstream portion of the site about 10-20 meters from the left downstream bank. Less than 15 % of the particles collected in this section were gravel.

At Site 3, mussels were not located in the deep portion of the stream which contained less than 15 % gravel (Figure 3). The substrate in this section was generally bedrock or silt. The majority of mussels were located in the shallow area near the bank where gravel comprised 35 % or more of the particles. The site contained woody debris including several large trees near the 100 meter mark on the y axis. The instream trees allowed for gravel bars to accumulate at distances greater than 10 meters across the stream and provided shallow water habitat. Mussels were located in these areas but they were not present in high densities.

At Site 4, mussels were highly clustered above the 140-meter mark on the left-downstream bank (Figure 4). The area was shallower and at least 55 % of the particles were gravel. As distance from the bank increased, the percentage of gravel decreased, and particle size increased. Very few mussels were located in that area. Below the 140 mark, mussels were clustered in patches where high percentages of gravel were present. The depth increased significantly with distance from the bank and percentage of gravel decreased. Mussels were not found past the 10 meter x-axis mark within the downstream portion of the site.

At Sites 1, 3, and 4 mussels were clustered in the shallowest portions of the site (Figure 5). At Site 1, two or more mussels per quadrat were found in depths less than 1.5 meters. Mussel density was high in the shallower portions of the site where depth ranged from approximately 0.25 to 0.75 meters. Mussels at Site 2 were present in depths up to 1.2 meters with the majority located where depth ranged from 0.4 to 1.0 meters. Mussels

were generally located in depth less than 2 meters at Site 3 with higher numbers in depth less than approximately 1.25 meters. At Site 4, mussels were found in depth up to 2.5 meters. Very high densities were found in the shallowest portions of the site at approximately 0.5 meters or lower. However, a high number of individuals per quadrat could be still be found in areas ranging up to 2 meters in depth at this site.

Higher densities of mussels were found in areas containing a higher percentage of gravel at Sites 1, 3, and 4 (Figure 6). In general, more individuals were located in quadrats where the particles were at least 45% gravel. At Site 2, the percentage of gravel was less of a factor.

Figure 5. Count of individuals in each quadrat plotted with depth (m) at the four sites (1-4).

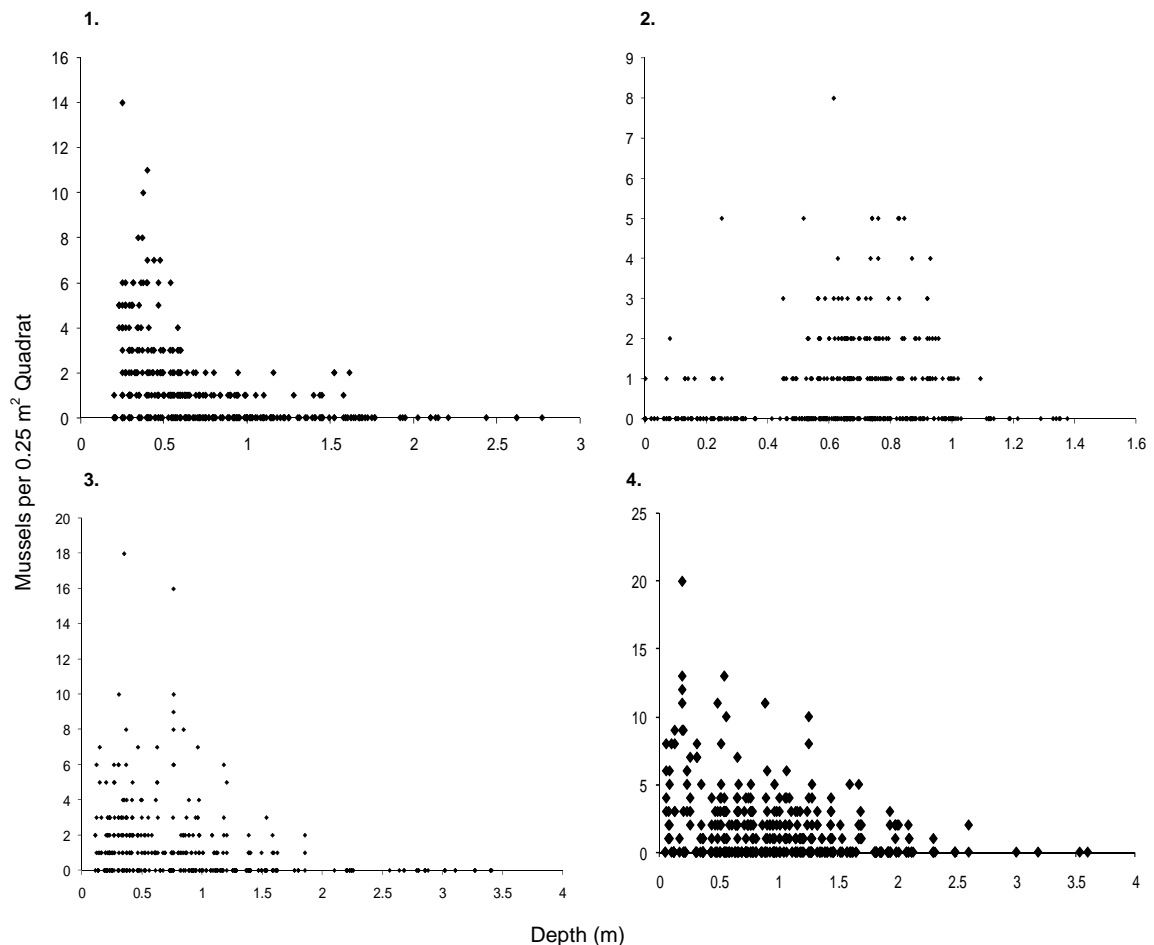
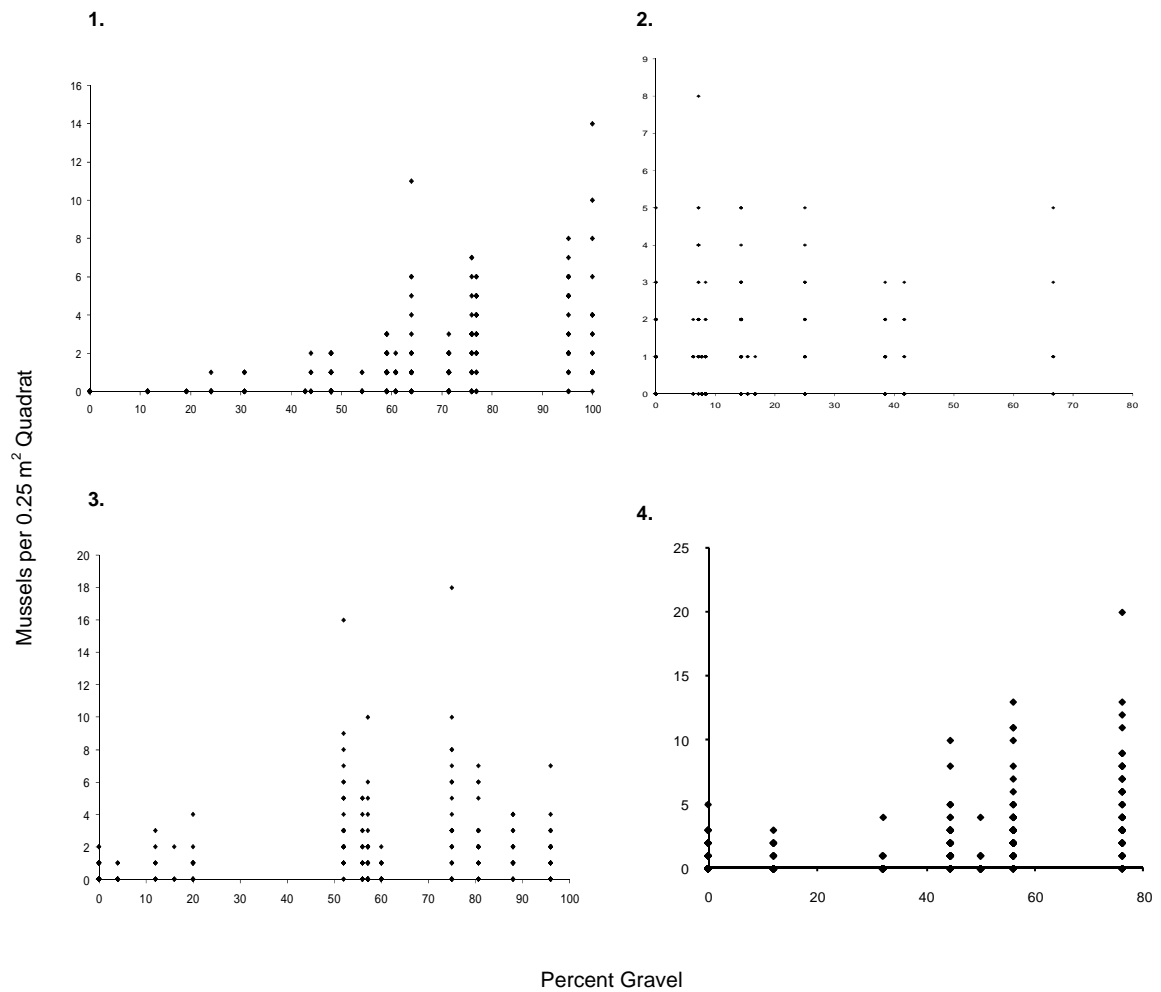


Figure 6. Count of individuals in each quadrat plotted with percent medium and coarse gravel at the four sites (1-4).



Site Comparisons

The tests for location and distribution indicated that percent gravel (medium and coarse) was significantly different between the high density strata of the four sites ($P <$

0.001, Figure 7A, Table 3). The Wilcoxon scores ranked Site 1 as the highest, followed by Site 3, Site 4 and Site 2. The median value at Site 1 was 71.4% gravel with a 25% quartile value of 59.1% and 75% quartile value of 76.9%. The median values for percent gravel at the other sites were significantly lower. In the high density stratum at Site 1, 90% of the quadrats were in areas that were composed of at least 48.0 % gravel and 10% of quadrats were comprised of at least 95.2% gravel. At Site 3, 90% of the quadrats were comprised of at least 16% gravel and 10% of quadrats contained at least 96% gravel. The standard deviation for percent gravel was highest at Site 3 and the mean (59.6%) and median (57.2%) were significantly lower than the mean and median percent gravel at Site 1 ($P < 0.0001$). Percent gravel was significantly lower within the high density stratum at Site 2 than the other sites ($P < 0.0001$).

Tests for location and distribution indicated that depth was significantly different between the four sites ($P < 0.001$, Figure 7B, Table 3). The median depth at Site 3 (0.40 m) was lower than the other sites. The site with the next lowest median depth was Site 1 (0.55 m), followed by Site 2 (0.67 m). The highest median depth was at Site 4 (0.73 m).

