

COMPARING LIFE HISTORY TRAITS AND RESPONSES TO ENVIRONMENTAL
VARIATION BETWEEN MUSSEL SPECIES IN THE SAN SABA RIVER

by

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A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Science
with a Major in Aquatic Resources
August 2020

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ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. Astrid Schwalb for her sage advice these past two years and for reading through countless drafts of this. In addition, I would like to thank my committee members Dr. Benjamin Schwartz and Dr. Clay Green for their input on this thesis.

A big thank you to my lab mates: Ericah Beason, Zachary Mitchell, Josi Robertson, Joshua Robledo, Montana Stevens, David Swearingen, and Leah Woolam, without whom this would not have been possible.

Finally, I would like to thank my Mom, Dad, and sister for their encouragement and support while I have attended Texas State.

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ABSTRACT

Freshwater mussel species (Unionidae) can vary considerably in their life history traits (e.g., longevity) and mussels with different life history strategies may dominate in different types of habitat. Unfortunately, data on life history traits are still missing for many species, and the factors that determine freshwater mussel distribution along a river are not well understood. Such information, however, is crucial for the conservation of this highly imperiled group of organisms. The objectives of my study were to (1) determine the maximum age and growth constant for three mussel species with different life history strategies; and (2) to perform translocation experiments at two spatial scales (within and between river segments) where mussels were moved to locations from which they were absent to determine if local habitat conditions were limiting their survival and performance. The translocation experiments were carried out in the San Saba River, Texas, using mussels with two different life history strategies (opportunistic and equilibrium). Thin sectioning of mussel shells and analyzing their size in relation to the number of annuli showed that the maximum lifespan differed considerably between species and was highest for *Cyclonaias petrina* (31 years), lowest for *Utterbackia imbecillis* (9 years), and intermediate for *Lampsilis bracteata* (16 years). In contrast, growth constants were rather similar between species. The translocation experiments showed that local habitat conditions are limiting the opportunistic species (*U. imbecillis*) more strongly than the equilibrium species (*C. petrina* and *pustolosa*). Detection was considerably and consistently lower and mortality higher for *U. imbecillis* that were

translocated to a habitat from which they were absent. Responses of *C. petrina* and *C. pustolosa* to translocation provided mixed evidence. Although several measures (e.g., mortality and glycogen within the lower segment, growth between segments) indicated that mussels performed better in the control habitat, it was not a consistent pattern. This suggests that other factors, which may affect earlier life stages not tested here, may limit their distribution. Detection of *Cyclonaias* declined considerably in a treatment pool in the upper segment after a flooding event, whereas it remained high in the upper riffle. Thus, disturbance may play an important role for the distribution of mussels, which should be investigated further by future studies. The study shows that suitable habitat is not necessarily indicated by presence of mussels especially those with different life history strategies, which needs to be considered in relocation efforts.

I. COMPARING GROWTH AND LONGEVITY OF THREE MUSSEL SPECIES WITH DIFFERENT LIFE HISTORY STRATEGIES IN THE SAN SABA RIVER, TEXAS

Abstract

Information on life history traits can be used to assess freshwater mussel (Unionidae) extinction probabilities and conduct species status assessments, though data on life history traits for many species are still unknown. The objective of this study was to estimate maximum age and growth constants of three mussels with distinct life history strategies: Two Texas endemic species, presumed to represent an equilibrium (*Cyclonaias petrina*) and a periodic life history strategy (*Lampsilis bracteata*), and a common opportunistic species *Utterbackia imbecillis*. We predicted that equilibrium species would have highest maximum age and the lowest growth constant, whereas opportunistic species would have the lowest maximum age and highest growth constant, and periodic species intermediate values. Thin sectioning of mussel shells and analyzing their size in relation to the number of annuli showed that the maximum lifespan differed considerably between species and was highest for *Cyclonaias petrina* (31 years), lowest for *Utterbackia imbecillis* (9 years), and intermediate for *Lampsilis bracteata* (16 years) as predicted. This is the first study to report growth constants for the three tested species and, in contrast to our prediction, growth constants were similar between species. Future studies should examine reproductive efforts alongside growth and longevity to better understand life history traits and potential trade-offs.

Introduction

Freshwater mussel species (Unionidae) can have very different life history strategies, and while knowledge on the subject has increased considerably in the past 20

years, data on life history traits is still missing for many species. Age structure, growth, and longevity are important factors that can be used in conservation strategies to determine extinction probabilities of mussel species and make species status assessments with the USFWS to inform ESA listings (Dennis et al. 1991, Morris et al. 2002, Haag and Rypel 2011, USFWS 2016). Similar to trees, mussels can be aged by counting annuli, i.e., rings they deposit annually in their shell in response to the drop-off of growth during winter (Haag and Commens-Carson 2008). Age-length relationships of unionid mussels can give insight into their population dynamics as well as reveal a species' Bertalanffy growth parameter or Brody's growth constant (K) (Ricker 1975, Kimura 1980, Haag 2012). K is the rate at which species approach their growth asymptote and can be used as a proxy measurement for growth rate although units of K are in time^{-1} rather than a change in length for a unit time as required by a growth rate (Kimura 1980, Ogle 2010). The growth asymptote (L_{∞}) of a species is the point at which growth slows even though the organism continues to age (Ricker 1975). A life history trade-off between the rate at which species approach their growth asymptote and their longevity, has previously been seen in mussels and other groups of organisms (Bauer 1992; Olsson and Shine 2002; Rollo 2002; Metcalfe and Monaghan 2003). Species that approach their growth asymptote quickly will exhibit a high K value but tend to have a shorter life span and vice versa (Haag 2009). This relationship holds especially true for high and extremely low K-values, but there also seems to be considerable variation in longevity for low to moderate K-values (e.g., ca. 0.1-0.3, Fig. 6.4 in Haag 2012).

Haag (2012) proposed three major groups of life history strategies, similar to the CSR (Competitors, Stress-tolerators, Ruderals) system in plants (Grime 1977). The

terminology was adapted from Winemiller and Rose (1992), who described three distinct strategies to fish: equilibrium, periodic, and opportunistic. For mussels, equilibrium species are characterized by a long life span, late maturity, and tend to have low fecundity similar to K-selected species (Haag 2012). They are presumed to have lower growth rates, but data is missing for many species and K was not used by Haag (2012) when he assigned different species to different life history groups based on their life history traits (Fig. 6.12 in Haag 2012). Conversely, opportunistic species tend to be fast-growing with shorter lifespans, early maturity, high fecundity similar to r-selected species, and presumably higher K values (Haag 2012). Periodic species show traits that fall between equilibrium and opportunistic species (Haag 2012). This strategy is characterized by moderate to high growth constants, low to intermediate life spans, and low to intermediate maturity age and fecundity (Haag 2012).

A better understanding of how life history strategies affect growth rate and longevity can give insight into certain mussel species' population dynamics. My objective was to estimate K based on age-length relationships, and to determine maximum length and age of three mussel species in the San Saba River: *Cyclonaias petrina*, *Lampsilis bracteata*, and *Utterbackia imbecillis*. Most mussels of the tribe Quadrulini are presumed to have an equilibrium life strategy, whereas most *Lampsilis* species are presumed to have a periodic life strategy. Therefore, we categorized *Cyclonaias petrina* as equilibrium and *Lampsilis bracteata* as a periodic species, while *Utterbackia imbecillis* had been previously categorized as opportunistic (Haag 2012). We predicted the equilibrium species to have the highest life span and lowest K-value, the

opportunistic species to have the lowest life span and highest K-value, and the periodic species to be intermediate. (Table 1.1).

Methods

Shells from dead mussels of varying sizes were collected from the San Saba river in Central Texas in 2017 and were thin-sectioned using the Fisheries and Ocean Protocol for Ageing Mussel Shells (Martin et al. 2018). Thin-sections were cut through the umbo of the shell to the longest exterior point using a diamond wafering blade and Buehler precision saw (Buehler Ltd., Lake Bluff, Illinois). Thin sections were then sanded using four increasing sandpaper grits and affixed to a microscope slide using clear epoxy. Two independent observers counted annuli under a compound microscope and compared ages. If an agreed upon age was unable to be established, the sample was not used in analysis. A lack of very small and young individuals can skew the growth curve, therefore the size of newly-released juvenile or age 0 was assumed to be 0.22 mm for *C. petrina* and *L. bracteata* and 0.31 for *U. imbecillis* based on the size of newly released juveniles (Barnhart et al. 2008, Haag 2009, Seagroves et al. 2019). Analyses were conducted with and without this value for age 0 to determine if it affected the K-value.

Mean age-length data of each species was used to determine K with the Bertalanffy growth equation:

$$L_t = L_\infty(1 - e^{-K(t-t_0)})$$

Solved for K:

$$K = \log(L_\infty - [L_t] L_\infty) / (t - t_0)$$

where L_t is length (mm) at time t (age in years), L_∞ is length (mm) at time infinity (the predicted mean maximum length for the population), t is age (years) and t_0 is the time at which length = 0 (Ricker 1975, Haag 2009).

Results

Maximum lifespan differed considerably between species and as expected, it was highest for *C. petrina* (31 years), lowest for *U. imbecillis* (9 years), and intermediate for *L. bracteata* (16 years, Table 1.2). In contrast, K-values were similar between species ranging between 0.17 for *C. petrina* and 0.23 for *L. bracteata* (Table 1.2, Fig. 1.1).

When age 0 was not included in calculations, growth constants were low for all three species (*C. petrina*: 0.13, *L. bracteata*: 0.14, *U. imbecillis*: 0.12).

Discussion

This is the first study that estimated growth constants for *C. petrina*, *L. bracteata*, and *U. imbecillis* and maximum life span for the endemic species *C. petrina* and *L. bracteata*. The maximum life span of the three species were in accordance with predictions based on their presumed life history strategies and other reported data of related species where available (Haag and Rypel 2011) (Table 1.3, 1.4). Comparison data was compiled by using only species that were described by Haag (2012, Table 6.3) to fit into one of three life history strategies. Maximum ages found in this study were very similar to those found for mussels with the same life history strategies (Table 1.3). Previously reported longevity for *U. imbecillis* (5 years, Haag 2012) was lower compared to our study (9 years), but the maximum size in our study was also larger (99 vs. 82 mm).

In contrast, all species showed moderate K-values; even the opportunistic species, for which high K-values were predicted (Table 1.1). However, all values were within the

range reported for other species of the same life history strategy (Table 1.4). The estimated K-value for *L. bracteata* from this study was most similar to the median value of periodic species K-values reported by other studies (0.23 vs. 0.24 respectively), albeit at the lower end of the reported range (Table 1.4). The estimated K-value for *U. imbecillis* was at the lower range of reported K-values for other opportunistic species and lower than the median value of reported values (0.22 vs. 0.46 respectively), whereas the estimated K-value for *C. petrina* was somewhat similar to the median value of reported values for equilibrium species (0.17 vs. 0.13 respectively).

The addition of size at age 0 was useful in determining growth constants as it allowed for growth from newly released juveniles to be accounted for in the Bertalanffy growth curves. Without the addition of age 0, k-values were quite low and growth curves were not as pronounced. Haag states the importance of smaller size-class individuals to avoid underestimating growth curves, which was seen when size at age 0 was not included in analysis.

