Multiple freshwater mussel species of the Brazos River, Colorado River, and Guadalupe River basins

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The findings and conclusions in this report presented by U.S. Fish and Wildlife Service project team members do not necessarily represent the views of the U.S. Fish and Wildlife Service.

Executive Summary

This comprehensive research project was initiated to quantify distributional, ecological, and biological information for five federal candidate freshwater mussel species in portions of the Colorado River basin, Brazos River basin, and Guadalupe River basin of central Texas. The five candidate freshwater mussel species were Smooth Pimpleback (*Cyclonaias houstonensis*, formerly *Quadrula houstonensis*), Texas Pimpleback (*Cyclonaias petrina*, formerly *Quadrula petrina*), Texas Fatmucket (*Lampsilis bracteata*), Texas Fawnsfoot (*Truncilla macrodon*), and False Spike (*Fusconaia mitchelli*). Field surveys were conducted in the lower Colorado River basin, middle Colorado River basin, upper Brazos River basin, Little River (a Brazos River tributary), and upper Guadalupe River to document current distribution, occurrence, abundance, population structure, and habitat associations of the candidate species. Applied research studies using respirometry, electron transport system (ETS) assays, and valve closure/movement experiments were conducted to evaluate the influence of thermal, hypoxia, suspended solids, salinity, and nitrogenous stressors on growth and survival of multiple candidate mussel species. Additional experimental trials evaluated the desiccation tolerances and behavioral responses to dewatering events for multiple candidate species. Stable isotope analysis was used to evaluate food resources being utilized by candidate mussel species and evaluate spatial and temporal variations in feeding across basins and seasons.

Previously established hydraulic model sites on the lower Colorado River were surveyed for freshwater mussels, and initial Habitat Suitability Criteria (HSC) were developed for multiple hydraulic variables and candidate species to be used in an ongoing environmental flow assessment. Robust design mark and recapture studies were initiated at two sites in the Colorado River basin to evaluate capture probabilities and assess population parameters such as abundance

and survival in relation to river discharge. Additionally, mark and recapture studies allowed for a quantification of movement and baseline growth for multiple candidate species. Lastly, development of facilities and protocols for captive propagation and rearing of candidate species was initiated at three separate U.S. Fish and Wildlife Service (USFWS) fish hatchery/research facilities. In aggregate, the research presented herein represents a substantial increase in available information on candidate freshwater mussel species. An overview of the more pertinent information for each species is provided below.

Smooth Pimpleback *Cyclonaias houstonensis*

Cyclonaias houstonensis was captured in three (lower Colorado River, middle Colorado River, and Little River) of the four survey basins where it had previously been documented. Although previously documented in the upper Brazos basin, including the Clear Fork Brazos River, live individuals were not observed during our surveys. In the lower Colorado River, *C. houstonensis* occurred at 31% of sites and ranked 3rd in relative abundance, accounting for 17% of all mussels observed. In the middle Colorado River, *C. houstonensis* occurred at 12% of sites, and ranked 10th in relative abundance (3%). In the Little River, *C. houstonensis* occurred at 40% of sites, and ranked first in relative abundance (42%). Among georegions, *C. houstonensis* was most common (15% occurrence among mesohabitats) and abundant (17% relative abundance) in Georegion 5 (Lowland). Across all survey basins, *C. houstonensis* was found in all mesohabitat types, although catch-per-unit-effort (CPUE; mussels/p-h) was lowest in riffles and backwaters and highest in mid-channel runs. Multivariate analysis of lower and middle Colorado River data demonstrated an association with run edge habitats and sand substrates. Initial habitat suitability criteria (HSC) generated for *C. houstonensis* generally support these results with the highest

utilization observed in moderate depths, moderate velocities, under low shear stress, with boulder, bedrock, and sand substrates.