The Wilcoxon rank sum tests indicated that the substrate composition differed between the high and low density strata at each site (Figure 8). At Site 1 and Site 4, the location of each substrate category was not similar between the high and low density strata ($P < 0.05$). The similarities between strata at Site 3 were in percent silt and percent very coarse gravel. At Site 2 there were no significant differences in percent very coarse gravel, percent small cobble and percent bedrock between the high and low density strata. At all four sites (with the exception of the bedrock category at Site 2), the three largest particle size categories (percent large cobble, percent boulder, and percent bedrock) had

significantly higher values in the low density strata. At Sites 1, 3, and 4 the low density strata also had higher values for percent small cobble. In the high density stratum at Site 1, the particle sizes consisted mainly of medium, coarse, and very coarse gravel. Small cobble and sand were also present but found in relatively low quantities. In the low density stratum at this site there was a shift to larger particle sizes and silt.

Within the high density stratum at Site 3 there was slightly lower amounts of gravel (including very coarse gravel) than at Site 1 but higher quantities of sand and silt. There was also a very low amount of large cobble in the high density stratum at Site 3. The shift to larger particle sizes in the low density stratum occurred at Site 3. Percent gravel decreased as percent small and large cobble increased. Boulders and bedrock were also found in the low density stratum at Site 3.

In the high density stratum at Site 4 there was less medium gravel and more small cobble than Sites 1 and 3. The low density stratum contained a high quantity of bedrock and little medium and coarse gravel. At Site 2, there was the highest percentage of silt and sand in the high density stratum compared to the other sites. There were also larger particles and less gravel. The low density stratum was comprised mainly of small cobble, large cobble, and boulders.

Table 3. Summary statistics for the high density strata at all four sites. The table displays the sample size, mean, median, standard deviation, quartiles, and 10th and 90th percentiles for the variables depth and percent gravel (medium and coarse).

Percent Gravel

Site	n	Mean	Median	Standard Deviation	Lower Quartile	Upper Quartile	10th Percentile	90th Percentile
1	297	69.6	71.4	17.6	59.1	76.9	48.0	95.2
2	405	13.4	8.3	14.5	0	16.7	0	38.5
3	252	59.6	57.2	26.2	52	80.6	16.0	96.0
4	290	52.9	50	16.3	44.4	56	32.1	76.0

Depth (m)

Site	n	Mean	Median	Standard Deviation	Lower Quartile	Upper Quartile	10th Percentile	90th Percentile
1	297	0.64	0.55	0.37	0.35	0.84	0.27	1.23
2	405	0.66	0.67	0.23	0.57	0.79	0.24	0.92
3	252	0.59	0.4	0.45	0.25	0.85	0.2	1.25
4	290	0.76	0.73	0.47	0.37	1.07	0.17	1.33

Figure 7. Box plots comparing percent medium and coarse gravel (A) and depth (B) within the high density strata at four sites on the Marais des Cynges River. Plots depict the mean (+ symbol), median, quartile, and range values for the variables at each site. Two sample non-parametric tests indicate that the location and distributions at the four sites are significantly different for both variables ($P < 0.05$).

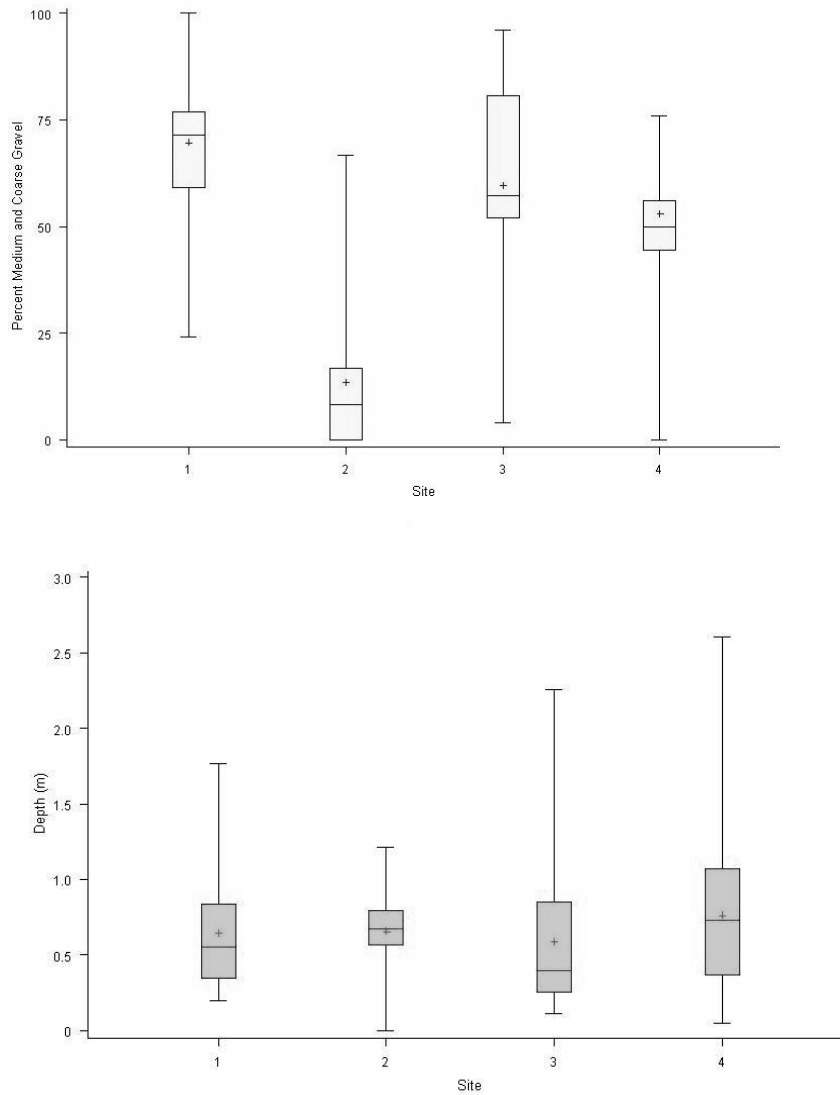
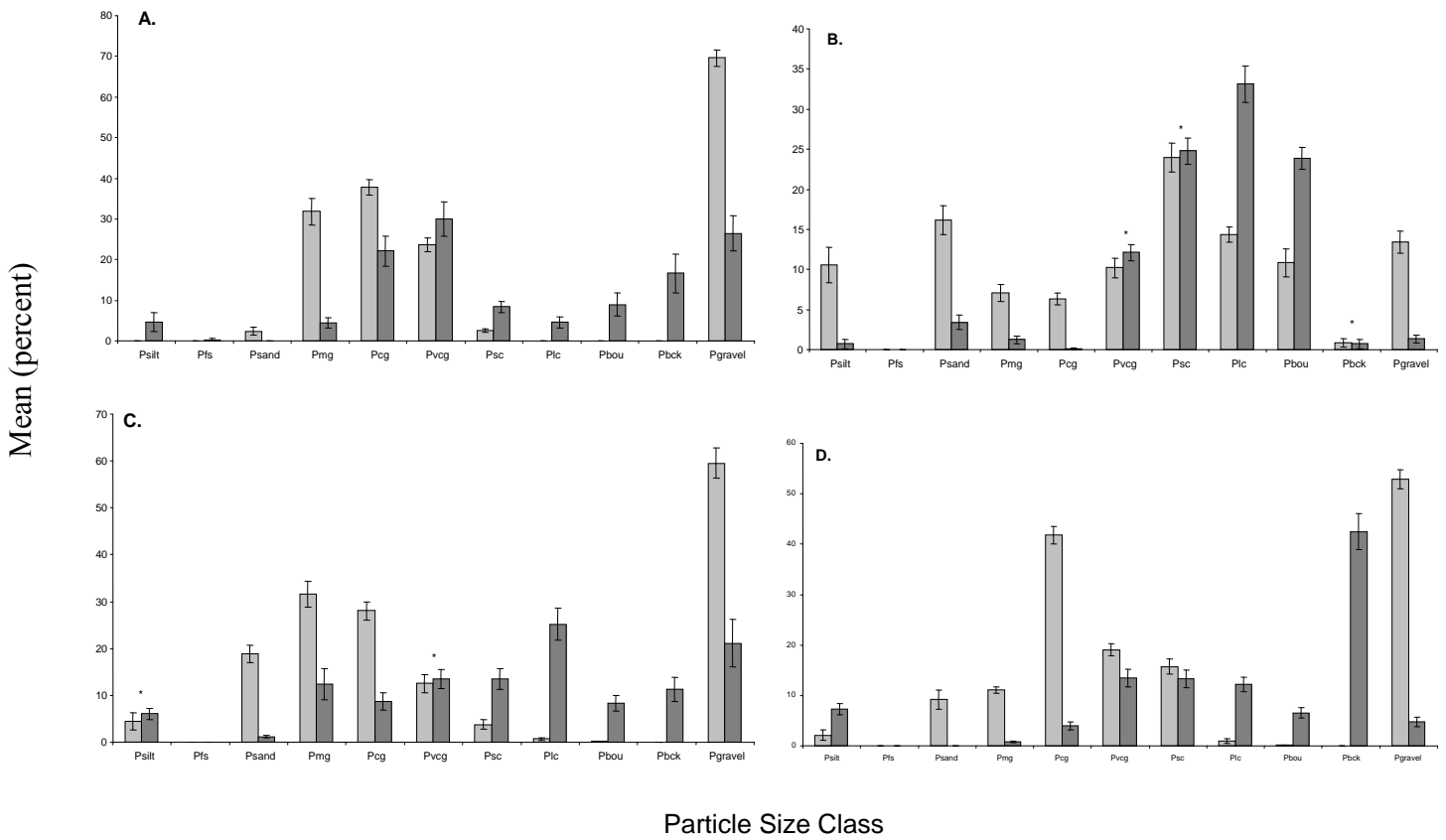


Figure 8. Mean percents of each particle size class in the high (light bars) and low (dark bars) density strata with 95% Confidence Intervals at the four sites (A=Site 1, B=Site 2, C=Site 3, D=Site 4). All differences between the high density stratum mean and the low density stratum mean for a given particle class are significant ($P < 0.05$) at the four sites with the exception of particle size classes marked with an asterisk (*). Particle size categories include; Psilt=percent silt, Pfs=percent fine sand, Psand=percent sand, Pmg=Percent medium gravel, Pcg=percent coarse gravel, Pvcg=percent very coarse gravel, Psc=percent small cobble, Plc=percent large cobble, Pbou=percent boulder, Pbck=percent bedrock, and Pgravel= percent medium gravel + percent coarse gravel.