Other studies have found populations of the same species can exhibit vastly different growth rates in different rivers (Haag and Rypel 2011, Sansom 2016). Thus, K-values may vary widely depending on local conditions. Productivity (Bauer 2002), substrate type (Kesler and Downing 1997), mussel density (Bolden and Brown 2002), and annual streamflow (Rypel 2008) of an environment have been shown to influence growth rates (Negishi and Kayaba 2010). Relatively stressful environmental conditions may limit the ability of mussels to grow quickly, including of opportunistic species, which may be able to grow quickly under optimal habitat conditions (Haag 2008). This

trend has been observed in marine mussels, where growth slowed in mussels transplanted to a stressful habitat (Petes et al. 2007).

Due to the difficulties of ageing mussel shells, it is possible that the estimated K-values are overestimated. Miscounting the number of annuli, through omission, may cause an overestimation of K as it will appear the mussel grew to the measured length much quicker than in actuality. Central Texas' mild winters may impact how defined annuli are making them more difficult to detect in warmer climates (Haddon 2001, Callil et al. 2018). If annuli are missed, more growth per year is assumed, although the growth may have occurred over a longer time period, causing an overestimation of K. A lack of smaller individuals in this study could also be influencing the growth constants as an abundance of larger individuals causes an overestimation of K (Haag 2009).

In contrast to other studies (Haag and Rypel 2011, Sansom et al. 2016), this study did not find a trade-off between K-values and longevity, and K-values may not be a good indicator to differentiate between different life history strategies. Haag and Rypel found that allocation to growth in mussels is highly plastic, and it may be the environment a mussel is found in that influences this trait (Bauer 2002, Haag and Rypel 2011). *C. petrina*, *L. bracteata*, and *U. imbecillis* all exhibiting low to moderate growth constants possibly indicates they are putting relatively more energy into other life history traits, and slow growth may be a successful strategy in the San Saba river. Low growth constants with low longevity may also indicate that a species is not reaching its maximum age due to the environment it inhabits, but the estimated longevity of the mussels tested here was as expected and similar to reported values (Dudding et al. 2020).

Future studies should examine reproductive efforts alongside growth and longevity to determine the life-history trade-offs of these species, as well as look at the local environmental conditions and see if they are causing a change in growth. Growth parameters from this study can be used to better understand the population dynamics of *C. petrina*, *L. bracteata*, and *U. imbecillis*, and fill in the gaps of growth and aging data.

Table 1.1: Tested species, their assumed life history strategy, and predicted differences in life span and K-values in accordance to Table 6.3 in Haag 2012.

Species	Life History Strategy	Life Span (years)	K
<i>C. petrina</i>	Equilibrium	high (>25)	low-moderate
<i>L. bracteata</i>	Periodic	moderate (8-30)	moderate-high
<i>U. imbecillis</i>	Opportunistic	low (<10)	high

Table 1.2: Sample size, size range, maximum age, K, and presumed life history strategy for three study species.

Species	Life History Strategy	n	Size Range (mm)	Max. Age	K
<i>C. petrina</i>	Equilibrium	52	23-77	31	0.17
<i>L. bracteata</i>	Periodic	40	32-86	16	0.23
<i>U. imbecillis</i>	Opportunistic	37	32-99	9	0.22

Table 1.3: Average and median values of maximum age for each life history strategy from different studies.

Life History Strategy	n	Average Max. Age	Median Max. Age	Range	Source
Equilibrium	23	30	26	14-54	1-7
Periodic	18	17	16	5-44	3, 8
Opportunistic	19	10	10	4-26	3, 4

Table 1.4: Average and median values of Brody's growth constant for each life history from multiple populations.

Life History Strategy	n	Average K	Median K	Range	Source
Equilibrium	23	0.14	0.13	0.074-0.292	1-7
Periodic	18	0.29	0.24	0.117-0.639	3, 8
Opportunistic	19	0.49	0.46	0.157-1.008	3, 4

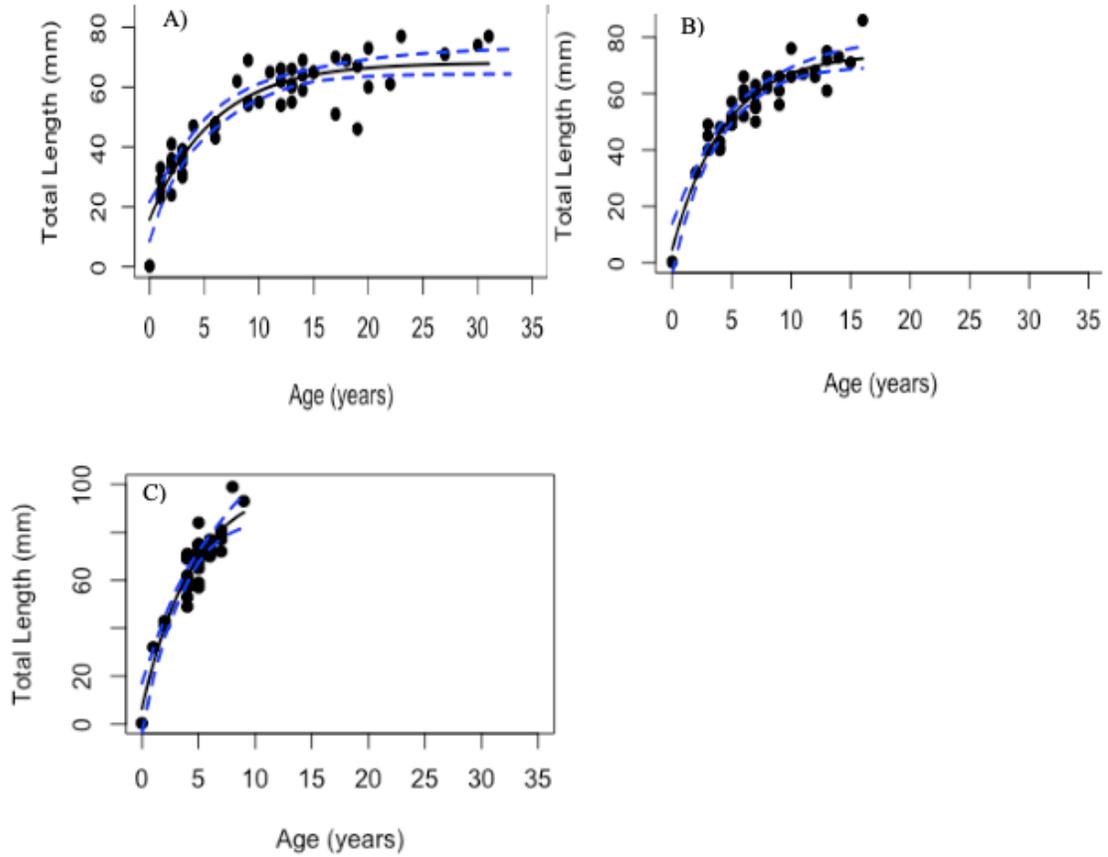


Figure 1.1: Growth curves for three study species based on von Bertalanffy growth parameters with 95% confidence intervals. A) *C. petrina*, presumed equilibrium species, $K: 0.17, L_{\infty}: 68.2, t_0: -1.56$. B) *L. bracteata*, presumed periodic species, $K: 0.23, L_{\infty}: 74.3, t_0: -0.29$. C) *U. imbecillis*, presumed opportunistic species, $K: 0.22, L_{\infty}: 101.5, t_0: -0.29$.

II. THE IMPACT OF LOCAL ENVIRONMENTAL FACTORS ON THE DISTRIBUTION OF FRESHWATER MUSSELS IN THE SAN SABA RIVER, TEXAS

Abstract

The factors that determine freshwater mussel (Unionidae) distribution along a river are not well understood. Such information, however, is crucial for the conservation of this highly imperiled group of organisms. The objective of this study was to perform translocation experiments at two spatial scales (within and between river segments) where mussels were moved to locations from which they were absent to determine if local habitat conditions were limiting their survival and performance. The translocation experiments were carried out in the San Saba River, Texas, using mussels with two different life history strategies (opportunistic and equilibrium). The translocation experiments showed that local habitat conditions are limiting the opportunistic species (*U. imbecillis*) more strongly than the equilibrium species (*C. petrina* and *pustulosa*). Detection was considerably and consistently lower and mortality higher for *U. imbecillis* that were translocated to a habitat from which they were absent. Responses of *C. petrina* and *C. pustulosa* to translocation provided mixed evidence. Although several measures (e.g., mortality and glycogen within the lower segment, growth between segments) indicated that mussels performed better in the control habitat, it was not a consistent pattern. This suggests that other factors, which may affect earlier life stages not tested here, may limit their distribution. Detection of *Cyclonaias* declined considerably in a treatment pool in the upper segment after a flooding event, whereas it remained high in the upper riffle. Thus, disturbance may play an important role for the distribution of mussels, which should be investigated further by future studies. The study shows that

suitable habitat is not necessarily indicated by presence of mussels, especially those with different life history strategies, which needs to be considered in relocation efforts.

Introduction

Freshwater mussels are important filter-feeders, bioturbators of sediment and nutrients, and indicators of aquatic health (Vaughn and Hakenkamp 2001; Haag 2012; Hoellein et al. 2017). Texas supports approximately 50 unionid mussel species, 14 of which are listed as state-threatened with 5 of those candidates for federal listing as endangered, and 1 already listed as endangered (*Popenaias popeii*) (USFWS; TPWD 2018). Freshwater mussel numbers have declined due to human activity; dams, groundwater pumping, *Dreissena polymorpha* invasion, in-stream disturbances, and pollution. (Bogan 1993; Vaughn and Hakenkamp 2001; Burlakova et al. 2011). A hierarchy of constraints affect the distribution of mussels, which occur in multi-species aggregations, called mussel-beds, in which densities are 10 to 100 times higher than outside of these beds (Strayer et al. 2004). The distribution of these mussel beds is patchy and they may occur in some reaches but not others (Vaughn and Taylor 2000, Strayer et al. 2004). Biogeographic history and climatic conditions determine regional differences (Vaughn and Taylor 2000; Dascher et al. 2018), and differences between river basins have been attributed to differences in host fish communities in some studies (Vaughn 1997; Schwalb et al. 2013), but not in others (Krebs et al. 2010; Daniel and Brown 2013). Within a river, mussel communities change along a longitudinal gradient, for which a conceptual framework was recently developed that associates these changes with life history strategies (Haag 2012), while another study associated them with landscape-scale

habitat variables (Atkinson et al. 2012). Still, the mechanisms behind these patterns are not well understood.

Attempts to explain patchiness in the distribution of mussels at a smaller-scale with traditional aquatic habitat measurements (e.g., substrate composition or water depth) have largely failed (Strayer 2008), although some unionids can exhibit microhabitat preference (Haag 2012). Less traditional factors such as measures of substrate stability have been identified to play a role by several studies (e.g. Morales et al. 2006; Allen and Vaughn 2010). Disturbance, such as flooding and drought, is known to play a large part in structuring aquatic communities (Resh et al. 1988). Flooding and drought can severely impact mussel abundance and mussel bed assemblages have been hypothesized to be structured by these disturbances (Strayer 1999, Hastie 2001, Haag 2012). Haag (2012) proposed that the relative abundance of mussels with different life history strategies would change with changes in habitat along a river. Equilibrium species, with traits similar to K-selected species (e.g., higher longevity, late maturity), are supposed to do well in stable habitats with lower disturbance, while opportunistic species, similar to r-selected species, can survive in disturbed habitat. Periodic species show traits that fall between equilibrium and opportunistic species and may dominate in small streams, likely better able to handle disturbance than equilibrium species, but not as well as opportunistic species (Haag 2012). A recent study in the San Saba River, a tributary of the Colorado River in Texas, found that periodic and opportunistic species co-dominated in the upper segment of the tributary, while equilibrium species dominated in the lower segment (Mitchell 2020) Opportunistic species dominated in the middle section, which has had an

intermittent flow regime in most recent summers with extensive drying and isolated pools.