Respirometry data suggests that increasing temperature to a maximum of 36 ºC results in increased metabolic demand which may cause *C. houstonensis* to be more susceptible to food limitation and subsequent growth limitations at higher temperatures. However, their ability to obtain oxygen from the water varied little with temperature, (*C. houstonensis* generally switched from aerobic to anaerobic respiration at around 2.0 mg/L dissolved oxygen [DO] regardless of temperature), and they were observed to have a short-term tolerance of low DO even at high temperatures. Optimal temperatures for respiratory enzymes (ETS) of temperature-acclimated mussels were 31.6°C for the Colorado River population and 27.6°C for the Navasota River population. Optimal ETS temperatures for non-acclimated animals were 30.5°C and 28.8°C for Colorado and Navasota river populations, respectively. Based on previous literature, optimal temperatures for mussel growth are likely a few degrees lower than those measured for enzymes. Onset of mussel mortality due to thermal stress was hypothesized to occur at 37.1°C for *C. houstonensis*, which was lower than the same value for *C. petrina* (38.9 ºC), but higher than values observed for *Lampsilis teres* (29.4 ºC), *Amblema plicata* (36.0 ºC), and *Fusconaia mitchelli* (29.4 ºC). Even at excessive concentrations (Turbidity ~75 NTU, TSS ~250 mg/L), there was little to no evidence that exposure to suspended solids resulted in valve closure. *Cyclonaias houstonensis* were more sensitive to salinity than turbidity, with salinities >2.5 ppt resulting in strong reductions in mussel gape. Total ammonia nitrogen (TAN) concentrations of 0.5 and 2 mg/L did not affect *C. houstonensis* respiration rates or ability to obtain oxygen from surrounding waters in the short term. However, frequency of valve closure did appear to increase at 2.0 mg TAN/L raising the possibility of negative impacts on filtration, respiration,

and fertilization efficiency during long-term exposure. A LT_{50} of 18.39 days during desiccation trials suggests *C. houstonensis* is moderately tolerant of desiccation. Currently available stable isotope data suggests the majority of the carbon assimilated by *C. houstonensis* is derived from coarse particulate organic matter (CPOM); however, whether this results from direct feeding on CPOM particles or a reliance on associated bacteria and fungi is unclear given current data.

Visual/tactile capture probability of *C. houstonensis* from the lower Colorado River mark recapture site ranged from 0.47 – 0.52. Population estimates suggest a population size of 350 – 400 individuals within one 300 m^2 area in Colorado County prior to flooding effects from Hurricane Harvey, and approximately 20 individuals after this event. Observed movement between primary sampling periods at both sites ranged from 0 – 24 meters (m), and averaged 3.96 – 12.4 meters. Observed growth rates averaged 1.3 – 1.4 mm/month in the lower Colorado River and 0.4 mm/month in the middle Colorado River. Although a wide variety of environmental parameters influence growth rates, growth rates were likely slower in the middle Colorado River due to the dominance of larger individuals.

Three USFWS hatchery/research facilities (San Marcos Aquatic Resource Center, SMARC; Inks Dam National Fish Hatchery, IDNFH; Uvalde National Fish Hatchery, UNFH) have developed infrastructure to house and propagate *C. houstonensis*, and attempts to collect gravid individuals from the wild and infest host fish will be initiated in spring 2018.

Texas Pimpleback *Cyclonaias petrina*

Cyclonaias petrina was observed in all three of the survey basins where it was historically documented. In the lower Colorado River, *C. petrina* occurred at approximately 13% of sites and represented 1.3% of all individuals captured, ranking 7th in relative abundance.

In the middle Colorado River, *C. petrina* was found at 15% of sites and ranked first in relative abundance (20%). The highest CPUE was within San Saba County downstream of the San Saba River confluence. In the upper Guadalupe River, *C. petrina* was observed at 20% of sites and ranked third in abundance (21%). Among georegions, *C. petrina* was most common (7.1% occurrence among mesohabitats) and abundant (37% relative abundance) in Georegion 3 (Llano Uplift). Across all survey basins, *C. petrina* was found in all mesohabitats, but mean CPUE was highest in run edge habitats and lowest in riffles. Multivariate analysis of lower and middle Colorado River data demonstrated an association with run habitats, swifter current velocities, and gravel and cobble substrates. Compared to all mussels in aggregate, initial HSC generated for *C. petrina* showed broader curves for mean column velocity, Froude number, Reynolds number, and shear stress, suggesting increased utilization of high energy environments.