Density Class Frequency Distribution at the Four Sites

When quadrats were classified into density groupings, approximately 50 % of quadrats in Sites 1, 3, and 4 contained no mussels and Site 1 had a slightly lower proportion of quadrats with no mussels than Sites 3 and 4 (Table 4). Mussels were absent from 68.4% of quadrats at Site 2. Site 2 also a lower proportion of quadrats containing two or more mussels than the other sites. The majority of quadrats at Sites 1, 3, and 4, where mussels were present contained two or more individuals.

Table 4. Description of class variables used to categorize quadrats based on density and the frequency of each group at the four sites.

Class		Proportion			
Description	Label	Site 1	Site 2	Site 3	Site 4
mussels absent	absent	0.465	0.684	0.524	0.518
one mussel present	one	0.234	0.173	0.193	0.136
two mussels present	two	0.18	0.057	0.160	0.221
three or more mussels present	three	0.121	0.086	0.124	0.125

Canonical Discriminant Analysis of Site 1

The STEPDISC procedure selected four microhabitat variables at Site 1 that could potentially discriminate between the four density groups. The variables were used to develop discrimination models. At Site 1, the four variables selected by the procedure

were percent gravel (medium and coarse gravel 2-32 mm), depth, percent bedrock, and average particle size. Density level increased with mean percent gravel and decreased as mean depth and mean percent bedrock increased. Density level increased as mean average particle size decreased (Table 5). Quadrats containing three or more mussels were located in a very narrow depth range (0.23-0.60 m). Additionally the high density quadrats were in areas where gravel comprised at least 59.09% of the particles. Quadrats containing three or more mussels were located in areas where average particle size ranged from 10 mm to 37.5 mm. All percentages were converted to decimals before further analysis. The univariate tests indicated that group means were not equal for the each of the four variables (F values: gravel=60.95, depth=49.24, bedrock=13.10, average particle size=13.69, $df=3$, $P < 0.0001$).

The multivariate one-way analysis of variance (MANOVA) indicated that not all of the mean class vectors of the variables were equal (Wilks' Lambda=0.59, $df=12$, $P < 0.0001$). The Mahalanobis distance matrix (Table 6) indicated that there was a significant difference between the absent group and the other three groups where mussels were present ($P < 0.001$). There were also significant differences between quadrats with one mussel and quadrats with three or more ($P < 0.001$), and differences between quadrats with two mussels and quadrats with three or more ($P < 0.05$).

The first canonical variable was correlated with the classification variable ($R=0.624$, Table 7). The likelihood ratio tested the null hypothesis that the canonical correlations are zero. At Site 1 only the first canonical correlation was significantly different than zero ($P < 0.0001$).

Table 5. Microhabitat variables identified by the stepwise discriminant procedure as significant in discriminating between the density groups at Site 1. The mean, standard deviation, 90% confidence levels for the mean, and maximum and minimum univariate statistics were computed for each density group.

Class	Variable	mean	stdev	lower 90%	upper 90%	max	min
Absent	Gravel (%)	46.51	24.27	43.46	49.56	100.00	0
	Depth (m)	1.0232	0.5395	0.9554	1.0911	2.7737	0.2000
	Bedrock (%)	7.16	15.97	5.15	9.16	60.00	0
	Average particle size (mm)	512.70	865.95	403.83	621.58	3072.00	10.00
one	Gravel (%)	66.59	18.07	63.37	69.82	100.00	24.13
	Depth (m)	0.6412	0.2936	0.5889	0.6936	1.5795	0.2000
	Bedrock (%)	0.10	0.63	0	0.21	4.17	0
	Average particle size (mm)	166.32	468.87	82.74	249.91	2064.50	10.00
two	Gravel (%)	73.39	17.05	69.12	77.66	100.00	44.00
	Depth (m)	0.5658	0.3239	0.4846	0.6469	1.6154	0.25
	Bedrock (%)	0	0			0	0
	Average particle size (mm)	69.43	302.95	0.00	145.31	2055.50	10.00
three	Gravel (%)	82.24	12.98	79.60	84.89	100.00	59.09
	Depth (m)	0.3675	0.1084	0.3454	0.3896	0.6000	0.2300
	Bedrock (%)	0	0			0	0
	Average particle size (mm)	20.74	9.06	18.90	22.59	37.50	10.00

Table 6. Squared Mahalanobis distance matrix between class means (bold) and F statistics (below each distance) for the variables: percent gravel, depth (m), percent bedrock, and average particle size (mm) at Site 1. The significance levels are: $P < 0.05^*$, $P < 0.01^{**}$, and $P < 0.001^{***}$.

	absent	one	two	three
absent		1.335*** 19.16	2.132*** 16.877	4.007*** 27.99
one			0.154 1.131	0.957*** 8.979
two				0.376* 2.153
three				

Table 7. Results of Canonical Discriminant Analysis with the first two canonical variables at Site 1. The canonical correlations with the classes, eigenvalues, and the likelihood ratio are displayed. The likelihood ratio tests the hypothesis that the canonical correlation and all that follow are zero.

Canonical Variable	Canonical Correlation	Approximate Standard Error	Eigenvalue	Proportion	Cumulative	Likelihood Ratio	F value	DF	Pr>F
1	0.624	0.0317	0.6376	0.965	0.965	0.5968	17.34	12	<.0001
2	0.1463	0.0508	0.0219	0.0331	0.998	0.9774	1.4	6	0.2109

Table 8. The table displays the total and pooled within class canonical structure (correlations between canonical variable 1 and the original variables either in the total sample or pooled within the classes), the pooled within-class standardized canonical coefficients, and the raw canonical coefficients at Site 1. Variable abbreviations located in Table 2.

	Total Canonical Structure	Pooled Within Canonical Structure	Pooled Within-Class Standardized Can. Coefficients	Raw Canonical Coefficients
Variable	Can1	Can1	Can1	Can1
pgravel	0.9231	0.8826	0.627	3.0172
depth	-0.8571	-0.7929	-0.537	-1.2983
pbck	-0.4671	-0.384	0.15	1.3742
avg. particle	-0.50167	-0.4133	-0.191	-0.0003

Table 9. Density class means of canonical variable 1 at Site 1.

Count	Can1
absent	-0.797
one	0.322
two	0.656
three	1.199

The total and pooled within canonical structure indicated that the first canonical variable had a strong positive correlation with gravel and a strong negative correlation with depth (Table 8). Bedrock and average particle size were negatively correlated the first canonical variable. The first canonical component consisted of the following linear combination of the centered variables and raw canonical coefficients:

$$\text{Can 1} = 3.0172 \text{ Gravel} - 1.2983 \text{ Depth} + 1.3742 \text{ Bedrock} - 0.0003 \text{ Avg. particle size}$$

The class mean on canonical variable 1 was negative for quadrats where mussels were absent (Table 9). Where mussels were present, the class mean on canonical variable 1 was positive with the mean increasing as count increased. The plot of the first two canonical variables indicates that canonical variable 1 does not completely separate the groups but the majority of samples where mussels were absent displayed a negative canonical 1 value (Figure 9). Quadrats where two and three or more individuals were found had positive canonical variable 1 values.

There were many samples misclassified into groups (53.01%, Table 10) with the first canonical component. The error rate was highest when attempting to classify samples that had one or two mussels in a quadrat. Quadrats with no mussels were misclassified as containing mussels 35.26% of the time. Quadrats with three or more mussels were misclassified 31.34 % of the time, but were not misclassified into the mussel absent group. The percentage of quadrats where mussels were present that were misclassified as absent based on the first canonical component was 13.57%. This percent error corresponds to the percentage of quadrats in areas of unfavorable favorable habitat (low canonical component 1 scores) that contained mussels. The 35.26 % of quadrats with no mussels that were misclassified as having mussels corresponds to the error associated with the percentage of quadrats in favorable habitat that did not contain mussels.

Figure 9. Plot of first two canonical variables at Site 1. Data points represent four class levels: quadrats where three or more individuals were located, quadrats containing two individuals, quadrats containing one individual, and quadrats where mussels were absent. Only the first canonical variable (x-axis) significantly distinguishes among density classes ($P < 0.05$).

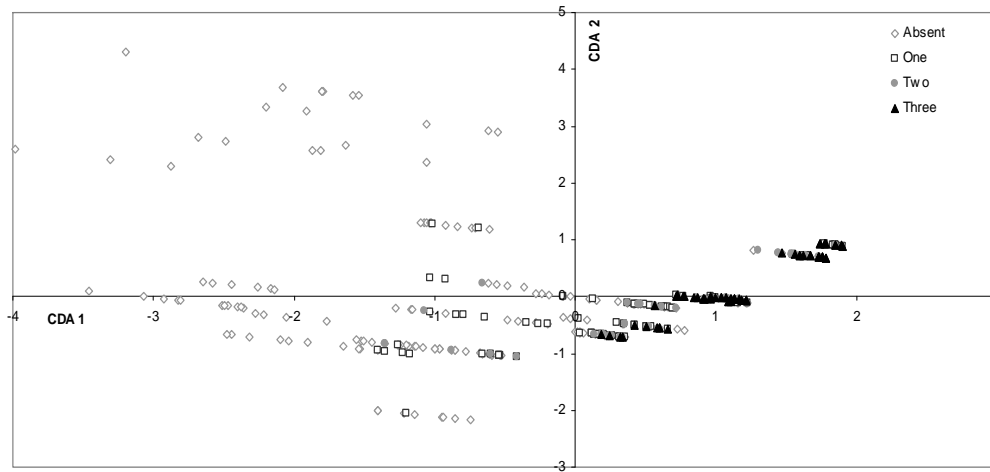


Table 10. A. Summary table with the number and proportion of quadrats classified into each group based on the linear discriminant function at Site 1. An equal prior probability of group membership was assumed. B. Error rate estimates for classifications.

A.						B.	
From Count	absent	one	two	three	total	Class	Error Rate
absent	112	31	15	15	173	none	0.3526
	64.74	17.92	8.67	8.67	100	one	0.6322
one	21	32	14	20	87	two	0.8222
	24.14	36.78	16.09	22.99	100	three	0.3134
two	6	12	8	19	45	total	0.5301
	13.33	26.67	17.78	42.22	100	Absent	0.3526
three	0	10	11	46	67	Present	0.1357
	0	14.93	16.42	68.66	100		

Canonical Discriminant Analysis of Site 2

The stepwise discriminant procedure identified five habitat variables that could potentially discriminate between the density groups at Site 2: percent small cobble, percent gravel, percent very coarse gravel, percent boulder, and depth. Quadrats where mussels were absent had a lower percent small cobble mean, a lower percent gravel and very coarse gravel mean, and a higher percent boulder mean than the other density groups (Table 11). The quadrats in this group also were also shallower on average than the other groups. The mean percentage of small cobble, gravel, and very coarse gravel increased when density group and the mean percentage of boulders decreased except between the two mussels in a quadrat group and three or more mussels in a quadrat group. Mean depth increased slightly with density group. Mussels were found in a wide range of habitat conditions at Site 2 but quadrats in the two and three or more classes had smaller ranges of percent boulder and depth than the other classes. The univariate test indicated that group means were not equal for the five variables (F values: small cobble=21.99, gravel=11.01, very coarse gravel=18.12, boulder=20.01, depth=9.60; df=3; $P < 0.0001$).