Due to the challenge of defining suitable habitat for mussels, the majority of relocation guidelines define suitable habitat by the presence of mussels (e.g. Cope and Waller 1995; Mackie et al. 2008). A number of studies that relocated unionid mussels to a habitat that was deemed suitable found that survival can be high when mussels are handled carefully (Bolden and Brown 2002; Hart et al. 2016; Tsakiris et al. 2017). The relocation of mussels is often used as a mitigation measure for construction projects affecting rivers, and is routinely carried out by consulting companies (Cope and Waller 1995). However, only few relocation experiments tested the survival of mussels in “unsuitable” habitat, an area where mussels are not present (but see Hamilton et al. 1997, Bolden and Brown 2002, Fernandez 2013). Such relocation or translocation experiments provide the unique opportunity to directly test the effect of local habitat conditions on mussel performance (e.g., survival and growth) to determine the importance of local environmental control on mussel communities. It also gives the opportunity to look at the possibility that low colonization rates are constraining mussels from occupying all possible suitable habitats (Leibold et al. 2004). This is an advantage to observational studies using statistical tools such as variation partitioning, which do not allow evaluation of the role of environmental factors versus spatial factors when environmental variation is spatially structured (which often is the case, e.g., (Henriques-Silva et al. 2018)). A large number of translocation studies have been conducted with plants to determine if dispersal limitation or environmental control is the main limiting factor in plant distribution (e.g. Ehrlén and Eriksson 2000; Marsico and Hellmann 2009). Like plants, mussels are also

considered sessile, and these types of experiments can be applied to mussels to gain a similar understanding of the relative importance of environmental controls on distribution of mussels.

Determining the role of local habitat conditions on mussel distribution will allow for a better understanding of what is driving their distribution, which is knowledge needed for conservation and management efforts. Our objective was to perform translocation experiments at two spatial scales (within and between river segments) to determine if local habitat conditions was limiting their survival or performance. For the experiments within segments mussels were moved to mesohabitat (pool or riffle) from which they were absent. For the experiments between segments mussels were moved to a segment from which they were absent or where they only occurred in low numbers. Mussels translocated into a different segment were moved to two different mesohabitats, one where other mussels were present (presumed suitable) and another where mussels were absent or only occurred in low numbers (presumed unsuitable). Growth, mortality, and glycogen levels were used to indicate mussel performance. Glycogen is the main store of carbohydrates in mussels and glycogen levels become depleted due to increased energy demands when they are stressed (Naimo et al. 1998). Changes in glycogen concentration can be seen as soon as 3-months, while changes in growth tend to be seen more clearly annually (Kesler and Downing 1997, Naimo 1998). Mortality was determined by the number of dead mussels that were found, i.e., empty tagged shells. Since only relatively few dead mussels were found the number of mussels detected with the pit-tag antenna was also used as a response variable, mostly as a measure of persistence in the translocated or control habitat. A decline in detection after a

disturbance event, e.g., flooding, was also used as an indicator for the impact of disturbance. We predicted that 1) mussels translocated to a nearby habitat where no other mussels were present (unoccupied) would result in lower performance than control mussels that remained in original habitat within a segment of the San Saba river (Table 2.1); 2) Mussels moved to a habitat in a new segment of the river with other mussels present (occupied) would have higher performance than mussels moved to an unoccupied habitat in the new segment, but would still have lower performance than control mussels (Table 2.1); 3) Mussels that were moved to unoccupied habitat would be dislodged more easily during flooding resulting in a greater decline of detection compared to the control.

Methods

Study Area

The San Saba river is a Central Texas river that runs from Menard to San Saba County where it eventually joins the Colorado river. The river is approximately 100 miles long and is predominantly surrounded by ranches (TPWD 2018). The upstream segment of the river in Menard varies in width from 20 to 50 feet and tends to be shallower than the downstream (TPWD 2018). Downstream San Saba is 50 to 70 feet wide (TPWD 2018). The river is largely undeveloped and natural with heavily vegetated banks. There is a mixture of riffles and pools throughout the San Saba River. Previous continuous surveys on the San Saba river identified that mussels tend to occupy riffles in the lower segment and pools in the upper segment (Mitchell 2018, unpublished data). *C. petrina*, *C. pustulosa*, *T. verrucosa* are most abundant in the lower segments, while *U. imbecillis* and *L. bracteata* tend to reside in large numbers in the upper.

Environmental Variables

Temperature (°C), specific conductivity ($\mu\text{S cm}^{-1}$), and dissolved oxygen (mg L^{-1}) were measured at each site using a YSI 143 556 MPS during monitoring trips. Average velocity (m/s) was measured at each mussel sampling site at 60% depth in the middle of the stream using an electromagnetic flow meter. Discharge data (m^3/s) for the lower sites were taken from USGS gauge 08146000 on the San Saba River at San Saba, TX and for the upper sites from USGS 08144500 San Saba River at Menard, TX. A Wolman pebble count was conducted at each site to find median substrate diameter (D50) and D10. D84 was calculated to attain bed roughness (k_s , cm), which was then used to calculate shear velocity (U^* , m^3/s). Shear velocity was used to calculate shear stress (t , dynes/cm^2). Critical shear stress (t_c , dynes/cm^2) was also calculated for each site using median substrate diameter. (Allen and Vaughn 2010).

$$k_s = 3.5 \times D_{84}$$

$$U^* = U / 5.75 \log_{10}(12d/k_s)$$

$$t = \rho(U^{*2})$$

$$t_c = \theta g D_{50}(\rho_s - \rho)$$

Where U is average velocity (m/s), d is depth, ρ is density of water (g/cm^3), θ is Shield's parameter, g is acceleration due to gravity (cm/s^2), and ρ_s is density of substrate (g/cm^3). Substrate from the lower segment pools were collected for sieve analysis. Sediment was dried for 24 hours at 105°C and sieved with a sieve shaker (Endecotts, Octagon 200) at different size classes (using a modified Wentworth scale). Data on visual substrate composition from another study (Mitchell 2020) in the San Saba river was also utilized.

Specimen Collection & Handling

Freshwater mussels were collected from the Upper and Lower segments of the San Saba River using tactile searches. Collected specimens were measured and marked with a shell tag and a uniquely numbered Passive Integrated Transponder (PIT) tag (8mm). Collections occurred during Spring and Fall to avoid exposing mussels to extreme temperatures (Dunn 1999). Mussels were kept in the river, contained in buckets with holes to keep emersion to a minimum during measuring and tagging (Mackie et al. 2008). Specimens were then placed back into the river along three to four transects, depending on number of mussels translocated, spaced 1.5 meters apart. 10 mussels were allocated to each transect that spread width of river segment. Specimens that were translocated to different segments of the river were transported in a cooler with substrate and water taken from origin site with aerators (Mackie et al. 2008). Once at the destination site, mussels were carefully placed along transects with their siphon facing upstream (Hamilton et al. 1997). Mussels were monitored and measured every couple of months when conditions allowed. Shell length was used to determine growth rate. Growth rate was computed as the difference between the measured length at the end and the beginning of the experiment divided by the duration (months) of the experiment. Antennas for PIT tag readers were used to detected mussels that were PIT-tagged.

Translocation experiments

Two different sets of translocation experiments were carried out, one experiment with *Cyclonaias pustolosa* (October 2018 to March 2020) and one fully crossed experiment with the congeners *C. pustolosa* and *C. petrina* and *Utterbackia imbecillis* (October 2019 to March 2020). For both sets of experiments translocations were carried

out within a segment, where mussels were translocated to a different nearby unoccupied mesohabitat (pool or riffle), and between segments, where mussels were translocated to a segment from which they were absent (i.e., *U. imbecillis* in lower segment) or in which segment they occurred in low numbers (i.e., *C. pustulosa* and *C. petrina*).

First experiment with C. pustulosa only

A total of 80 individuals of *Cyclonaias pustulosa*, were collected in a riffle in the lower segment of the San Saba River on October 6th, 2018 (Figure 2.1), 30 individuals from that riffle were handled and placed back into their origin habitat to act as a control, and 30 were moved to a nearby pool (within segment translocation) where mussels were absent. Mussels were monitored 5-months, 9-months, and 1-year after translocation (Table A2.1).

For the between translocation 10 individuals were moved to a riffle (unoccupied habitat) and another 10 in a pool (occupied habitat) in the upper segment of the San Saba. To increase the number of individuals that were translocated between segments, an additional 29 mussels were moved from the lower control riffle to the upper segment, 14 to the pool, and 15 to the riffle on March 25th, 2019 (Figure 2.1). In the upper segment, the mussels that were translocated in October 2018 were monitored 5-months, 6-months, 9-months, and 1-year after translocation (Table A2.2). The mussels translocated in March 2019 were monitored 1 month, 4-months, and 7-months after translocation (Table A2.3).

Fully Crossed Experiment

C. pustulosa and C. petrina

A total of 102 mussels (43 *C. pustulosa*, 59 *C. petrina*) were collected on September 27th, 2019 in two nearby riffles in the lower segment, 30 of which (10 *C.*

pustulosa, 20 *C. petrina*) were kept at the original site (first riffle) to act as a control (Figure 2.1). For the within segment translocation, 20 mussels (7 *C. pustulosa*, 13 *C. petrina*) were moved to a nearby pool, where no mussels were found (unoccupied). For the between segment translocation, 26 mussels (13 *C. pustulosa*, 13 *C. petrina*) were translocated to an unoccupied riffle in the upper segment and another 26 (13 *C. pustulosa*, 13 *C. petrina*) were moved to an occupied pool (control pool of *Utterbackia*, see below Figure 2.1). These were the same pools and riffles used for the within segment experiments. Mussels were monitored 1-month and 5-months after translocation (Table A2.6).

Utterbackia imbecillis

A total of 52 individuals of *Utterbackia imbecillis* were collected from a pool in the upper segment of the Saba River (upper treatment pool for *Cyclonaias*, Figure 2.1) on October 5th, 2019, where a larger number of *U. imbecillis* was found (but they were less abundant than *Cyclonaias* in the lower segment). Sixteen were placed in the original pool to act as a control, and 16 were translocated to a nearby riffle (within segment translocation). For the between segment translocation, 10 individuals were translocated to a riffle in the lower segment (control riffle for *Cyclonaias*, see above *fully crossed experiment*) that was occupied by other mussel species and another 10 to an unoccupied pool in the lower segment (treatment riffle for *Cyclonaias* within segment translocation, Figure 2.1). Detection of mussels (both *Cyclonaias* and *Utterbackia*) was measured three weeks after the translocation (26 and 27th of October 2019) and both detection and growth were measured five months after translocation (6 and 7th of March 2020).

Glycogen Analysis

Glycogen was used as a supplemental measure of mussel condition as measurement of length is variable, needs long-term monitoring, and repeated handling of mussels can limit growth (Haag 2009). Glycogen was used as a cumulative measure of stress, as glycogen will deplete over time and can be an indicator of energetic status (Naimo et al. 1998). During the final check-up on mussels, a 4-11 mg sample of their foot was cut and transported in a cooler with dry-ice for glycogen analysis. Also during the final check-up on mussels, random mussels from both lower riffles were collected to establish baseline glycogen concentrations of mussels not handled during the experiment.