Respirometry data suggests that increasing temperature to a maximum of 36ºC results in increased metabolic demand which may cause *C. petrina* to be more susceptible to food limitation and subsequent growth limitations at higher temperatures. Increased valve closure was noted at higher temperatures. However, their ability to obtain oxygen from the water varied little with temperature, and they were observed to have a short-term tolerance of low DO even at high temperatures. Optimal temperatures for electron transport system (ETS) enzymes of temperature-acclimated mussels were 35.3°C for the Colorado River population and 34.6°C for the Navasota River population. Optimal ETS temperatures for non-acclimated animals were 30.2°C and 28.5°C for Colorado and Guadalupe river populations respectively. Optimal temperatures for mussel growth are likely a few degrees lower than those measured for enzymes. Onset of mussel mortality due to thermal stress was hypothesized to occur at 38.9°C for *C. petrina*, which was higher than all other species tested (*C. houstonensis*, *Lampsilis teres*,

Amblema plicata, and *Fusconaia mitchelli*). Even at excessive concentrations (Turbidity ~75 NTU, TSS ~250 mg/L), exposure to suspended solids had little influence on valve closure. *Cyclonaias petrina* were more sensitive to salinity than turbidity, with salinities >2.0 ppt resulting in major decreases in gape and nearly complete valve closure by 4ppt. Total ammonia nitrogen (TAN) concentrations of 0.5 and 2 mg/L resulted in frequent valve closure events precluding the ability to measure effects on respiration rates and ability to extract oxygen from ambient water. High frequency of valve closure raises the possibility of negative impacts on filtration, respiration, and fertilization efficiency during long-term exposure to a greater degree than for *C. houstonensis*. Desiccation and dewatering trials suggest *C. petrina* is tolerant of short-term desiccation (LT_{50} of 32.04 days) and increases movement in response to dewatering, thus reducing their propensity for stranding. Currently available stable isotope data suggests the majority of the carbon assimilated by *C. petrina* is derived from coarse particulate organic matter (CPOM); however, whether this results from direct feeding on CPOM particles or a reliance on associated bacteria and fungi is unclear given current data.

Visual/tactile capture probability of *C. petrina* from the lower Colorado River mark recapture site ranged from 0.54 – 0.58. Population estimates suggest a population size of approximately 127 individuals within one 300 m^2 area in Colorado County prior to flooding effects from Hurricane Harvey, and approximately eight individuals after this event. Population estimates at the middle Colorado mark recapture site ranged from 255 – 490 individuals within 300 m^2. Observed movement between primary sampling periods at both sites ranged from 0 – 24 meters (m), and averaged 3.9 – 7.8 meters. Observed growth rates averaged 1.9 – 2.3 mm/month in the lower Colorado River and 0.6 – 0.8 mm/month in the middle Colorado River. Although a

wide variety of environmental parameters influence growth rates, growth rates were likely slower in the middle Colorado River due to the dominance of larger individuals.

The same three USFWS hatchery/research facilities have developed infrastructure to house and propagate *C. petrina*, and attempts to collect gravid individuals from the wild and infest host fish will be conducted in spring 2018.

Texas Fatmucket *Lampsilis bracteata*

Live *L. bracteata* were observed in two of the three survey basins where they were historically documented. In the upper Guadalupe River, they were observed at 40% of the sites sampled and ranked 1st in relative abundance (34%). Gravid females were observed in the upper reaches in Kerr County. In the middle Colorado River basin, *L. bracteata* was the rarest mussel encountered (0.4% relative abundance) and occurred at 2% of sites. None were observed in the mainstem, but a previously undocumented population was located at one site sampled in Cherokee Creek. In the lower Colorado River basin, *L. bracteata* were previously reported from lower Onion Creek but were not located at one site within Onion Creek in this study. *Lampsilis bracteata* was only found in Georegion 3 (Llano Uplift). Across basins, highest mean CPUE was in run edge habitats, and the species was not located in backwaters. Overall, they occupied swift current velocities in comparison to other species. In the upper Guadalupe River, they were found more often than expected over bedrock and gravel substrates.

Although laboratory studies are ongoing for this species, initial data demonstrates lower optimal temperatures than those observed for *C. houstonensis* and *C. petrina*, suggesting this species may be more thermally sensitive than the two *Cyclonaias* species. Of the three species, *L. bracteata* was also the quickest to succumb to desiccation (LT_{50} 2.86 days) and did not exhibit

a movement response to dewatering. In aggregate, this suggests that *L. bracteata* populations would be expected to exhibit greater impacts than the other two species from extreme low flows, which often result in high temperatures and potential desiccation. Not surprisingly, the distribution of *L. bracteata* seems to be associated with relatively persistent spring inputs. Currently available stable isotope data suggests that the majority of carbon assimilated by *L. bracteata* is derived from CPOM. However, carbon sources were much more variable for *L. bracteata* than for the other species examined, and were dominated by suspended particulate organic matter at certain sites/seasons. Stable isotope analysis is ongoing and additional data may help elucidate spatial and temporal patterns.