The multivariate one-way analysis of variance (MANOVA) indicated that not all of the mean class vectors were equal (Wilks' Lambda=0.7363, df=15, $P < .0001$). The Mahalanobis distance matrix (Table 12) indicated that there was a significant difference between the absent group and the other three groups where mussels were present ($P < 0.001$). There was also a significant difference between quadrats with one mussel and quadrats with three or more ($P < 0.01$).

Table 11. Microhabitat variables identified by the stepwise discriminant procedure as significant in discriminating between the density groups at Site 2. The mean, standard deviation, 90% confidence levels for the mean, maximum and minimum univariate statistics were computed for each density group.

Class	Variable	mean	stdev	lower 90%	upper 90%	max	min
none	Small cobble (%)	20.69	16.03	19.32	22.06	53.85	0
	Gravel (%)	8.13	12.51	7.06	9.20	66.67	0
	Very Coarse Gravel (%)	8.69	8.77	7.94	9.44	42.86	0
	Boulders (%)	17.64	18.13	16.09	19.19	75.00	0
	Depth (m)	0.5345	0.3397	0.5055	0.5635	1.3767	0
one	Small cobble (%)	28.76	15.88	26.04	31.48	53.85	0
	Gravel (%)	14.37	15.57	11.70	17.03	66.67	0
	Very Coarse Gravel (%)	12.51	13.44	10.21	14.82	42.86	0
	Boulders (%)	9.24	12.58	7.09	11.40	61.54	0
	Depth (m)	0.6611	0.2278	0.6220	0.7001	1.0933	0.0013
two	Small cobble (%)	34.44	13.81	31.06	37.82	53.85	0
	Gravel (%)	14.58	12.06	11.62	17.53	41.67	0
	Very Coarse Gravel (%)	16.68	15.31	12.93	20.43	42.86	0
	Boulders (%)	3.09	4.85	1.90	4.27	16.67	0
	Depth (m)	0.7089	0.1505	0.6720	0.7457	0.9567	0.0810
three	Small cobble (%)	36.49	12.54	32.67	40.32	53.85	0
	Gravel (%)	17.64	16.44	12.63	22.65	66.67	0
	Very Coarse Gravel (%)	20.15	14.28	15.80	24.50	42.86	0
	Boulders (%)	4.46	6.34	2.52	6.39	20.00	0
	Depth (m)	0.7019	0.1470	0.6571	0.7467	0.9300	0.2500

Table 12. Squared Mahalanobis distance matrix between class means (bold) and F statistics (below each distance) for the variables: percent small cobble, percent gravel, percent very coarse gravel, percent boulders, and depth (m) at Site 2. The significance levels are: $P < 0.05^*$, $P < 0.01^{**}$, and $P < 0.001^{***}$.

	absent	one	two	three
absent		0.867*** 12.918	2.022*** 16.753	2.991*** 16.993
one			0.302 1.876	0.792*** 3.666
two				0.258 0.958
three				

The first canonical component was correlated with the class variable ($R = 0.5053$, Table 13). The likelihood ratio test of the null hypothesis that the canonical correlation and all that follow were equal to zero indicated that the null should be rejected ($P < 0.0001$, $df=15$) for the first component. The second canonical correlation was not significantly different from zero and was excluded from further analysis.

The analysis of the total and pooled with-in canonical structure indicated that the canonical variable was positively correlated with percent small cobble, percent very coarse gravel, percent gravel, and depth (Table 14). Percent boulder was negatively correlated with the canonical variable. The first canonical component consisted of the following linear combination of the centered variables and raw canonical coefficients:

$$\text{Can 1} = 2.8848 \text{ Small Cobble} + 4.2873 \text{ Gravel} + 3.9359 \text{ Very Coarse Gravel} - 1.946 \text{ Boulder} + 0.8105 \text{ Depth}$$

The class mean on canonical variable 1 was negative for quadrats where mussels were absent (Table 15). Where mussels were present, the class mean on canonical variable one was positive with the mean increasing as count increased. The plot of the first two canonical variables indicates that canonical variable one does not completely separate the groups but the majority of samples where mussels were absent displayed a negative canonical 1 value (Figure 10). Quadrats where two and three or more individuals were found tended to have positive canonical variable 1 values.

There were many samples misclassified into groups (56.59%, Table 16) with the first canonical component. The error rate was highest when attempting to classify

Table 13. Results of Canonical Discriminant Analysis with the first two canonical variables at Site 2. The canonical correlations with the classes, eigenvalues, and the likelihood ratio are displayed. The likelihood ratio tests the hypothesis that the canonical correlation and all that follow are zero.

Canonical factor	Canonical Correlation	Approximate Standard Error	Eigenvalue	Proportion	Cumulative	Likelihood Ratio	F value	DF	Pr>F
1	0.5053	0.0319	0.3429	0.9682	0.9682	0.7363	11.59	15	<.0001
2	0.0888	0.0425	0.0079	0.0224	0.9906	0.9888	0.76	8	0.6414

Table 14. The table displays the total and pooled within class canonical structure (correlations between canonical variable 1 and the original variables either in the total sample or pooled within the classes), the pooled within-class standardized canonical coefficients, and the raw canonical coefficients at Site 2. Variable abbreviations located in Table 2.

	Total Canonical Structure	Pooled Within Canonical Structure	Pooled Within-Class Standardized Can. Coeff.	Raw Canonical Coefficients
Variable	Can1	Can1	Can1	Can1
Psc	0.6517	0.5967	0.4515	2.8848
PGravel	0.4660	0.4142	0.5699	4.2873
Pvcg	0.5852	0.5297	0.4225	3.9359
Pbou	-0.6192	-0.5632	-0.3123	-1.9460
Depth	0.4389	0.3888	0.2350	0.8105

Table 15. Density class means of canonical variable 1 at Site 2.

Count	Can1
Absent	-0.3769
one	0.5396
two	1.0349
three	1.3298

samples that had one or two mussels in a quadrat. Quadrats with no mussels were misclassified as containing mussels 28.15% of the time. Quadrats with three or more mussels were misclassified 32.26 % of the time. The percentage of quadrats where mussels were present that were misclassified as absent based on the first canonical component was 24.42 %. This percent error corresponds to the percentage of quadrats in areas of unfavorable habitat (low canonical component 1 scores) that contained mussels. The 28.15 % of quadrats with no mussels that were misclassified as having mussels corresponds to the error associated with the percentage of quadrats in favorable habitat that did not contain mussels.

Figure 10. Plot of first two canonical variables at Site 2. Data points represent four class levels: quadrats where three or more individuals were located, quadrats containing two individuals, quadrats containing one individual, and quadrats where mussels were absent. Only the first canonical variable (x-axis) significantly distinguishes among density classes ($P < 0.05$).

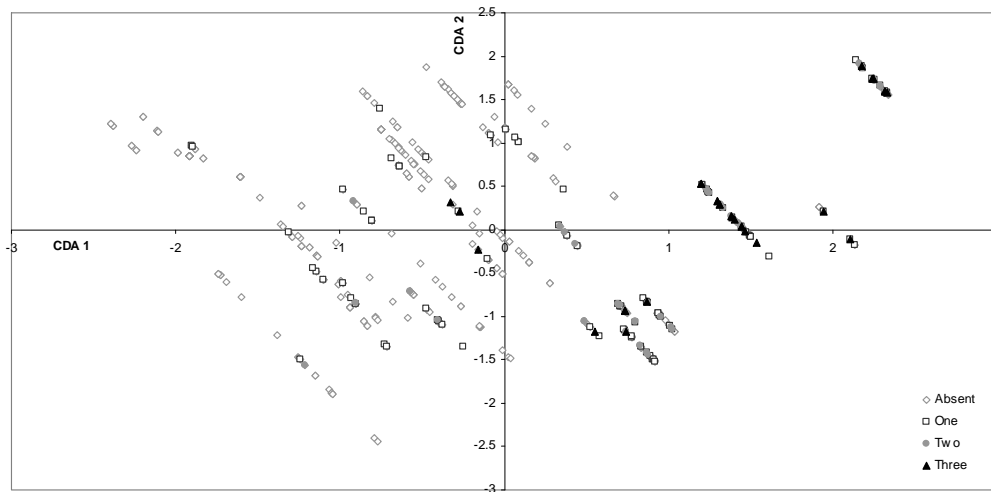


Table 16. A. Summary table with the number and proportion of quadrats classified into each group based on the linear discriminant function at Site 2. An equal prior probability of group membership was assumed. **B.** Error rate estimates for classifications.

A.						B.	
From Count	absent	one	two	three	total	Class	Error Rate
absent	268	57	25	23	373	none	0.2815
	71.85	15.28	6.70	6.17	100	one	0.766
one	34	22	11	27	94	two	0.8936
	36.17	23.40	11.70	28.72	100	three	0.3226
two	5	16	5	21	47	total	0.5659
	10.64	34.04	10.64	44.68	100	Absent	0.2815
three	3	5	2	21	31	Present	0.2442
	9.68	16.13	6.45	67.74	100		

Canonical Discriminant Analysis of Site 3

The stepwise discriminant procedure identified three variables that could potentially discriminate between density groups at Site 3. The variables are percent gravel, depth, and percent very coarse gravel. Mean percent gravel was higher for quadrats that contained mussels and increased with count (Table 17). Mean depth was higher where mussels were absent and decreased with count. Mean percent very coarse gravel was higher where mussels were present but was similar between the three 'present' classes. Depth at the site ranged from 0.11 to 3.41 meters but quadrats where mussels were present ranged from 0.110 to 1.8549 meters. The range of percent gravel and percent very coarse gravel was similar between the four classes. The univariate test indicated that group means were not equal for the variables gravel and depth (F values: gravel=40.43, depth=26.22; df=3; $P<0.0001$).

Table 17. Microhabitat variables identified by the stepwise discriminant procedure as significant in discriminating between the density groups at Site 3. The mean, standard deviation, 90% confidence levels for the mean, and maximum and minimum univariate statistics were computed for each density group.