Glycogen analysis was conducted using a procedure modified from a method to quantify glucose in potatoes (Bethke and Busse 2008). First, glycogen was extracted from the tissues through homogenization in 10% ethanol and centrifuging to get supernatant of samples. Supernatants were then placed in vacufuge for 8-10 hours. Second, glycogen content of samples was quantified by adding amylogucosidase into one replicate of sample wells to transform glycogen into glucose monomers. An enzyme mix of glucose oxidase, 10-acetyl-3,7-dihydroxyphenoxazine (ampliflu Red), and horseradish peroxidase (HRP) was then added to cause a color change in the samples to a pinkish resorufin in the microplate well. The resorufin has an absorbance of 560 nm and is proportional to glucose concentration. Spectrophotometer absorbance values of sample wells with amylogucosidase were subtracted from wells without amylogucosidase to compute the amount of glycogen (mg/g) in mussel tissue.

Data Analysis

Differences in environmental parameters, growth rates, and glycogen concentration, between treatment and control sites were examined by Analysis of Variance (ANOVA) when data were normally distributed. Data were tested for normality using a shapiro-wilks test and homogeneity of variances was confirmed through a Levene's test. Tukey's honest significance test was utilized when ANOVA showed significant difference between sites to determine which sites differed. *C. petrina* and *C. pustulosa* were tested for differences by adding species as a factor in ANOVA, and since there was no significant effect, data from both species was pooled.

Results

Environmental Variables

In the first and fully crossed experiment, environmental conditions did not differ significantly between sites except for average velocity (m/s) and depth (m). As expected, the riffles had significantly higher velocity and lower depths compared to the pools (Table 2.2). Geomorphic variables also differed between sites used in the first and fully crossed experiments, with the riffles having the smaller channel widths but higher median substrate diameters (D50) than the pools. The Wolman pebble count resulted in median particle diameters ranging from 36 mm (large pebble) to 68 mm (small cobble, Table 2.3), where the values ranged between 8 mm (coarse gravel) and 33 mm (large pebble) in the pools. Visual estimates for proportion of substrate sizes per habitat indicated upper pools were majority bedrock (70 and 80%) (Mitchell 2020). Upper riffles were mix of cobble (50%-60%), pebble (30%), and gravel (10%-20%). Lower riffle of the fully crossed experiment was a mix of pebble (35%), gravel (30%), and sand (20%), while

lower pool consisted of gravel (10%), sand (45%), and silt (45%). Critical shear stress for the pools were considerably lower compared to the riffles (Table 2.3). Upper pools had an abundance of bedrock, rock shelves, and emergent vegetation, while lower pools were more sandy without any vegetation. The pool in the lower segment of the fully crossed experiment had a considerably higher proportion of silt (2%) and very fine sand (28%) compared to the pool in the first experiment (silt: 0.2%, very fine sand: 15%).

Growth Rates

In the first experiment, after 1 year of translocation, mean within-segment growth rate of *C. petrina* at the control site (0.14 ± 0.1 , mean \pm SD mm per month) was slightly higher than the pool (0.11 ± 0.1 mm per month), but the difference was not statistically significant (Figure 2.2, Table 2.4, $T_{24} = -0.48$, $p = 0.65$). Mussels (*Cyclonaias petrina* and *pustulosa*) translocated to the upper riffle (0.06 ± 0.1 mm per month) in March 2019 and measured in March 2020 showed significantly higher growth rates compared to the upper pool (0.02 ± 0.03 mm per month) ($T_{12} = -3.4$, $p < 0.01$), but even the higher growth rates in the upper riffle were significantly lower compared to the control site in the lower segment (Figure 2.2, Table 2.4).

Detection

During the first experiment one flooding event occurred in October 2018 immediately after the translocation experiment was initiated. Discharge peaked at ~ 707 m^3/s for the USGS gauge at San Saba (Figure A2.1) in the lower section and at $566 m^3/s$ in the upper section for the USGS gauge at Menard, 307 and 446 times median flow rate for that month respectively ($2.3 m^3/s$, $1.3 m^3/s$). Additional major flood events occurred in May 2019, peaking at $424 m^3/s$ in the lower section and at $538 m^3/s$ in the upper

section (Figure A2.4). During the fully crossed experiment no major flood events occurred, but slightly higher flow occurred in March 2020 up to 2.8 m³/s, 3.3 times the median flow rate in the upper and 5.6 m³/s, 2.2 times median flow rate in the lower section (Figure A2.2, Figure A2.3).

Detection within segment

No differences in detection (at least longer term) were found within the lower segment when major flooding occurred, whereas differences were found during the fully crossed experiment in the absence of major flooding (Table 2.4, Table 2.5). In the first experiment, differences in detection within the lower segment were found after 5 months, but not after 9 or 12 months (Figure A2.1). However, the difference detected after 5 months 87% (26 out of 30) in control riffle vs. 60% (18 out of 30) in the pool) was likely due to a fallen tree that covered a transect in the pool in which 10 mussels had been placed. After one year, 47% and 44% were detected in the riffle and pool respectively (Figure A2.1). Detection may have been ultimately higher in the pool, where shear stress was slightly lower compared to the riffle (Table 2.3) compared to the riffle, when considering that some mussels may have been trapped and inaccessible under the tree in the pool.

In contrast to the first experiment, there were differences in detection within the lower segment during the fully crossed experiment, when no major flooding occurred (Table 2.4). After 3 weeks of translocation, 80% (24 out of 30) of *C. pustulosa* and *C. petrina* were detected in the control riffle and 45% (9 out of 20) in the pool (Figure A2.2). Although detection decreased in the riffle after 5-months of translocation (63%, 19 out of 30), it remained higher compared to the pool (45%).

Detection between segments

When mussels were moved to the upper segment, no differences in detection were found in the mussels translocated in October 2018, but distinct differences were detected before and after flooding of those moved in March 2019 during the first experiment. This difference did not occur in the absence of major flooding 5 months after translocation in October 2019 during the fully crossed experiment.

In the first experiment where individuals of *C. pustulosa* were moved from the lower to the upper San Saba, detection in the upper segment varied in time (Figure A2.4). After one year, detection in the upper riffle (50%, 5 out of 10) and pool (30%, 3 out of 10) were similar to detection in the lower control riffle (47%).

There was a distinct difference in detection before and after flooding occurred for the additional mussels that were moved from the lower control riffle to the upper segment in March 2019. A high percentage of mussels (80-86%) was detected a month after translocation before any flooding occurred (Figure 2.5). After a large flooding event in May (see above) and 4-months after being translocated, detection in the upper riffle remained high (87%), while detection dropped in the upper pool (43%). This trend continued during the 7-month and 1-year check where detection in the riffle was 93%, which was higher than at the control riffle in the lower segment at 9-months (60%), while detection in the pool was considerably lower (36%). None of the mussels were found dead.

In the fully crossed experiment *Cyclonaias sp.* moved from the lower control riffle had high detection in both the upper pool (92%) and upper riffle (100%) after 5-months of translocation without flood events (Figure A2.5).

Mortality

A small number of mussels were found dead in the lower segment, but the percentage of translocated mussels that were found dead during the first experiment was lower in the control riffle compared to the pool in both the first experiment (0 and 3% respectively) and fully crossed experiment (6 and 10% respectively, Table 2.4). The differences between control riffle and treatment pool became larger when calculating the percentage of detected mussels that were found dead in the first experiment (0% and 8% for control riffle and pool respectively) and fully crossed experiment (11 and 22% respectively, Table 2.6). Dead mussels were found within the habitat they were translocated, and not on the shore. It should be noted that major flooding may have also removed dead mussels.

Mortality in the upper segment was higher, especially in the upper riffle. The percentage of detected mussels that were found dead were similar in the upper pool (the habitat occupied by other mussels, 0 and 4% for the first and fully crossed experiment respectively) and the control riffle in the lower segment (0 and 11% for the first and fully crossed experiment respectively, see above), whereas it was considerably higher in the riffle in the upper segment (50 and 23% for the first and fully crossed experiment respectively, Table 2.6).

Opportunistic species-Utterbackia

For the within-segment translocation, *U. imbecillis* showed a considerably higher detection in the control pool (100%) than in the treatment riffle (19%) after 5-months of translocation (Figure A2.3). Mussels translocated to the lower segment showed low detection in the lower riffle (10%, 1 out of 10) and moderate detection in the pool (50%,

5 out of 10) after 5-months of translocation (Figure A2.6). The percentage of translocated mussels that were found dead ranged from 10% (1 out of 10, lower pool) to 12.5% (2 out of 16 in the upper control pool and the upper riffle). Differences were more extreme when looking at the percentage of detected mussels that were found dead. Of the detected mussels, the lowest mortality was found in the upper control pool (12.5%), followed by 20% in the pool in the lower segment and, similar to *Cyclonaias*, was highest (66%) in the upper riffle (note only 1 dead individual total was detected in the lower riffle, Table 2.6).

Glycogen

Glycogen concentration (mg/g) significantly differed between sites in the first experiment but not during the fully crossed experiment (Table 2.4). For the first experiment, glycogen concentration were significantly higher in the mussels that were collected randomly in the control riffle and that had not been handled and were not utilized in the experiment (15.3 ± 1.3 mg/g, mean \pm SE) compared to the experimental control mussels in the lower control riffle (10.85 ± 0.92 mg/g) (Figure 2.3). There were also significant differences between mussels in the lower control riffle and mussels translocated to the lower pool (5.1 ± 2.3 mg/g), however only 4 samples of glycogen were analyzed for the pool. No significant differences in glycogen concentrations were detected for mussels in the lower control riffle and mussels moved to the upper segment. However, the mean glycogen concentration for the upper riffle (10.1 ± 1.2 mg/g) was slightly higher compared to the upper pool (8.4 ± 0.7 mg/g) (Figure 2.3).

There were no significant differences in glycogen concentrations between sites for the fully crossed experiment. The random mussels not utilized during the experiment

from the lower control riffle had the highest mean glycogen concentration (14.7 ± 3.2 mg/g), while the mussels handled in the lower riffle had a slightly lower average (12.1 ± 2.3) (Figure 2.4). The lower control riffle had a higher average concentration than the lower pool (9.4 ± 0.5 mg/g), but differences were not statistically significant (Table 2.4). For *U. imbecillis*, there were only a sufficient number of samples for glycogen analysis from the upper control pool (8.21 ± 1.22), where *U. imbecillis* had a lower mean glycogen concentration compared to *Cyclonaias* except for those in the lower pool in the first experiment.

Discussion

This is the first study that used translocation experiments to examine the role of local habitat conditions for the distribution of freshwater mussels and to compare the performance of mussels with two different life history strategies. The opportunistic species (*U. imbecillis*) appeared to be strongly limited by local habitat conditions. The results were less clear for the equilibrium species (*C. petrina* and *pustulosa*), but there were several indications that their performance was better in riffle compared to pool habitat. In addition, the detection of *Cyclonaias* declined considerably in a treatment pool in the upper segment after a flooding event, indicating the importance of disturbance events (see below).

Consistent and sometimes considerable differences in detection and mortality of *U. imbecillis* between control and translocation sites suggested strong environmental control as detection was only high (and mortality was low) in the pool it originated from in the upper San Saba River. *Utterbackia* performed especially poorly in riffles, but even detection in the pool of the lower segment was considerably lower compared to the

control pool in the upper segment. Pools in the upper segment are distinct from pools in the lower segment. *U. imbecillis* was typically found in the roots of vegetation or under rock shelves in the upper pool, which may act as flow refuge. Such habitat structures are missing in the lower segment. Other studies have found that mussel beds tend to be in areas with the least potential for bed disturbance (Strayer 1999, May and Pryor 2016). Also, the pools in the upper segment tended to have a higher critical shear stress (requiring more shear stress, more flow before substrate starts moving) compared to the lower segment, suggesting that substrate would be less stable in the lower segment.