The same three USFWS hatchery/research facilities have developed infrastructure to house and propagate *L. bracteata*. Gravid females collected in spring and summer 2017 were used to infest host fish at both the SMARC and IDNFH facilities. Initial studies conducted at SMARC evaluated three potential host fish (i.e., Green Sunfish, Bluegill, and Blacktail Shiner) and found Green Sunfish to be the most suitable and efficient host. Subsequent inoculations using Green Sunfish resulted in production of 1,533 live juveniles. However, growth rates were slow and 100% mortality was observed within six weeks. As a result, future propagation efforts at SMARC will use filtered pond water instead of well water, and holding temperature will be increased from 21 to 25°C. Both Bluegill and Green Sunfish were inoculated at IDNFH and staff estimated 2,300 metamorphosed juveniles were produced. However, near total mortality was observed due to an issue with reduced source water oxygen content. This issue has been resolved by adding the ability for supplemental aeration to the system. Two juveniles which survived this event exhibited good growth rates over the course of the study. Although

propagation success was low in 2017, changes implemented at both facilities are anticipated to promote successful propagation of a larger number of *L. bracteata* in 2018.

Texas Fawnsfoot *Truncilla macrodon*

Live *T. macrodon* were observed in two of the four basins where they were historically documented. In the lower Colorado River, nine individuals (0.4% relative abundance) were present at seven survey sites (15% occurrence) in Colorado, Wharton, and Matagorda counties. In the middle Colorado River, *T. macrodon* were not captured during surveys. However, recently dead shells were found (with tissue still present) in San Saba County, and one juvenile individual was captured in this basin during mark recapture studies. This suggests the species persists in the middle Colorado basin. Although *T. macrodon* were documented in both the Little River and upper Brazos River basin, no live individuals were located in these areas during this study. Recent drought impacts in the Clear Fork Brazos River, an upper Brazos tributary where *T. macrodon* was previously reported, warrant the need for additional surveys to assess the current status of the species in this area. Among georegions, *T. macrodon* were mainly found in Georegion 5 (Tertiary). Across all basins, *T. macrodon* were found in run edge, pool edge, and backwater habitats with the highest mean CPUE observed in run edge habitats. Multivariate analysis of lower and middle Colorado River data demonstrated an association with run edges and clay, silt, and sand substrates. A total of 16 individual *T. macrodon* were captured from the mark recapture site in the lower Colorado River and one individual was captured from the mark recapture site in the middle Colorado River. Recapture data were insufficient to examine capture probability, population estimates, or growth rates. Observed movements between primary

periods ranged from 1 m to 20 m and averaged 6.6 m – 10.2 m, similar to that of larger bodied *Cyclonaias* mussels.

The same three USFWS hatchery/research facilities have developed infrastructure to house and propagate *T. macrodon*. However, given the difficulty in locating large numbers of individuals at any location, finding gravid females is challenging. Efforts to locate gravid females for propagation studies will continue in coming years.

False Spike *Fusconaia mitchelli*

Fusconaia mitchelli was previously reported in three of the five survey basins (i.e., middle Colorado River basin, upper Guadalupe River basin, and Little River basin), but no live individuals were observed during surveys as part of this project. The species is currently known to be extant in portion of the Little River basin, the Llano River, the San Saba River, and the lower Guadalupe River, with the highest abundances occurring in the lower Guadalupe River.

Ten individual *F. mitchelli* were collected from the lower Guadalupe River and used for non-acclimated ETS enzyme experiments. The optimal range of ETS enzyme activity for *F. mitchelli* was 26.5 – 28.8ºC, with a hypothesized onset of lethal effects at 31.0ºC. This estimated onset of lethal effects was second lowest among the five species examined, being slightly higher than that observed for *L. teres* (29.4ºC) and considerably lower than *C. petrina* (38.9ºC), *C. houstonensis* (37.1ºC), and *A. plicata* (36.0ºC).

The same three USFWS hatchery/research facilities have developed infrastructure to house and propagate *F. mitchelli*. However, given the difficulty in locating large numbers of individuals at any location, finding gravid females is challenging. Efforts to locate gravid females for propagation studies will continue in coming years.