Class	Variable	mean	stdev	lower 90%	upper 90%	max	min
absent	Gravel (%)	29.65	33.77	25.89	33.41	96.00	0
	Depth (m)	1.1701	0.8290	1.0778	1.2624	3.4100	0.1100
	Very Coarse Gravel (%)	11.50	12.64	10.09	12.90	44.00	0
one	Gravel (%)	49.23	33.69	43.00	55.46	96.00	0
	Depth (m)	0.7086	0.4166	0.6315	0.7856	1.8549	0.1200
	Very Coarse Gravel (%)	14.78	14.80	12.05	17.52	44.00	0
two	Gravel (%)	66.66	24.80	60.90	72.42	96.00	0
	Depth (m)	0.5940	0.3910	0.5031	0.6848	1.8549	0.1100
	Very Coarse Gravel (%)	13.95	16.94	10.01	17.88	44.00	0
three	Gravel (%)	68.38	17.09	64.89	71.86	96.00	12.00
	Depth (m)	0.5159	0.3117	0.4523	0.5794	1.5350	0.1200
	Very Coarse Gravel (%)	14.64	17.96	10.98	18.30	44.00	0

Table 18. Squared Mahalanobis distance matrix between class means (bold) and F statistics (below each distance) for the variables: percent gravel, depth (m), and percent very coarse gravel at Site 3. The significance levels are: $P < 0.05^*$, $P < 0.01^{**}$, and $P < 0.001^{***}$.

	absent	one	two	three
absent		0.734*** 14.421	1.718*** 23.975	1.986*** 33.842
one			0.345* 3.620	0.403** 4.900
two				0.018 0.177
three				

The multivariate one-way analysis of variance (MANOVA) indicated that not all of the mean class vectors were equal (Wilks' Lambda=0.7245, df=9, $P < 0.0001$).

The Mahalanobis distance measures (Table 18) indicated that there was a significant difference between the absent group and the other three groups where mussels were

present ($P < 0.001$). There were also significant differences between quadrats with one mussel and quadrats with three or more mussels ($P < 0.01$), and between quadrats with one individual and quadrats with two individuals ($P < 0.05$).

The first canonical component was correlated with the classification variable ($R = 0.5172$, Table 19). The null hypothesis that the correlation was equal to zero was rejected ($P < 0.0001$). The second canonical component was not significantly correlated to the classification variable and was not used in further analysis.

The first canonical variable was positively correlated with percent gravel and negatively correlated with depth (Table 20). Both the total and pooled within canonical structure indicated that the correlations with these two variables were strong. Percent very coarse gravel had a very weak positive correlation with canonical component 1.

Table 19. Results of Canonical Discriminant Analysis with the first two canonical variables at Site 3. The canonical correlations with the classes, eigenvalues, and the likelihood ratio are displayed. The likelihood ratio tests the hypothesis that the canonical correlation and all that follow are zero.

Canonical Variable	Canonical Correlation	Approximate Standard Error	Eigenvalue	Proportion	Cumulative	Likelihood Ratio	F value	DF	Pr>F
1	0.5172	0.0358	0.3652	0.9708	0.9708	0.7245	15.85	9	<.0001
2	0.1042	0.0483	0.011	0.0292	1	0.9891	1.14	4	0.3385

Table 20. The table displays the total and pooled within class canonical structure (correlations between canonical variable 1 and the original variables either in the total sample or pooled within the classes), the pooled within-class standardized canonical coefficients, and the raw canonical coefficients at Site 3. Variable abbreviations located in Table 2.

	Total Canonical Structure	Pooled Within Canonical Structure	Pooled Within- Class Standardized Can. Coeff.	Raw Canonical Coefficients
Variable	Can1	Can1	Can1	Can1
pgravel	0.9156	0.8906	0.7713	2.5155
depth	-0.7654	-0.7143	-0.3599	-0.5493
pvcg	0.1853	0.1595	0.3511	2.4085

Table 21. Density class means of canonical variable 1 at Site 3.

Count	Can1
absent	-0.5458
one	0.2796
two	0.7609
three	0.8635

The first canonical component consisted of the following linear combination of the centered variables and raw canonical coefficients:

$$\text{Can 1} = 2.5155 \text{ Gravel} - 0.5493 \text{ Depth} + 2.4085 \text{ Very Coarse Gravel}$$

The class mean on canonical variable 1 was negative for quadrats where mussels were absent (Table 21). Where mussels were present, the class mean on canonical variable one was positive with the mean increasing as count increased. The plot of the first two canonical variables indicates that canonical variable one does not completely separate the groups but the majority of samples where mussels were absent displayed a negative canonical 1 value (Figure 11). The majority of quadrats where two and three or more individuals were found had positive canonical 1 values.

At Site 3, there were many samples misclassified into groups (55.22%, Table 22) with the first canonical component. The error rate was highest when attempting to classify samples that had one mussel in a quadrat. Quadrats with no mussels were misclassified as containing mussels 43.18 % of the time. Quadrats with three or more mussels were misclassified 50.75 % of the time but were not misclassified into the ‘absent’ group. Quadrats with two mussels were rarely misclassified into the ‘absent’ group (7.69%). The percentage of quadrats where mussels were present that were misclassified as absent based on the first canonical component was 13.16 %. This percent error corresponds to the percentage of quadrats in areas of unfavorable habitat (low canonical component 1 scores) that contained mussels. The 43.18 % of quadrats with no mussels that were misclassified as having mussels corresponds to the error associated with the percentage of quadrats in favorable habitat that did not contain mussels.

Figure 11. Plot of first two canonical variables at Site 3. Data points represent four class levels: quadrats where three or more individuals were located, quadrats containing two individuals, quadrats containing one individual, and quadrats where mussels were absent. Only the first canonical variable (x-axis) significantly distinguishes among density classes ($P < 0.05$).

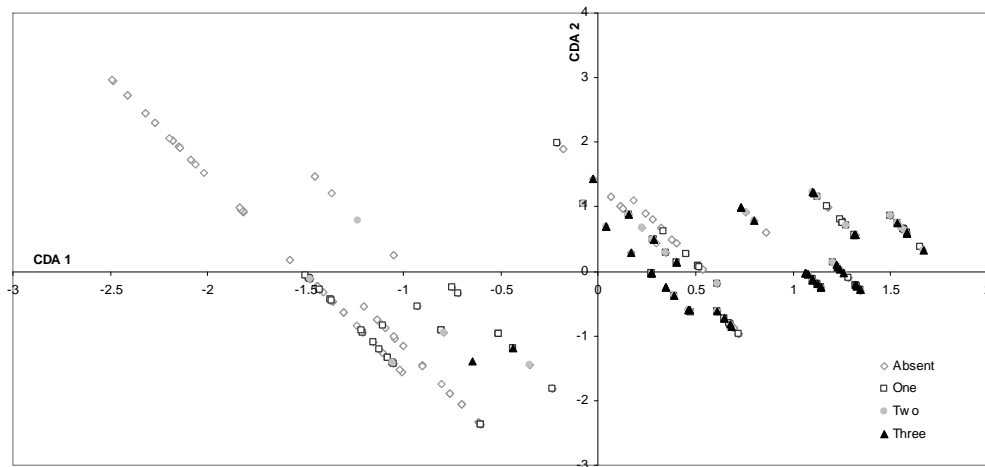


Table 22. A. Summary table with the number and proportion of quadrats classified into each group based on the linear discriminant function at Site 3. An equal prior probability of group membership was assumed. B. Error rate estimates for classifications.

A.						B.	
From Count	absent	one	two	three	total	Class	Error Rate
absent	125	24	52	19	220	none	0.4381
	56.82	10.91	23.64	8.64	100	one	0.8272
one	21	14	26	20	81	two	0.4423
	25.93	17.28	32.10	24.69	100	three	0.5075
two	4	4	29	15	52	total	0.5522
	7.69	7.69	55.77	28.85	100	Absent	0.4318
three	0	7	27	33	67	Present	0.1316
	0	10.45	40.33	49.25	100		

Canonical Discriminant Analysis of Site 4

Seven variables were selected by the stepwise discriminant analysis procedure. The variables were percent gravel, percent sand, percent small cobble, percent very coarse gravel, particle standard deviation, percent silt, and depth. Mean percent gravel was lowest in quadrats not containing mussels and increased with count where mussels were present (Table 23). Mean percent small cobble and very coarse gravel was higher where mussels were present. Mean particle standard deviation decreased with count and was highest for the 'absent' group. Both mean percent silt and mean percent sand were lowest where mussels were present particularly where count was two or more. Mean depth was lowest in the three or more mussel category, but mean depth was similar among the other three classes. The depth ranged from 0.050 meters to 3.600 meters at Site 4 and mussels were present in depths ranging from 0.058 meters 2.600 meters. Silt did not exceed 14 % of the particles in areas where two or more mussels were found. The univariate test indicated that group means were not equal for each of the seven variables (F values: gravel=53.41, sand=12.39, small cobble=12.70, very coarse gravel=16.65, standard deviation=26.44, silt=12.85, depth=13.14, df=3, $P < 0.0001$).

The multivariate one-way analysis of variance (MANOVA) indicated that not all of the mean class vectors were equal (Wilks' Lambda=0.4995, df=21, $P < 0.0001$). The Mahalanobis distance measures (Table 24) indicated that there was a significant difference between the absent group and the other three groups where mussels were present ($P < 0.001$). There were also significant differences between quadrats with one

mussel and quadrats with three or more mussels ($P < 0.001$), and between quadrats with two individuals and quadrats with three or more individuals ($P < 0.01$).

Table 23. Microhabitat variables identified by the stepwise discriminant procedure as significant in discriminating between the density groups at Site 4. The mean, standard deviation, 90% confidence levels for the mean, and maximum and minimum univariate statistics were computed for each density group.

Class	Variable	mean	stdev	lower 90%	upper 90%	max	min
Absent	Gravel (%)	24.60	23.66	22.01	27.18	76.00	0
	Sand (%)	9.71	17.56	7.78	11.63	46.43	0
	Small Cobble (%)	11.67	11.32	10.43	12.90	64.00	0
	Very Coarse Gravel (%)	13.88	11.21	12.66	15.11	33.33	0
	Particle Standard Dev. (mm)	609.55	793.63	522.75	696.36	2047.97	0
	Silt (%)	6.200	10.67	5.03	7.36	44.00	0
	depth (m)	1.1209	0.6722	1.0473	1.1944	3.600	0.0500
one	Gravel (%)	39.36	23.10	34.38	44.34	76.00	0
	Sand (%)	4.90	11.50	2.41	7.38	46.43	0
	Small Cobble (%)	19.13	13.28	16.27	22.00	64.00	0
	Very Coarse Gravel (%)	21.49	10.79	19.16	23.81	33.33	3.57
	Particle Standard Dev. (mm)	158.81	430.85	65.86	251.76	2047.97	0
	Silt (%)	3.10	8.89	1.18	5.02	44.00	0
	depth (m)	1.0384	0.5271	0.9247	1.1521	2.3033	0.0783
two	Gravel (%)	43.44	24.66	37.88	49.01	76.00	0
	Sand (%)	0.81	1.54	0.46	1.16	3.70	0
	Small Cobble (%)	20.22	11.35	17.66	22.78	64.00	0
	Very Coarse Gravel (%)	20.80	9.12	18.74	22.86	33.33	0
	Particle Standard Dev. (mm)	148.92	383.97	62.28	235.57	2047.97	0
	Silt (%)	1.52	4.38	0.53	2.51	14.00	0
	depth (m)	1.0651	0.5637	0.9379	1.1923	2.6000	0.0783

Table 23 cont.