The low detection of this lentic thin-shelled species in riffles only three weeks after translocation is most likely a result of the inability of the species to remain in place at higher flow velocities and higher shear stress (Table 2.3). Even without any major flooding event occurring during the fully crossed experiment, *U. imbecillis* was likely dislodged. Similarly, a transplant study using *Lampsilis teres*, another opportunistic species, found that most translocated individuals were washed out of habitats after translocation; even without flooding (Hamilton 1997). Alternatively, mussels may have moved away from the riffles and also the pool in the lower segment, however, we searched up to 0.5 km upstream and 2 km downstream of each site. Only three mussels were found about 20 m downstream of their transect in the upper pool in a new bed of vegetation. Horizontal movement in mussels has previously been hypothesized as an adaptation to flow conditions in rivers and as a way for mussel to find suitable habitat or to avoid adverse conditions (Waller et al. 1999, Schwalb and Pusch 2007, Borden and Brown 2002) and opportunistic species may be especially mobile (Waller et al. 1999, Mitchell et al. 2018).

Handling has been shown to be stressful for mussels (Haag and Commens-Carson 2008). This was also found in this study where randomly collected *Cyclonaias pustulosa* in the control habitat had significantly higher glycogen concentrations compared to the experimental mussels. Opportunistic species, especially thin shelled species, may be even more sensitive to handling than thick-shelled equilibrium species. Indeed, *Utterbackia* had significantly lower glycogen concentration in their control habitat of compared to *Cyclonaias*. The increased stress after handling may have resulted in the higher mortality and the lower detection compared to *Cyclonaias* but detection in the control habitat was higher for *Utterbackia* compared to *Cyclonaias*. In addition, a higher proportion of detected *Utterbackia* was found dead in the riffles compared to the pools, suggesting that habitat conditions in riffles were unsuitable, likely stressful. In summary, *U. imbecillis* may not occur in the lower part of the San Saba River and in the riffle of the upper San Saba due to a lack of suitable habitat.

Results for *Cyclonaias sp.* were more complex and also included results from the first experiment, which allowed us to determine differences in growth. Within the lower segment, results for, mortality, detection in the absence of flooding (fully crossed experiment), and glycogen (after 1 year of translocation, first experiment) indicated that mussels performed better in control riffles than in pools, but no significant differences were found for growth, detection in the first experiment (when major flooding occurred, Table 2.4), and glycogen in the fully crossed experiment (absence of flooding). Thus, although differences in mortality and partly glycogen suggested suboptimal habitat conditions in nearby pools within the lower segment for adults, growth did not. There may be greater differences in glycogen after 1-year of being translocated (first

experiment), than after 5-months (fully crossed) due to differences in sampling times or the duration of the study. Glycogen concentration changed relatively quickly in response to stress, so variable annual cycles of food availability can highly influence glycogen reserves (Albentosa et al. 2007, Cordeiro et al. 2016, Vodakova and Doua 2019). Lower food availability can also deplete glycogen concentration of mussels, and mussels in first experiment may have been more starved than mussels in fully crossed experiment at least in unsuitable habitat (e.g., lower pool, Patterson et al. 1997).

The mixed evidence suggests that other additional factors may explain the absence of *Cyclonaias* from pools and may include factors acting on different life stages not included in this study. Even though the detection of adults did not differ between pool and control riffle within the lower segment when major flooding occurred, juveniles (especially 1-2 yo when size is limited to a few mm) may get easily washed out of the pools with their lower critical shear stress, where finer substrate with similar sizes to juvenile mussels are transported downstream. Other studies have found that a lack of stable substrate (Strayer 2008), lack of flood refuge (Strayer 1999), higher shear stress (Daraio et al. 2010, French and Ackerman 2014), and disturbance (Layzer and Madison 1995) can prevent juvenile settlement.

Cyclonaias is generally found more commonly in riffles than in pools and several results of our study suggest a better performance of *Cyclonaias* in riffles compared to pools. Although *Cyclonaias* had similar growth rates in the lower riffle and pool, higher growth rates were found in the upper riffle compared to the upper pool. Results from the first experiment also suggested a better performance in the upper riffle compared to the upper pool, suggesting that pools are sub-optimal habitat for *Cyclonaias*. This is

consistent with findings from a translocation experiment, in which *Margaritifera hembeli* was moved to different riffles and pools in Jordan and Beaver creek in Louisiana, where resident mussels in pools are uncommon and found growth rates were greater in riffles than pools (Bolden and Brown 2002). An important factor could be higher substrate stability of riffles (Johnson and Brown 2000). For example, the upper pools only had a thin layer of sediment that do not allow mussels to burrow deeply. It has been suggested that pools may also experience poor food delivery because of lower flow velocities (Bolden and Brown 2002, Strayer 2008).

Although riffles may be generally better habitat for *Cyclonaias* compared to pools, *Cyclonaias* (*pustulosa* and *petrina*) are more frequently and in higher abundances found in riffles of the lower segment of the San Saba River (Mitchell 2020). The lower growth rate and higher mortality in the upper riffles compared to the lower riffles indicate that riffles in the upper segment may be less optimal habitat compared to the lower segment, although detection in the upper riffle was high. The high detection in the upper riffle is possibly due to the remote location (i.e., far from urban influence) of the upper riffle, where they are less likely to be predated by raccoons, also supported by the fact no raccoon piles were found in the upper segment. Interestingly, *Cyclonaias* species have been found in moderate abundances only at riffle sites in the upper segment, which resemble habitat conditions in the lower segment (Mitchell, pers. Comm.), especially in terms of flow and substrate. Conditions in the upper riffle may be similar enough to conditions in the lower that mussels are able to remain in that mesohabitat, even with severe flooding. Mussels in the upper riffle may be experiencing lower growth than the lower riffle due to flashier flow regime of upper, one that is not suited for equilibrium

species that may be better adapted to more stable environments (Haag 2012). Another possible explanation is there may be less food available in the upper riffle than in lower segment. There was an abundance of *Corbicula* occupying the upper riffle that could be competing with translocated mussels.

The impact of disturbance on the performance of mussels in different mesohabitats became evident when detection of *Cyclonaias* declined considerably in the upper pool after a flooding event, whereas it remained high in the upper riffle. In contrast, detection of *Utterbackia* in the same riffle was extremely low even in the absence of flooding. While *Cyclonaias* were likely to anchor and burrow themselves in the gravel of the upper riffle, *Utterbackia* likely did not do this and probably either moved or were swept away from the riffle. On the other hand, *Utterbackia* was able to persist in the upper pool, probably actively moving to the next habitat structure acting as flood refuge (see below), whereas *Cyclonaias* has low mobility (Mitchell et al. 2018) and may not be able to anchor in the bedrock dominated upper pool with only a smaller layer of gravel and finer substrate. Disparities in mussel detection in the two different mesohabitats in the upper segment after flooding suggest disturbance as a potentially important process of environmental control. Other studies have shown that flooding eliminates mussel beds or relocates them further downstream, depending on their location in the river, and difference in hydrology within the same river can possibly determine suitability of sites (Hastie 2001, Cope et al. 2003; Stodola et al. 2017). Flooding made a noticeable difference in detection for the upper segment (decline of *Cyclonaias* in the upper pool see above), where flow conditions are more flashy (Mitchell et al., in prep), but did not appear to affect detection in the lower segment. However, in the absence of flooding

(fully crossed experiment) the detection in the control habitat was higher in the upper segment and the upper pool had high detection for both species (*U. imbecillis* 100%, *Cyclonaias* 92%).

Although no flooding occurred during the translocation experiment with *U. imbecillis*, the species may be able to persist in pools because they provide more habitat structure that can act as flood refugia (e.g. vegetation, rock shelves), and other studies have found mussel beds tend to be in areas with the least potential for bed disturbance (Strayer 1999, May and Pryor 2016). In addition, pools may not dry out as easily, compared to riffles that may go dry or water temperatures may become high during low flow conditions in summer. Drying did not occur during this study, but future studies should look at the impact of drought in the San Saba and other rivers as a potential driving factor for distribution. *Cyclonaias* never occurs in pools in the upper segment, although mortality for *Cyclonaias* was lower in the upper pool, suggesting it could potentially persist there. However, the severe decline in detection after flooding (see above) suggest that displacement during flooding may prevent this equilibrium species from residing in the upper pools.

Mussels that were not detected may have been flushed downstream, predated by raccoons, or failed to be located. However, the area up to 0.5 km upstream and 2 km downstream of each site was searched intensively with a PIT tag reader and at the shores to detect any mussels possibly predated by raccoons. Three mussels were found about 20m downstream of their transect in the upper pool in a new vegetation patch (see above) and no mussels were found at the shore. Other studies have showed displacements can occur due to flooding and mussels horizontally moving from habitat (Kurth et al. 2007;

Peck et al. 2007, Bolden and Brown 2002, Villella et al. 2004, Fernandez 2013), but future studies should examine displacement distances of mussels especially potentially displacement of larger distances caused by flooding.

While effects of local environmental conditions have been a focus in this study, dispersal limitation may be another mechanism that could limit the distribution of mussels. Movement of fish during the parasitic stage of mussels are crucial for the dispersal of mussels. Fish populations have been observed to be similar in the lower and upper San Saba river (Mitchell, pers. Comm.). Nevertheless, dispersal limitation could be occurring if a physical barrier was blocking a mussels' host fish from reaching another segment. The mid-reach of the San Saba river can dry out and become intermittent during the summer preventing fish migration. This could prevent host-fish from travelling between segments during the reproductive time of *Cyclonaias* (mid-May to mid-July) and *Utterbackia* (early June to late July) when they release their glochidia (larvae) (Lefevre and Curtis 1912, van der Schalie 1938, Watters 1995). Although the middle San Saba tends to dry out toward the end of the mussels' reproductive period (July to September), host-fish could still navigate between segments before then (Mitchell pers. Comm.).

Findings from this study indicate suitable habitat for a particular species is not necessarily indicated by presence of mussels, especially if different species with different life history strategies are present. Mitigating the effects of in-stream construction on mussels through translocation to another mussel bed, deemed suitable habitat by presence of other mussel species, may be ill-suited depending on the environmental conditions and flow regime the habitat exhibits. Environmental control appeared to be stronger for the opportunistic species compared to equilibrium species in this study, but this could be due

to more the unique habitat requirements of *U. imbecillis*, and this should be tested with other species in the future. Adaptation to local flow conditions also appears to be important for mussel distribution, which may only become relevant during disturbance events such as flooding. There should be more studies to better understand how the disturbance regime of river can play a role in their distribution, including long-term translocation experiments.

Table 2.1: Prediction table for translocation experiments within and between segments. In the lower segment, riffles were the occupied and pools the unoccupied habitat, whereas in the upper segment pools were occupied habitat and riffles unoccupied.

Translocation	Prediction
Within Segment	control (occupied) > treatment (unoccupied)
Between Segment	control (occupied) > treatment (occupied) > treatment (unoccupied)

Table 2.2: Environmental parameters measured at the experimental sites for A) the first experiment with *Cyclonaias pustulosa* and B) the fully crossed experiment with *Cyclonaias* and *Utterbackia*. Mean value of all sampling events given with range in parentheses Parameters with an asterisk indicate that there was a significant difference between sites ($P \leq 0.05$).