Class	Variable	mean	stdev	lower 90%	upper 90%	max	min
three	Gravel (%)	58.78	19.4	55.51	62.05	76.00	0
	Sand (%)	1.27	5.04	0.43	2.12	46.43	0
	Small Cobble (%)	16.68	12.28	14.61	18.75	64.00	0
	Very Coarse Gravel (%)	20.09	8.31	18.69	21.5	33.33	0
	Particle Standard Dev. (mm)	30.17	100.1	13.29	47.05	994	0
	Silt (%)	0.28	1.96	0	0.61	14.00	0
	depth (m)	0.673	0.4449	0.5979	0.7480	1.9383	0.0583

The first two canonical variables were retained in the analysis at Site 4. Both correlations were significantly different than zero (Table 25). Canonical variable 1 was highly correlated with the classification variable ($R=0.6757$).

The canonical structure indicated that percent gravel had a high positive correlation with canonical variable 1 (Table 26). Percent small cobble and percent very coarse gravel had weaker positive correlations with canonical variable 1. Percent sand, percent silt, particle size standard deviation, and depth were all negatively correlated with variable 1. Canonical component 2 has positive correlations with depth, percent small cobble, percent silt, and percent very coarse gravel. It was negatively correlated with percent gravel, percent sand, and particle size standard deviation. The first two canonical components consisted of the following linear combinations of the centered variables and raw canonical coefficients:

Can 1 = **2.8755** Gravel – **5.3630** Sand + **1.5479** Sc + **0.0928** Vcg - **0.0007** Stdev – **3.0945** Silt – **0.1303** Depth

Can 2 = **-1.0630** Gravel + **3.5671** Sand + **2.9437** Sc + **7.5932** Vcg - **0.0004** Stdev + **1.3153** Silt + **1.0126** Depth

The class mean on canonical variable 1 was negative for quadrats where mussels were absent (Table 27). Where mussels were present, the class mean on canonical variable one was positive with the mean increasing as count increased.

Table 24. Squared Mahalanobis distance matrix between class means (bold) and F statistics (below each distance) for the variables: percent gravel, percent sand, percent small cobble, percent very coarse gravel, particle size standard deviation (mm), percent silt, and depth (m) at Site 4. The significance levels are: P < 0.05*, P < 0.01**, and P < 0.001***.

	absent	one	two	three
absent		1.855*** 12.416	2.810*** 17.540	4.649*** 44.574
one			0.320 1.293	1.614*** 8.430
two				0.762** 3.770
three				

Table 25. Results of Canonical Discriminant Analysis with the first two canonical variables at Site 4. The canonical correlations with the classes, eigenvalues, and the likelihood ratio are displayed. The likelihood ratio tests the hypothesis that the canonical correlation and all that follow are zero.

Canonical Variable	Canonical Correlation	Approximate Standard Error	Eigenvalue	Proportion	Cumulative	Likelihood Ratio	F value	DF	Pr>F
1	0.6757	0.02594	0.8403	0.9059	0.9059	0.4995	16.09	21	<.0001
2	0.2711	0.04420	0.0793	0.0855	0.9914	0.9191	3.09	12	0.0003
3	0.0892	0.04735	0.0080	0.0086	1	0.9921	0.69	5	0.6295

Table 26. The table displays the total and pooled within class canonical structure (correlations between the first two canonical variables and the original variables either in the total sample or pooled within the classes), the pooled within-class standardized canonical coefficients, and the raw canonical coefficients at Site 4. Variable abbreviations located in Table 2.

Variable	Total Canonical Structure		Pooled Within Canonical Structure		Pooled Within-Class Standardized Can. Coeff.		Raw Canonical Coefficients	
	Can1	Can2	Can1	Can2	Can1	Can2	Can1	Can2
pgravel	0.7758	-0.3029	0.6515	-0.341	0.6568	-0.2481	2.8755	-1.063
psand	-0.4063	-0.1016	-0.312	-0.102	-0.728	0.4836	-5.363	3.5621
psc	0.3497	0.5705	0.2688	0.5726	0.1829	0.3479	1.5479	2.9437
pvcg	0.4288	0.4714	0.33	0.4782	0.0096	0.8151	0.0928	7.5932
stdev	-0.5733	-0.1975	-0.4595	-0.2067	-0.4030	-0.3289	-0.0007	-5E-04
psilt	-0.4214	0.1220	-0.324	0.01222	-0.2647	0.1125	-3.0945	1.3153
depth	-0.3616	0.5076	-0.2783	0.5107	-0.0777	0.6040	-0.1303	1.0126

The plot of the first two canonical variables indicates that canonical variables do not completely separate the groups but the majority of samples where mussels were absent displayed a negative canonical 1 value (Figure 12). The majority of quadrats where two and three or more individuals were found had positive canonical 1 values.

At Site 4, there were many samples misclassified into groups (49.43%, Table 28) with the first two canonical components. The error rate was highest when attempting to classify samples that had one or two mussels in a quadrat. Quadrats with no mussels were misclassified as containing mussels 23.25 % of the time. Quadrats with three or more mussels were misclassified 28.87 % of the time but only 4.12 % of the total quadrats where three or more were found were misclassified into the ‘absent’ group. The percentage of quadrats where mussels were present that were misclassified as absent based on the first canonical component was 16.04 %. This percent error corresponds to the percentage of quadrats in areas of unfavorable favorable habitat (low canonical component 1 scores) that contained mussels. The 28.87 % of quadrats with no mussels that were misclassified as having mussels corresponds to the error associated with the percentage of quadrats in favorable habitat that did not contain mussels.

Table 27. Density class means at Site 4 on the first two canonical variables.

Count	Can1	Can2
Absent	-0.8387	-0.0803
one	0.3701	0.5296
two	0.775	0.3299
three	1.303	-0.3258

Figure 12. Plot of first two canonical variables at Site 4. Data points represent four class levels: quadrats where three or more individuals were located, quadrats containing two individuals, quadrats containing one individual, and quadrats where mussels were absent. Both canonical variables are significant ($P < 0.05$).

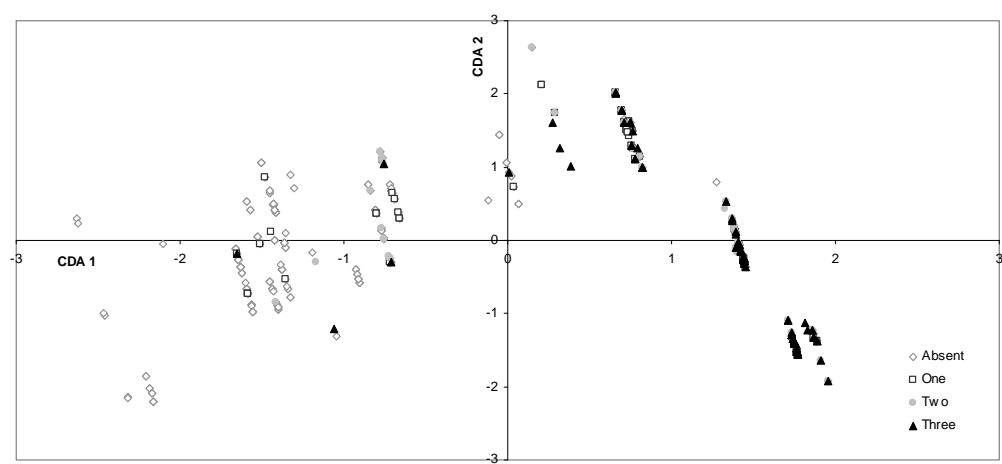


Table 28. A. Summary table with the number and proportion of quadrats classified into each group based on the linear discriminant functions at Site 4. An equal prior probability of group membership was assumed. B. Error rate estimates for classifications.

A.						B.	
From Count	absent	one	two	three	total	Class	Error Rate
absent	175	31	1	21	228	none	0.2325
	76.75	16.60	0.44	9.21	100	one	0.5833
one	18	25	2	15	60	two	0.8727
	30.00	41.67	3.33	25.00	100	three	0.2887
two	12	14	7	22	55	total	0.4943
	21.82	25.45	12.73	40.00	100	Absent	0.2325
three	4	21	3	69	97	Present	0.1604
	4.12	21.65	3.09	71.13	100		

Discussion

The ability of discrimination models to predict mussel density from habitat

Research on the role of microhabitat conditions in structuring mussel communities has produced conflicting results. Studies have determined that microhabitat measures including local current velocity, depth, or substrate are correlated with mussel density or preference (Vannote and Minshall 1982, Salmon and Green 1983). However others have found that microhabitat variables explain little of the variation in mussel distribution (Holland-Bartels 1990, Strayer and Ralley 1993, Brim Box et al. 2002). The current study found that density classes at the four sites could be distinguished from each other based on depth and substrate variables, but discrimination models based on these variables alone were limited in their ability to predict mussel presence or abundance in a given quadrat, particularly in habitats that were considered favorable.

The significant predictor variables differed among sites and a separate discriminant model was computed for each site. This finding indicates that mussels may select certain habitats but are constrained by site-specific conditions in a given site. For instance, the percent of small cobble had a high positive influence on density at Site 2, but had no significant influence on density at Site 1. In addition, each variable selected by the stepwise discriminant procedure when considered by itself was significant in distinguishing between density groupings but had low predictive power signified by a low R-square value. This finding is similar to the results from other studies of unionids that indicate microhabitat variables are correlated with mussel density but have low predictive power because they are flow-conditional (Layzer and Madison 2005).

High variability in the spatial distribution of freshwater mussels has been described in other studies (Layzer and Madison 1995, Haag and Warren 1998, Strayer 1999, Howard and Cuffey 2003, Morales et al 2006). At Sites 1, 3, and 4, high variation in substrate composition, mussel density, and depth was found along the majority of transects. Because the data contained a large quantity of quadrats in the low density stratum outside the immediate mussel bed, the models could compare the habitat conditions where mussels are simply present, where mussels are in abundance (3 or more), and where mussels are absent.

The variables that distinguished between density groups at Site 1 were percent gravel, depth, bedrock, and average particle size. Quadrats that contained three or more mussels were in areas with a higher percentage of gravel (mean=82.24%), lower depth (mean= 0.37 m), less bedrock (absent), and a smaller average particle size (mean=20.74 mm) than quadrats where mussels were present in smaller quantities or absent. The vector comprised of these variables could distinguish between all density groupings except between quadrats that contained one mussel and quadrats that contained two mussels. Percent gravel and depth were the variables most highly correlated with the canonical variable at this site.

The percentage of misclassifications indicates that the model had a high total error rate when used to predict density group from the canonical variable (53.01%). Much of the error was found in the misclassifications of quadrats that contained one or two mussels. The discriminant function based on habitat classified quadrats where mussels were absent with ~ 65% accuracy. The function classified quadrats where mussels were present with ~86% accuracy. These results indicate that there were about 35% of

quadrats where mussels were absent located in seemingly favorable habitat conditions (low depth, high gravel). Only about 9% of these were in areas that were classified as containing three or more mussels. Approximately 14 % of the quadrats where mussels were present were found in areas of unfavorable habitat (misclassified as absent) and these quadrats contained either one or two mussels. The discriminant function classified quadrats containing three or more mussels with ~69% accuracy and did not misclassify these quadrats into the absent category. The model error rate indicates that microhabitat alone cannot predict the exact spatial distribution of individuals within a mussel bed as certain quadrats located in favorable habitat did not contain mussels. The model was more effective at correctly predicting mussel absence in unfavorable habitat. The implications are that unfavorable habitat (low ratio of gravel to other particle sizes, higher depth, bedrock, and larger average particle size) limits the extent of the mussel bed as mussels tend to cluster in shallow areas with gravel.

There may be some hydrological influences on habitat selection that can be inferred from substrate composition as well which will be discussed in the next section. The findings also indicate that although mussels select certain habitat within a site not all available preferred habitat patches are being used by mussels, causing the models to be less accurate. There may be other abiotic or biotic factors such as temperature, flow, bed stability, or food availability that are influencing their distribution at the microscale. For example, a consideration with smaller particle sizes in certain areas of the stream is the question of whether they are a fixed, stable part of the stream bottom or whether they have been recently deposited after the recession of high water during the last flood event.

This may influence the presence of mussels in certain patches. Bed scouring will be addressed in the next section.

At Site 2, the significant variables that were able to distinguish between density groups were percent small cobble, percent gravel, percent very coarse gravel, percent boulder, and depth. The quantitative variables percent gravel, depth, and percent boulder were similar to the variables in the model derived for Site 1 which contained bedrock rather than boulder. The most important difference was the role of small cobble in distinguishing between density groups. Percent gravel was noticeably less at this site and small cobble was more frequent. This site also had a higher proportion of quadrats where mussels were absent (~68%) than the other sites. On average, quadrats containing three or more mussels had a higher percentage of small cobble and gravel, a lower percentage of boulders, and a greater depth. The vector of the variables was significant in distinguishing between all the density groups except between quadrats that contained one mussel and quadrats that contained two mussels and in distinguishing between areas that contained two mussels and areas that contained three mussels. The canonical structure analysis revealed that density was correlated with higher percentages of small cobble and gravel, lower percentages of boulder, and greater depth.

The percentage of misclassifications indicates that the model for Site 2 also had a high total error rate when used to predict density group from the canonical variable (56.59%). The total error rate was similar to the error rate associated with the Site 1 model. Like the Site 1 function, much of the error was found in the misclassifications of quadrats that contained one or two mussels. The discriminant function based on habitat classified quadrats where mussels were absent with ~ 72% accuracy. The function

classified quadrats where mussels were present with ~76% accuracy. These results indicate that there were about 28% of quadrats where mussels were absent located in seemingly favorable habitat for mussels. Only about 6% of these were in areas that were classified as containing three or more mussels. Approximately 24 % of the quadrats where mussels were present were found in areas of unfavorable habitat (misclassified as absent). The discriminant function classified quadrats containing three or more mussels with ~67% accuracy.

At Site 2, the misclassification rate of quadrats where mussels were absent was lower than this misclassification error rate at Site 1, indicating mussels may be using available preferred habitat patches to a greater extent at this site. The misclassification rate of quadrats where mussels were present was slightly higher than the rate at Site 1 which could indicate that more patches considered unfavorable by the model at Site 2 can still support mussels (typically the ‘one mussel’ density group).

The significant variables that were able to distinguish between density groups at Site 3 were percent gravel and depth. These two variables were selected for the discriminant functions at all the other sites. Very coarse gravel was initially selected but was not significant in discriminating between density groups but I included it in the final discriminant function. Mean percent gravel was similar between the ‘two mussels’ and ‘three or more mussels’ groups (67%-68%) but significantly higher in these groups than the ‘one mussel’ and ‘absent’ groups. Depth tended to decrease with higher density. The vector of variables detected significant differences between all density classes except between the ‘two mussels’ and ‘three or more mussels’ groups. Canonical analysis revealed that percent gravel had a strong positive correlation and depth had a strong

negative correlation with canonical variable 1. Quadrats with two or more mussels had high average canonical 1 values.

The percentage of misclassifications indicates that the model for Site 3 also had a high total error rate when used to predict density group from the canonical variable (55.22%). The total error rate was similar to the error rate associated with the other models. The majority of the error was associated with classifying quadrats that contained one mussel and distinguishing between quadrats that contained two mussels and quadrats containing three or more. The discriminant function based on habitat classified quadrats where mussels were absent with ~ 57% accuracy. The function classified quadrats where mussels were present with ~87 % accuracy. These results indicate that there were about 43 % of quadrats where mussels were absent located in seemingly favorable habitat. Only about 9% of these were in areas that were classified as containing three or more mussels. Approximately 13 % of the quadrats where mussels were present were found in areas of unfavorable habitat (misclassified as absent). No quadrats in unfavorable habitat contained three or more mussels and only about 2.7% of quadrats in unfavorable habitat (n=150) contained two mussels. The discriminant function classified quadrats containing three or more mussels with ~49 % accuracy. I combined the “two mussels” and “three or more mussels” and the model predicted this new group with ~87 percent accuracy. When comparing the results at Site 3 to Sites 1 and 2, it seems that there are more favorable habitat patches that are not being used at this site. In addition, there are slightly fewer mussels found in ‘unfavorable habitats’ than at Site 2.

At Site 4, stepwise discriminant analysis selected seven variables that were significant in distinguishing between density groups: percent gravel, percent sand,

percent small cobble, percent very coarse gravel, particle standard deviation, and depth. Greater densities where particle size deviation is lower in gravel habitats indicates that mussels may be preferring areas that consist of homogenous substrate at this site. The vector of these variables was significant in distinguishing between all density groups except between the 'one mussel' and 'two mussels' groups. In contrast to the three other sites two canonical variables rather than one had correlations that were significantly different from zero. The first canonical variable had a much stronger correlation with density group and the structure indicated that percent gravel had the highest correlation with canonical variable 1. Percent small cobble and percent very coarse gravel were positively correlated with canonical variable 1 and percent sand, percent silt, depth, and particle standard deviation had negative correlations. On average, higher values of canonical variable 1 were associated with higher density levels.

The discriminant function at Site 4 had a slightly lower total error rate (49%) than the other sites when distinguishing the density groups. The majority of the error rate was associated with the misclassification of quadrats containing one or two mussels into the correct group. The discriminant function based on habitat classified quadrats where mussels were absent with ~ 77% accuracy. The function classified quadrats where mussels were present with ~84 % accuracy. These results indicate that there were about 23% of quadrats where mussels were absent located in seemingly favorable habitat. Only about 9% of these were in areas that were classified as containing two or more mussels. Approximately 16 % of the quadrats where mussels were present were found in areas of unfavorable habitat (misclassified as absent). The discriminant function classified quadrats containing three or more mussels with ~71 % accuracy. When comparing the

results at Site 4 to the other sites, the classifications indicate that there are fewer ‘favorable’ habitat patches that are devoid of mussels at this site.

Discrimination models based on the microhabitat variables substrate and depth are useful in assessing the extent of a mussel bed in a given area on the Marais des Cygnes River. While models based on the high percentage of gravel relative to the percentage of very coarse or very fine substrate and low depth may be inaccurate at predicting mussel absence in a given quadrat containing ‘favorable habitat’, they are accurate in predicting the absence of mussels in ‘unfavorable’ habitats on the river. The models were accurate at predicting the location of quadrats containing three or more mussels (two or more mussels at Site 3) based on habitat conditions. However, these conditions were site-specific. Whereas mussels could be found in different habitats (higher percents of small cobble and coarser substrate at Site 2), they were densest in areas with shallow water and a high percentage of medium and coarse gravel if available. I suggest in the next section that the fixed presence of large beds composed of primarily gravel along one bank indicates that specific past hydrological patterns and diverted flow during high water events may have allowed the bedload in these areas to remain fairly stable through time.

Comparison of habitat conditions between high and low density strata

There was a significant difference in substrate composition between strata at all four sites. More gravel and sand were found in high density areas. In addition these areas were shallower at Sites 1, 3, and 4. At Site 1, the high density stratum was comprised of primarily gravel with approximately 70% of particles on average consisting of medium and coarse gravel. The substrate in certain areas of the high density stratum was comprised of at least 95% medium and coarse gravel. Percent gravel was lower in the

upstream part of the site and decreased with distance from the bank in the downstream portion of the site. In the low density area the substrate was coarser on average and was comprised primarily of coarse gravel, very coarse gravel, and bedrock. Boulders, cobble, and silt particles were also present.

The same pattern was quantified at Site 3 with finer particle sizes comprising the majority of the substrate in the high density strata. Several areas contained a significant portion of sand in addition to gravel. The low density stratum at Site 3 was comprised of primarily large cobble with considerable amounts of small cobble, very coarse gravel, boulder, and bedrock. The low density stratum was deeper on average. An interesting qualitative observation at this site was the presence of large woody debris in the center of the channel in the downstream section. This debris caused a large quantity of gravel to accumulate in the area downriver from the debris and this portion of the stream was shallower. The gravel bar extended well into the 'low density stratum' at this lower portion of the site and several mussels were located there.

At Site 4, the high density stratum was primarily composed of coarse gravel with considerable amounts of very coarse gravel, small cobble, medium gravel, and sand. The pattern of increasing particle size with distance from the bank was evident at Site 4 as well. The low density stratum was composed of primarily bedrock with smaller amounts of boulders, cobble, and very coarse gravel.

The high density stratum at Site 2 contained primarily small cobble with lesser amounts of large cobble, boulders, very coarse gravel, and sand. Boulders and large cobble comprised a greater percentage of the substrate in the low density region.

The fact that the mussel beds or what I designated as the high density strata at the sites were primarily located in shallower areas adjacent to the bank and the majority of the substrate in these areas contained smaller particle sizes allows for inferences to be made about the hydrological regime and the concept of shear stress acting on the substrate during higher flows.