A	Lower Riffle 1	Lower Pool 1	Upper Riffle 1	Upper Pool 1
Temp. °C	22.1 (16.7- 28.7)	22.0 (16.2- 28.9)	22.8 (17.3- 26.3)	22.4 (17.1- 27.4)
DO (mg/L)	7.2 (6.2-8.2)	6.9 (6.0-7.9)	7.4 (6.9-8.05)	7.0 (6.4-7.55)
Sp. Cond. ($\mu\text{S}/\text{cm}$)	614 (570- 655)	620 (586- 640)	611 (545- 632)	627 (580- 652)
Average Velocity (m/s)*	0.68 (0.56- 0.84)	0.18 (0.1- 0.3)	0.47 (0.24- 0.62)	0.03 (0.01- 0.05)
Depth (m)*	0.48 (0.31- 0.78)	0.9 (0.62- 1.21)	0.25 (0.19- 0.31)	0.76 (0.6- 1.08)

B	Lower Riffle 2	Lower Pool 2	Upper Riffle 2	Upper Pool 2
Temp. °C	23.6 (16.2- 27.8)	24.4 (16.1- 28.5)	23.2 (17-25.4)	22.8 (16.4- 26.7)
DO (mg/L)	6.5 (5.0-7.59)	6.0 (5.32-6.4)	6.7 (4.5-7.8)	5.9 (4.8-6.5)
Sp. Cond. ($\mu\text{S}/\text{cm}$)	562 (543-588)	576 (533-598)	614 (601-635)	612 (598- 634)
Average Velocity* (m/s)	0.81 (0.67- 0.92)	0.04 (0.02- 0.06)	0.57 (0.45- 0.72)	0.03 (0.02- 0.04)
Depth (m)*	0.6 (0.3-0.75)	1.5 (1.2-1.6)	0.6 (0.23-1.2)	0.7 (0.31-0.9)

Table 2.3: Geomorphic parameters measured at experimental sites for A) the first experiment with *Cyclonaias pustulosa* and B) the fully crossed experiment with *Cyclonaias* and *Utterbackia* D10 and D50 were obtained through Wolman pebble count.

A	Lower Riffle 1	Lower Pool 1	Upper Riffle 1	Upper Pool 1
D10 (mm)	12	5.9	19.9	9.9
D50 (mm)	35.5	16	64	22
Channel Width (m)	13.6	13.4	7.1	32.3
Bed Roughness (k_s , cm)	16.8	15.4	34.3	22.8
Shear Velocity (U^* , cm/s)	0.34	0.31	0.94	0.06
Shear Stress (t , dynes/cm ²)	0.12	0.10	0.88	0.004
Critical Shear Stress (t_c , dynes/cm ²)	373.6	168.4	673.5	231.5
Relative Shear Stress (RSS)	0.003	0.0006	0.001	0.000002

B	Lower Riffle 2	Lower Pool 2	Upper Riffle 2	Upper Pool 2
D10 (mm)	24	4	20	13
D50 (mm)	68	8	43	33
Channel Width (m)	12.6	22.8	4	25.5
Bed Roughness (k_s , cm)	39	7	29.4	18.6
Shear Velocity (U^* , cm/s)	0.76	0.003	0.41	0.012
Shear Stress (t , dynes/cm ²)	0.57	0.000009	0.17	0.01
Critical Shear Stress (t_c , dynes/cm ²)	715.6	84.19	452.5	347.3

Table 2.4: Results for all translocation experiments. S: results supported prediction, PS: partial support, NS: not supported. Results with fully or partially supported results are bolded. Major flooding occurred during the first experiment (duration: 1 year), but not during the fully crossed experiment (5 months).

Translocation	Species	Growth	Prediction: Control > Treatment		
			Glycogen	Detection	Survival (1-mortality)
Within lower segment (First experiment)	<i>C. pustulosa</i>	NS (riffle =pool)	S (riffle > pool)	NS (riffle =pool)	S (riffle > pool)
Within lower segment (Fully Crossed)	<i>C. petrina, and C. pustulosa</i>	N/A	PS (riffle > pool, but not sign.)	S (riffle > pool)	S (riffle > pool)
Lower to upper segment (First experiment)	<i>Cyclonaias pustulosa</i>	S (lower control riffle > upper riffle > upper pool)	NS (upper riffle> upper pool, but not sign.)	NS (upper riffle> lower control riffle > upper pool)	PS (lower control riffle = upper riffle > upper pool)
Lower to upper segment (Fully Crossed)	<i>C. petrina, and C. pustulosa</i>	N/A	NS (upper riffle> upper pool, but not sign.)	NS (upper riffle ≥ upper pool > lower control riffle)	NS (upper pool ≥ lower control riffle > upper riffle)
Within upper segment (Fully Crossed)	<i>U. imbecillis</i>	N/A	N/A	S (pool >> riffle)	S (upper pool >> lower pool)
Upper to lower segment (Fully Crossed)	<i>U. imbecillis</i>	N/A	N/A	S (upper pool>> lower pool > lower riffle)	S (upper pool > lower pool)

Table 2.5: Detection of mussels in each habitat at the end of the first and fully crossed translocation experiments (see methods for details on dates).

Species and experiment	Percentage of detected mussels			
	Upper riffle (unoccupied habitat)	Upper pool	Lower riffle	Lower pool (unoccupied habitat)
<i>Cyclonaias</i> First experiment 1 year, major flooding	76 (19 out of 25)	33 (8 out of 24)	47 (14 out of 30)	44 (13 out of 30)
<i>Cyclonaias</i> Fully crossed (5 months)	100 (26 out of 26)	92 (24 out of 26)	63 (19 out of 30)	45 (9 out of 20)
<i>Utterbackia</i> 5 months)	19(3 out of 16)	100 (16 out of 16)	10 (1 out of 10)	50 (5 out of 10)

Table 2.6: Mortality of mussels detected in each habitat for the first and fully crossed translocation experiments. Note: The first experiment was started in October 2018, additional mussels were added to the upper segment in March 2019). See methods for details on experimental dates.

Species and experiment	Percentage of detected mussels found dead (number of dead out of number of detected mussels)			
	Upper riffle (unoccupied habitat)	Upper pool	Lower riffle	Lower pool (unoccupied habitat)
<i>Cyclonaias</i> First experiment 1 year, major flooding	40 (2 out of 5) 0 (0 out of 13, added March 2019)	0 (0 out of 3,) 0 (0 out of 5, added March 2019)	0 (0 out of 14)	8 (1 out of 13)
<i>Cyclonaias</i> Fully crossed (5 months)	23 (6 out of 26)	4 (1 out of 24)	11 (2 out of 19)	22 (2 out of 9)
<i>Utterbackia</i> 5 months)	66 (2 out of 3)	12.5 (2 out of 16)	NA (only 1 mussel detected)	20 (1 out of 5)

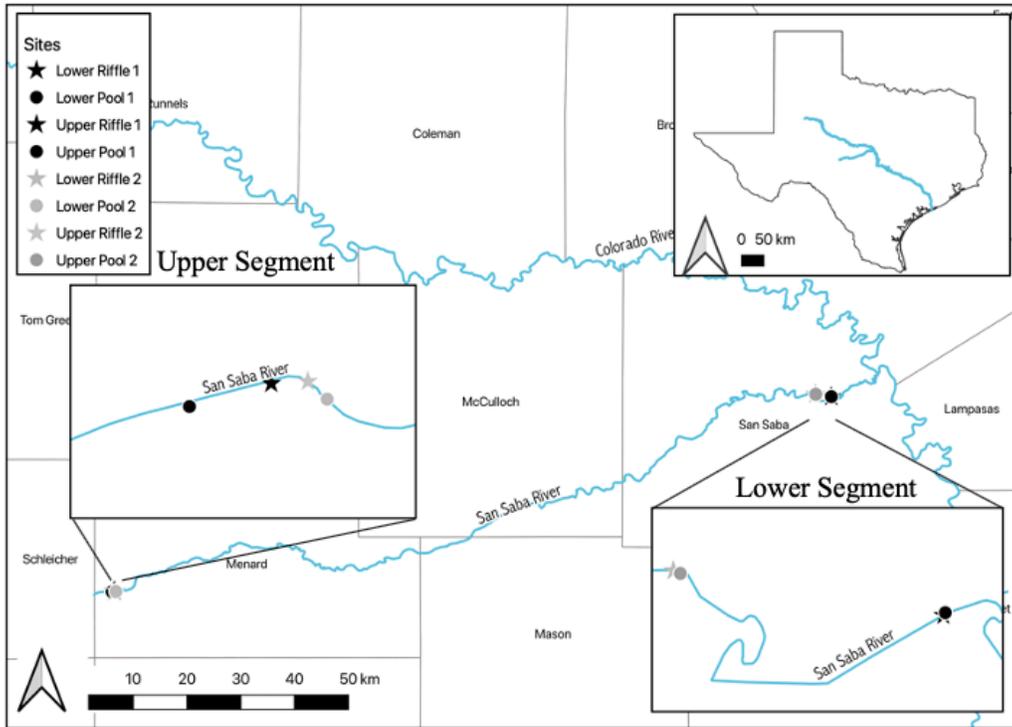


Figure 2.1: Map of experimental sites in the San Saba River.

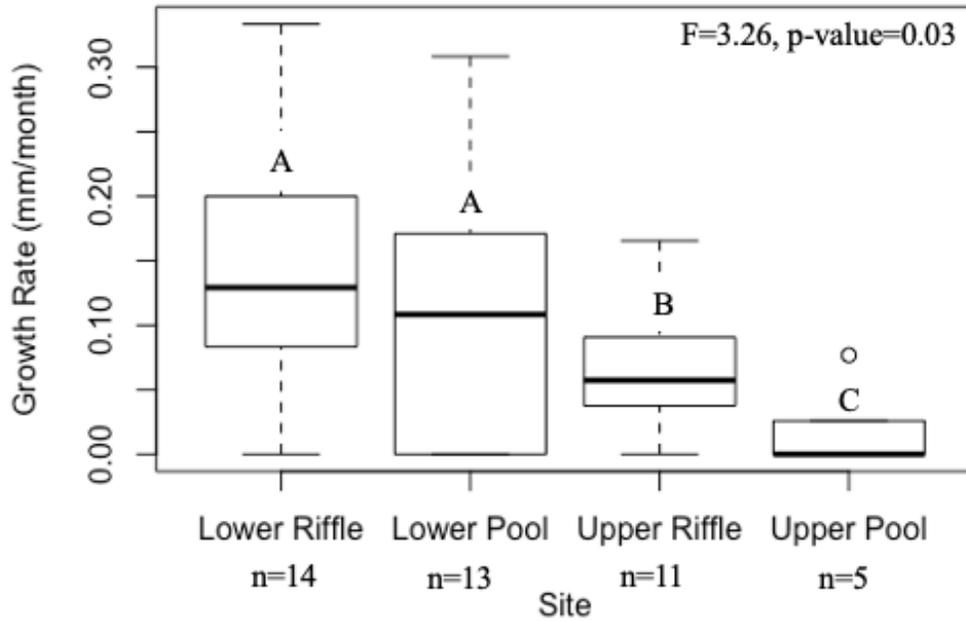


Figure 2.2: Growth rates (mm/month) of *C. pustulosa* in the control riffle and pool in the lower segment of the San Saba River (October 2018 to October 2019) and of *C. petrina* and *C. pustulosa* in riffle and pool of the upper segment of the San Saba River (March 2019 to March 2020). Different letters indicate significant differences (Tukey test, $P < 0.05$).