The substrate composition in an area was determined from transect pebble counts rather than sorting or cores that can describe the substrate composition within a given quadrat at a finer scale and more precisely. Wolman pebble counts are biased towards the larger surface particles, but this bias may have implications regarding general bed stability in the vicinity of the quadrat. Larger particles on the surface have a higher tractive force which is proportional to particle diameter and require a higher shear velocity acting on them for entrainment (Newbury 1984). In an area of heterogeneous particle sizes, the larger particles may stabilize finer grain sizes and are more hydrologically significant for determining the general stability of the bed as a whole during higher flows (Leopold 1994). Typically, the finer particles can become entrained in the water current as velocity over the area increases slightly. During bankfull discharge, one can expect the majority of finer particles to become entrained in the water column and set in motion. As discharge decreases these particles are deposited as the energy required to carry them is no longer available. The presence of gravel bars along one bank and large cobble, boulders, and bedrock composing the substrate in the center of the channel and opposite bank indicates that the flow and forces acting on the substrate are not distributed evenly across the stream.

The findings in this study suggest that high quality mussel beds or high density strata at Sites 1, 3, and 4 are found in areas of high concentrations of gravel particles (2-32 mm). Presumably if shear force acting on the substrate was uniform in the cross-stream direction, during long periods of high discharge the majority of these particles would not be stable and the bed would suffer from extreme scouring. In addition, as the flow decreases finer particles would settle out in a somewhat random pattern along the cross-section (Leopold 1994). The presence of bars where the majority of particles are gravel or finer indicates that either the shear stress acting on particles is insufficient to entrain the majority of the particles or that gravel carried in the water column is more likely to settle out in bars as the velocity is lower proportionally over these areas during depositional conditions.

These two scenarios which may both be occurring to a certain extent in the vicinity of the mussel beds have important implications for mussel survival and recruitment. Mussels have adaptations that allow them to become anchored in the sediment either by burrowing deeper or extending their foot into the substrate during high water (Di Maio and Corkum 1995). Researchers believe that the presence of mussels can indicate the stability of substrate at higher flows (Vannote and Minshall 1982, Amyot and Downing 1998, Di Maio and Corkum 1995) or that dense clusters of mussels can add to the overall stability of the substrate (Johnson and Brown 2000). However, if the majority of gravel particles composing the substrate become entrained during bankfull discharge, it is difficult to see how mussels could remain in place as the bed is scouring. Juveniles may be especially vulnerable to higher forces acting on the bed and significant substrate movement (Layzer and Madison 1995, Morales et al. 2006). During bankfull discharge it

is expected that some of the gravel particles will become entrained and move downstream even if flow is reduced in the area near the bank compared to the flow in the rest of the channel (Leopold 1994). The gravel bar will become replenished as the particles are replaced with gravel and fine sediment that settles out over the bed from upstream areas when flow decreases. Potentially, the current could also be carrying displaced or newly excysted juvenile mussels (which are ~ 0.2 mm in size) from upstream (Morales et al. 2006). As velocity decreases over the mussel bed, juveniles may become deposited in these locations. Several studies have found that the low values of sheer stress acting on the substrate during high flows can predict the occurrence of mussels (Layzer and Madison 1995, Strayer 1999, Hardison and Layzer 2001, Howard and Cuffey 2003). Mussels will select “flow refugia” or areas in the channel where shear stress during floods is too low to displace mussels or the sediments where they are embedded (Strayer 1999, Howard and Cuffey 2003, Morales et al. 2006).

Morales and others (2006) incorporated a dimensionless parameter termed shear stress ratio (RSS) which combined shear force and the shear stress at the onset of sediment motion into simulation models to predict mussel occurrence based on habitat. The equation used to derive this equation is similar to the relative bed stability equation developed by Jowett (1989):

$$\text{Relative bed stability (RBS)} = V_c/V_b$$

The stability of the bed is the ratio of critical velocity required to move a particle of a given size (V_c) to the actual or predicted water velocity near the bed (V_b). The bed is assumed to be stable for RBS values less than 1. When they tested the concept of RSS

with data by Layzer and Madison (1995), Morales and others determined that water depth and substrate preferences for unionids can be explained largely in terms of substrate stability as RSS was consistently less than 1 for gravel substrates across all water depths when using this data. They conclude that the relative number of mussels using gravel was disproportionately greater than the number of mussels using other substrates type.

On the Marais des Cygnes River, the presence of these 'flow refugia' where mussel beds are present could occur from the influence of channel morphology including the geometry of the river banks or large woody debris. The Marais des Cygnes is a meandering, low-gradient river with quality floodplain hardwood forests in the riparian areas along the portion that flows through the refuge. The stream portion which contained the four sites has several meanders of notable size and a large quantity of woody debris from fallen trees. These instream fallen trees are present along the banks and in the center of the channel. Lisle (1986) observed that the formation of gravel bars tended to occur 3-4 bed widths downstream and one bed width upstream from large obstructions and river bends. The large obstructions could include woody debris, bedrock, or artificial obstructions and could serve to stabilize gravel bars by affecting secondary currents and backwater effects (Lisle 1986, Gordon et. al. 2004). Typically gravel bars are located on the same side of the stream channel as the upstream river bend (Leopold 1994).

It has been suggested that the annual peak flows will most often limit the distribution of mussel communities (Morales et al. 2006). Juveniles may be particularly sensitive to bed stability and shear stress during high flows (Hardison and Layzer 2001, Hastie et al 2001, Morales et al. 2006). Therefore the absence of juveniles from a mussel

bed which has an abundance of adults may indicate that the host fish are not abundant at the site during reproduction, the relative bed stability has been altered as scour from high flows becomes more frequent, or that smaller particle sizes and juveniles carried in the water column from upstream are no longer settling in favorable habitat.

Several habitat features associated with Site 1 may be allowing for the establishment of juveniles at this site. The mussel bed is located immediately upstream from an island composed of gravel, very coarse gravel, and cobble and it is on the inner downstream region of a bend. Further upstream is a group of large trees residing in the center of the channel and along the banks. The bank on the opposite site is high and severely eroded and the substrate on this side of the stream consists of bedrock and boulders. The gravel bar and high density mussel stratum extended 13 meters across the stream. I suggest that the bed remains stable during high discharges as a result of the channel morphology in this region and the upstream and downstream obstructions which divert flow to the thalweg and outside bank. Some surface gravel particles may become dislodged during these events if the flooding is of a long duration but when flow decreases in magnitude gravel particles and possibly juvenile mussels may be more likely to settle out in this area. Another feature at Site 1 is its proximity to Site 3, a large abundant mussel bed upstream from the site. If the bed is scoured at Site 3 during floods, some of the flow which has finer particles or even displaced juveniles entrained in the water column may be significantly reduced over Site 1 as frictional forces from obstructions causes the flow to lose kinetic energy and these particles drop in favorable gravel habitat.

The absence of recruitment at the other sites may indicate that the mussel bed is no longer as stable during floods or that high water events (which occur with an increase in frequency and duration since flow modifications) has scoured some of the gravel substrate, leaving a stable but coarser stream bottom.

Because recruitment has occurred recently at Site 1, this site could serve as a reference site for future studies on the river. Reintroduction of young juveniles into the sites may be affected by the relative stability of the substrate and different values of shear force acting on the bedload at the four sites. The host fish movements and their habitat preferences also should be investigated as the host fish could prefer the abiotic and biotic conditions at Site 1, during the time of mussel reproduction. The answer to the question of why recruitment occurs at one mussel bed but is reduced or nonexistent at the others in a given stream segment has very important implications to the long-term survival of mussel populations. As many mussel beds are composed of a high abundance of old individuals and relatively few new recruits (this pattern was present in 3 out of the 4 mussel beds I examined), the conditions that could be contributing to the recent recruitment success at a particular site should be investigated.

Appendix

Table 1. Reported fish host species (scientific and common names) for the glochidia of three freshwater mussels: *Amblema plicata*, *Quadrula pustulosa*, and *Ellipsaria lineolata*. Fish species in bold type are known to occur in the West Osage River Basin in Kansas (Dent et al. 1997). Citations are indicated by a superscript above the scientific name of each mussel species. Citation # 2 is listed above the bottom portion of the *A. plicata* host list and the species below that reference are included under citation 2. The citations are; 1= Watters 1994, 2=Weiss and Layzer 1995, 3=Fuller 1974, 4= Fuller 1978. This information is reported in Parmalee and Bogan

Mussel species	Scientific name	Common name	Scientific name	Common name	Scientific name	Common name
	<i>Amblema plicata</i> ¹	Threeridge	<i>Quadrula pustulosa</i> ³	Pimpleback	<i>Ellipsaria lineolata</i> ⁴	Butterfly
Reported fish hosts for glochidia	<i>Pomoxis nigromaculatus</i>	black crappie	<i>Ameiurus melas</i>	black bullhead	<i>Aplodinotus grunniens</i>	freshwater drum
	<i>Lepomis macrochirus</i>	bluegill	<i>A. nebulosus</i>	brown bullhead	<i>Stizostedion canadense</i>	sauger
	<i>Pylodictis olivaris</i>	flathead catfish	<i>Ictalurus punctatus</i>	channel catfish	<i>Lepomis cyanellus</i>	green sunfish
	<i>L. cyanellus</i>	green sunfish	<i>Pyodictis olivaris</i>	flathead catfish		
	<i>Micropterus salmoides</i>	largemouth bass	<i>Pomoxis annularis</i>	white crappie		
	<i>Esox lucius</i>	northern pike	<i>Scaphirhynchus platyrhynchus</i>	shovelnose sturgeon		
	<i>L. gibbosus</i>	pumpkinseed				
	<i>Ambloplites rupestris</i>	rockbass				
	<i>Stizostedion canadense</i>	sauger				
	<i>Lepisosteus platostomus</i>	shortnose gar				
	<i>Morone chrysops</i>	white bass				
	<i>Pomoxis annularis</i>	white crappie				
	<i>Perca flavescens</i>	yellow perch				
	<i>Hiodon tergisus</i> ²	goldeye				
	<i>Notropis atherinoides</i>	emerald shiner				
	<i>Cyprinella spiloptera</i>	spotfin shiner				
	<i>C. whipplei</i>	steelcolor chiner				
	<i>Erimystax dissimilis</i>	streamline chub				
	<i>Moxostoma duquesnei</i>	black redhorse				
	<i>M. erythrurum</i>	golden redhorse				
	<i>Hypentelium nigricans</i>	northern hog sucker				
	<i>Ictalurus punctatus</i>	channel catfish				
	<i>Percina caprodes</i>	logperch				
	<i>Aplodinotus grunniens</i>	freshwater drum				

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