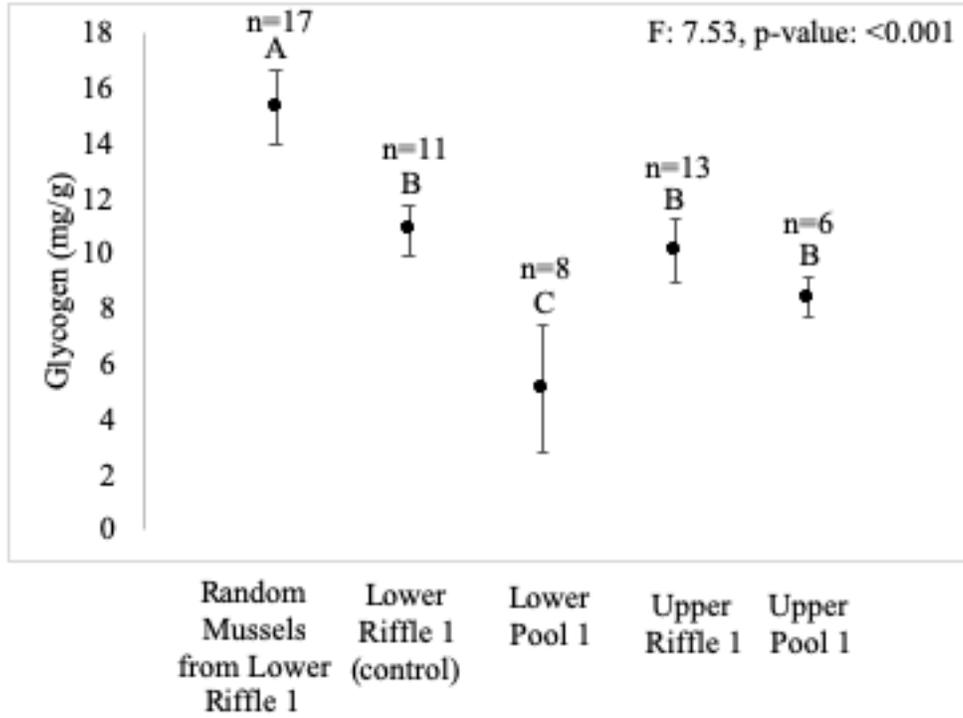


Figure 2.3: Glycogen concentration (mg/g) of *C. pustulosa* for first experiment from lower riffle 1 and lower pool 1 (October 2018- October 2019) and upper riffle 1 and upper riffle 1 (October 2018- October 2019 and March 2019-March 2020).

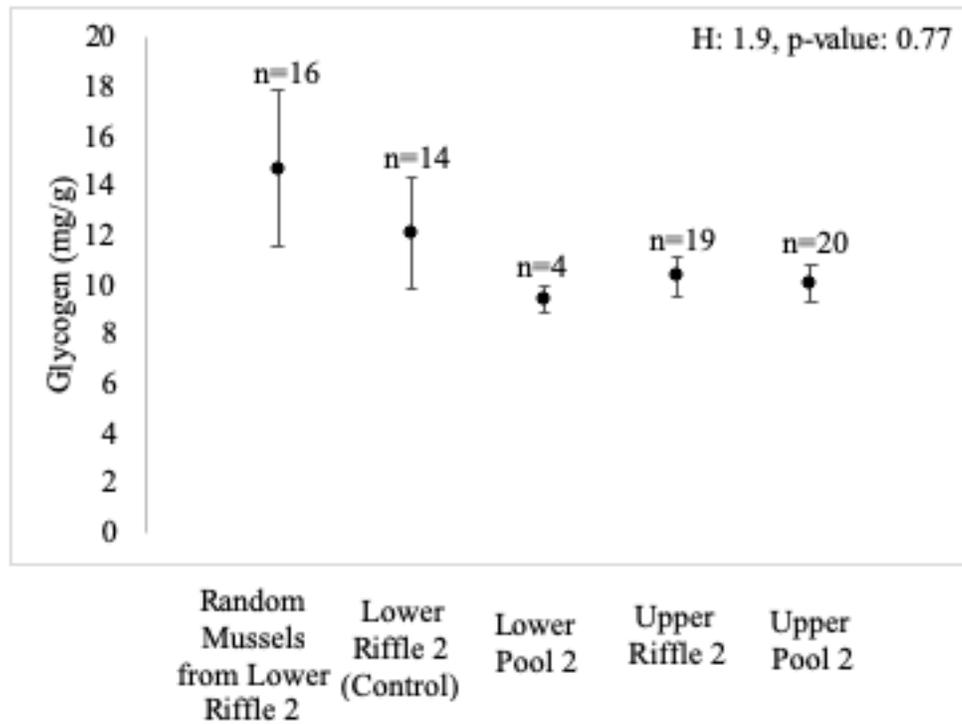


Figure 2.4: Glycogen concentration (mg/g) of *C. pustulosa* and *C. petrina* for fully crossed experiment (October 2019-March 2020).

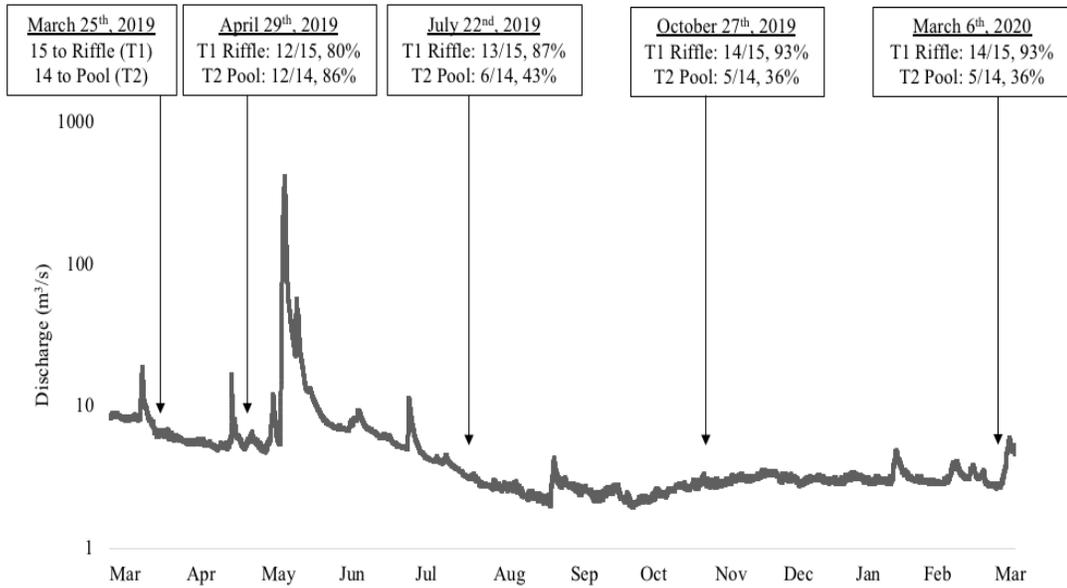


Figure 2.5: Detection of *C. pustulosa* for the additional mussels moved to the upper in March 2019 to supplement the first experiment's between segment treatments (lower to upper segment). Detection in the upper riffle declined considerably after major flooding occurred end of April 2019.

APPENDIX SECTION

Table A1.1: Sources for tables 3 and 4 (Haag and Rypel 2011, Table 1, Haag 2012, Table 6.3).

	Source
1	Bruenderman and Neves (1993)
2	Christian et al. (2000)
3	Haag and Rypel (2011)
4	Haukioja and Hakala (1978)
5	Hove and Neves (1994)
6	Jones and Neves (2002)
7	Jones et al. (2004)
8	Michaelson and Neves (1995)

Table A2.1: Dates of Performance measures for first within segment experiment in the lower San Saba.

	October 6th, 2018	March 25th, 2019 5-month check	July 18th, 2019 9-month check	October 14th, 2020 1-year check
Translocated	x			
Detected		x	x	x
Measured	x	x	x	x
Clipped for Glycogen				x

Table A2.2: Dates of performance measures for first between segment experiment (lower to upper segment) for *C. pustulosa* moved October 2018.

	October 6th, 2018	March 25th, 2019 5-month check	April 29th, 2019 6- month check	July 22nd, 2019 9-month check	October 27th, 2020 1-year check
Translocated	x				
Detected		x	x	x	x
Measured			x	x	x
Clipped for Glycogen					x

Table A2.3: Dates of performance measures for additional between segment mussels (lower to upper segment) for *C. pustulosa* moved March 2019.

	March 25th, 2019	April 29th, 2019 1-month check	July 22nd, 2019 4- month check	October 27th, 2019 7-month check	March 6th, 2020 1-year check
Translocated	x				
Detected		x	x	x	x
Measured		x	x	x	x
Clipped for Glycogen					x

Table A2.4: Dates of performance measures for fully crossed within segment experiment with *C. pustulosa* and *C. petrina* in the lower San Saba.

	September 27th, 2019	October 26th, 2019 1-month check	March 7th, 2020 5-month check
Translocated	x		
Detected		x	x
Measured			x
Clipped for Glycogen			x

Table A2.5: Dates of performance measures for fully crossed within segment experiment with *U. imbecillis* in the upper San Saba.

	October 5th, 2019	October 27th, 2019 1-month check	March 6th, 2020 5-month check
Translocated	x		
Detected		x	x
Measured			x
Clipped for Glycogen			x

Table A2.6: Dates of performance measures for fully crossed between segment experiment of *C. pustulosa* and *C. petrina* moved from the lower segment to upper segment of the San Saba river.

	September 27th, 2019	October 27th, 2019 1-month check	March 6th, 2020 5-month check
Translocated	x		
Detected		x	x
Measured			x
Clipped for Glycogen			x

Table A2.7: Dates of performance measures for fully crossed between segment experiment of *U. imbecillis* moved from the upper segment to the lower segment of the San Sab river.

	October 5th, 2019	October 26th, 2019 1-month check	March 7th, 2020 5- month check
Translocated	x		
Detected		x	x
Measured			x
Clipped for Glycogen			x

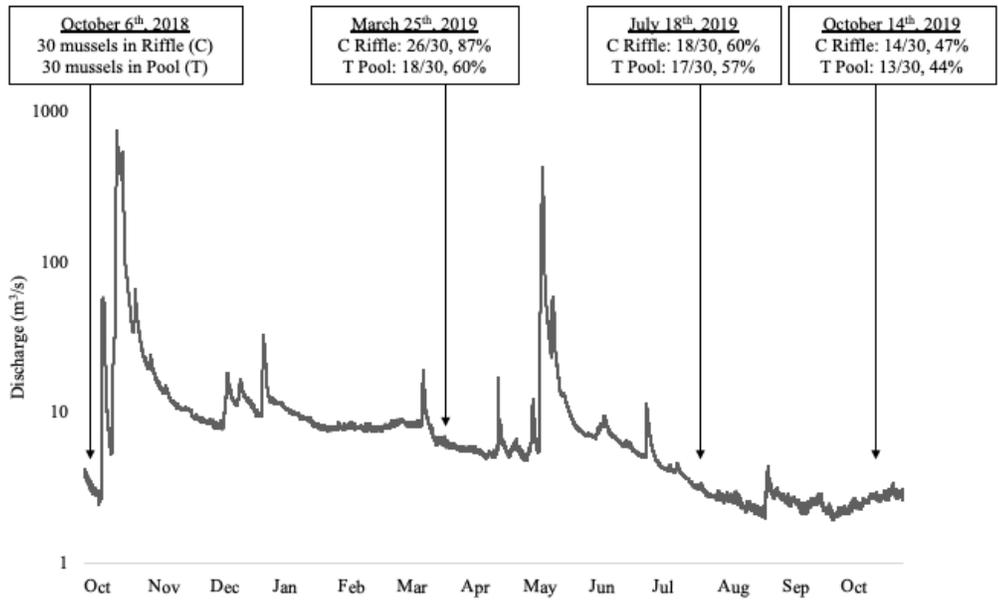


Figure A2.1: Detection of *C. pustulosa* for the first within segment experiment in the lower San Saba along hydrograph.

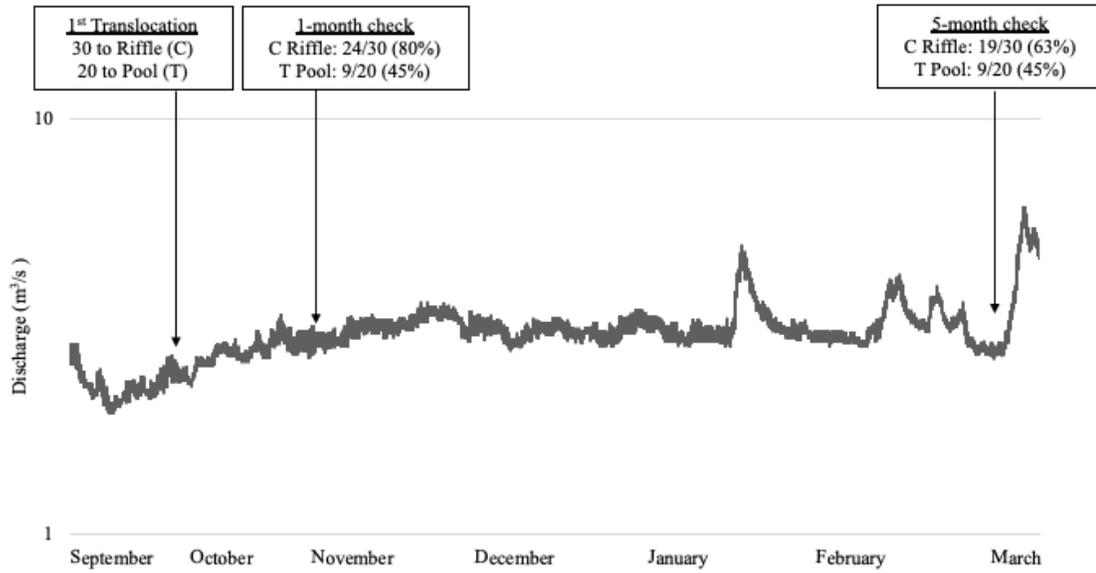


Figure A2.2: Detection of *Cyclonaias sp.* for fully crossed within segment experiment in the lower San Saba along hydrograph.

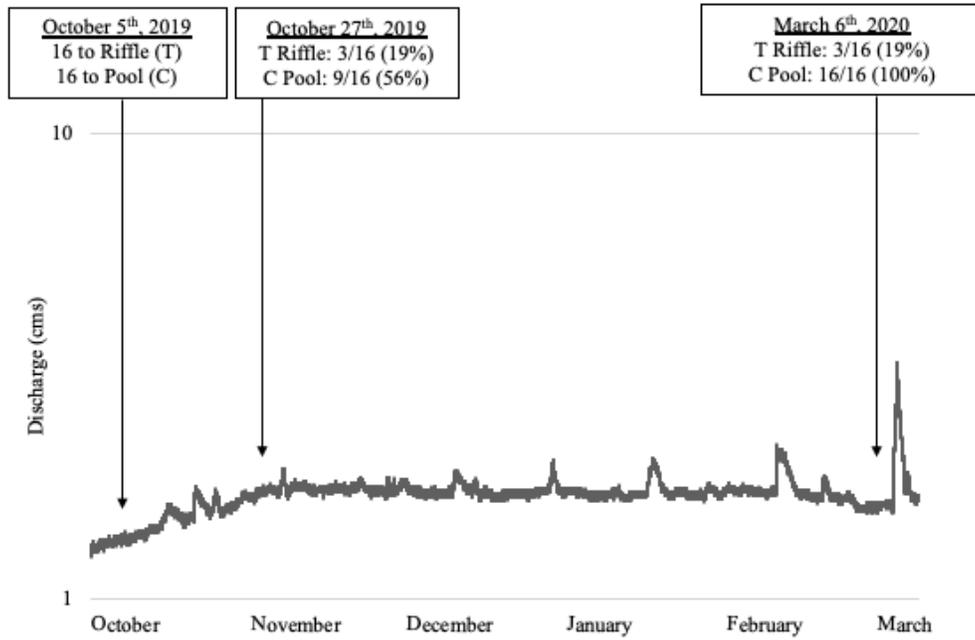


Figure A2.3: Detection of *U. imbecillis* for fully crossed within segment experiment in the upper San Saba along hydrograph.

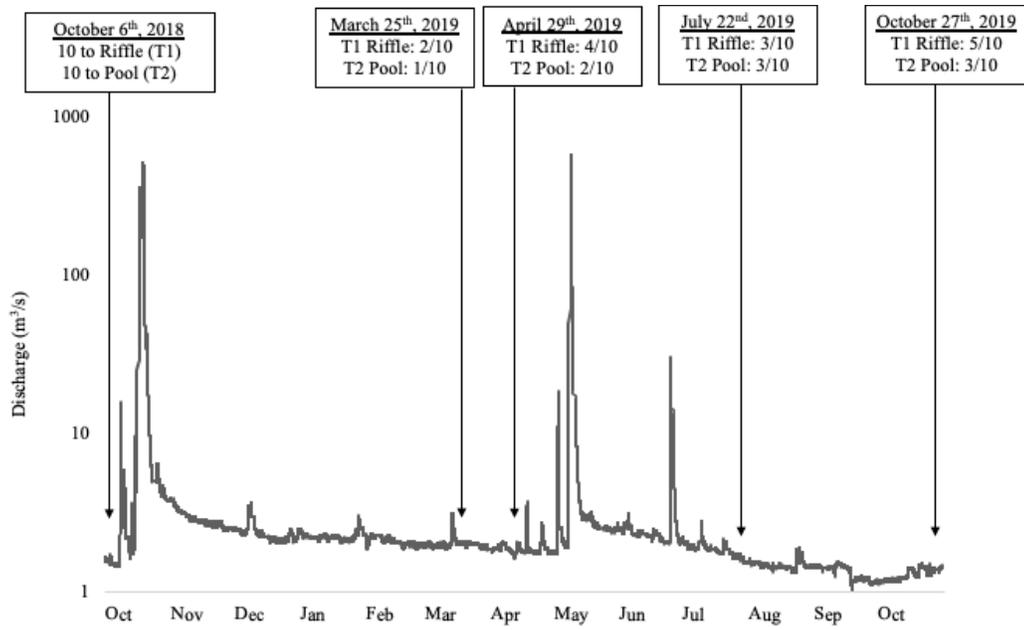


Figure A2.4: Detection of *C. pustulosa* for the first between segment experiment (lower to upper segment) along a hydrograph of upper San Saba.

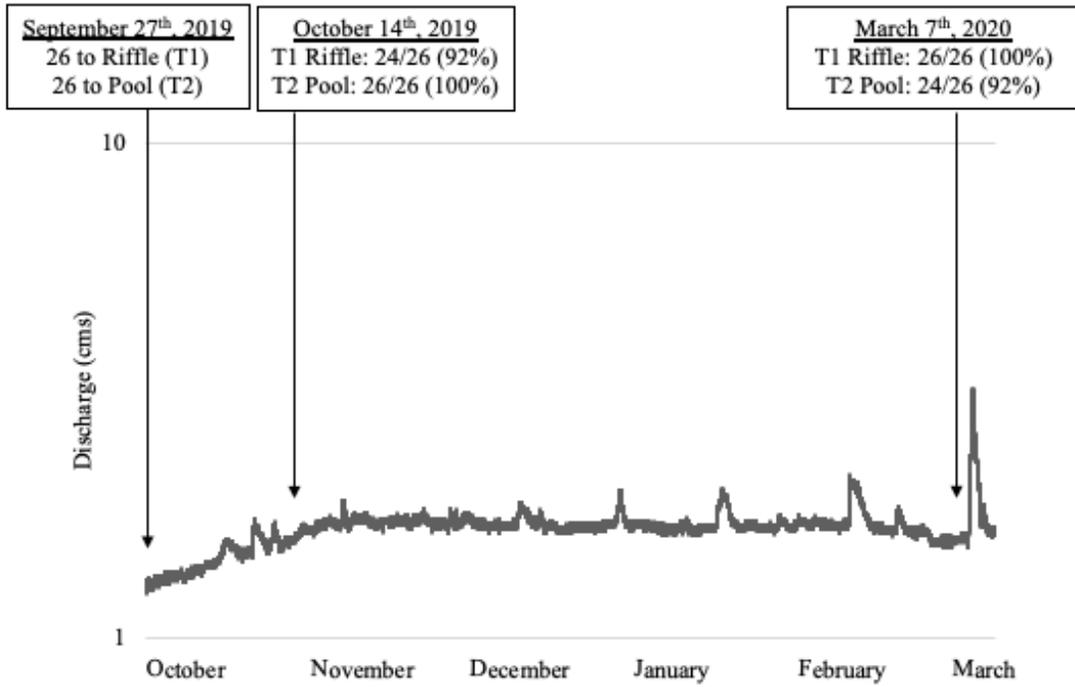


Figure A2.5: Detection of *Cyclonaias sp.* for the fully crossed experiment where mussels were moved from the lower segment of the San Saba river to the upper segment along hydrograph of upper San Saba

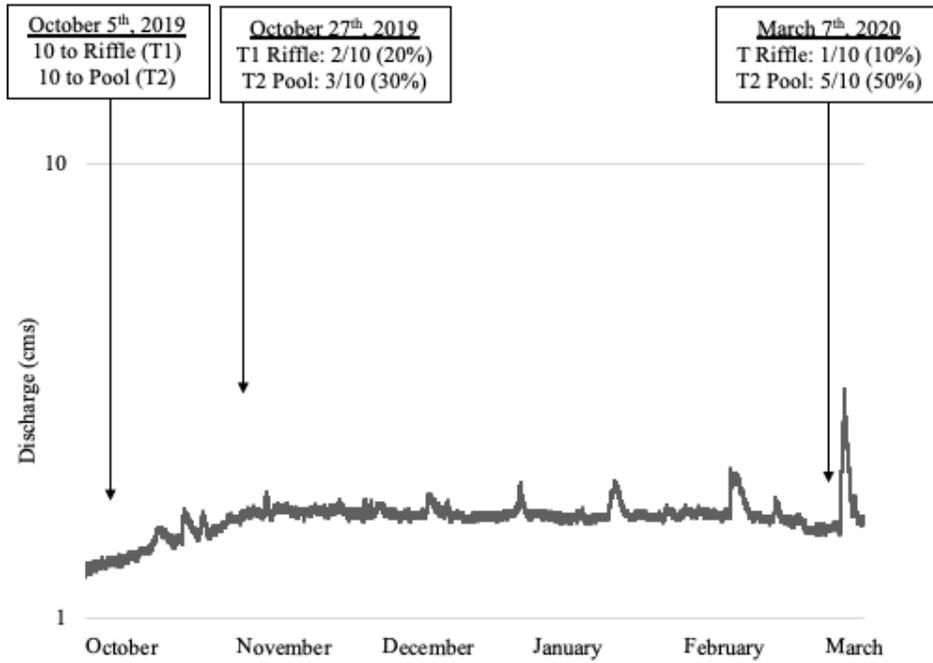


Figure A2.6: Detection of *U. imbecillis*. for the fully crossed experiment where mussels were moved from the upper segment of the San Saba river to the lower segment along hydrograph of lower San Saba.

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