In conclusion, this comprehensive research project was conducted to quantify distributional, ecological, and biological information for five federal candidate freshwater mussel species. Field surveys, applied research, mark-recapture studies, and captive propagation activities presented herein filled several key data gaps, which will facilitate upcoming state and federal conservation assessments.

Task 1: Freshwater mussel field surveys

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Literature review of target mussel distributions in Texas

Approximately 50 species of freshwater mussels reside in Texas, 15 of which are listed as state threatened (TPWD 2010). Of these 15 state threatened species, 14 are endemic to the region (Burlakova et al. 2011). In the central Texas province, which includes the Nueces-Frio, Guadalupe-San Antonio, Colorado, and Brazos basins, 6 endemic species are known to occur, 5 of which are pending review by the U.S. Fish and Wildlife Service (USFWS) for federal listing (USFWS 2011; Howells 2014). The most recent studies on the unionid assemblages of central Texas have focused on tributaries of each major basin, with less emphasis on the mainstem portions of these systems (Burlakova & Karatayev 2010; Randklev et al. 2017). Though several studies have begun to tackle these larger systems, such as the mainstem Guadalupe and Brazos rivers, a large data gap is present within the mainstem Colorado River (Burlakova & Karatayeu 2010; Randklev et al. 2009; Tsakiris & Randklev 2016; Tsakiris & Randklev 2016). Burlakova and Karatayeu (2010) surveyed several sites on the mainstem Colorado, but only did so in close proximity to public access sites. For the USFWS to make appropriate decisions on these candidate species, it is crucial to fill data gaps where adequate survey efforts are lacking.

Cyclonaias houstonensis (formerly in the genus *Quadrula*; Williams et al. 2017), Smooth pimpleback, is known to have occurred in the Colorado and Brazos River basins. Historically, *C. houstonensis* was considered rare where it occurred based on expert's inability to find large populations that persisted throughout its native range (USFWS 2011). In more recent studies, *C. houstonensis* was found in high numbers within the Brazos River basin, including tributaries such as the Little River and Yegua Creek, among others (Tsakiris & Randklev 2016; Randklev et al. 2017). Within the Colorado River basin, *C. houstonensis* was thought to be much less common. The majority of survey efforts have been focused within tributaries of the Colorado River, which includes the Llano, San Saba, Pedernales, Concho Rivers, with less emphasis on the mainstem Colorado (Randklev et al. 2017). Burlakova and Karatayeu (2010) found low densities of *C. houstonensis* in the Lower Colorado River, though few sites (N = 14) were surveyed.

Cyclonaias petrina (formally in the genus *Quadrula*; Williams et al. 2017), Texas pimpleback, is known to have occurred in the Guadalupe-San Antonio River and Colorado River basins. The range of *C. petrina* was historically believed to have been reduced substantially, and only occurred in four streams, the San Saba, Concho, Guadalupe, and San Marcos Rivers (USFWS 2011). Most recent surveys found live *C. petrina* in these four streams, as well as the Llano River (Randklev et al. 2017) and lower Guadalupe River (Tsakiris & Randklev 2016). Although Burlakova and Karatayeu (2010) surveyed a few sites in the mainstem Colorado River and observed no live *C. petrina*, extensive surveys within the Colorado River mainstem are lacking.

Lampsilis bracteata, Texas fatmucket, is known to have occurred in the upper reaches of the Colorado and Guadalupe-San Antonio basins within the Edwards Plateau. In the Colorado

River system, *L. bracteata* was historically found in the mainstem and tributaries, which includes the Pedernales River, Llano, San Saba, and Concho Rivers, and Onion, Jim Ned, and Elm Creeks (USFWS 2011). Based on the most current survey efforts, *L. bracteata* appears to be extirpated from the mainstem Colorado River, and is restricted to its tributaries, including the Concho, Llano, and San Saba Rivers (Burlakova & Karatayeu 2010; Randklev et al. 2017). Survey efforts by Randklev et al. (2017) found the San Saba River to have the densest populations where *L. bracteata* persists. In the Guadalupe River, *L. bracteata* was historically found from the lower reaches in Gonzales County to the headwaters in Kerr County, though individuals from the lower reaches were most likely misidentified *Lampsilis hydiana*, Louisiana fatmucket (USFWS 2011). Furthermore, *L. bracteata* was also historically found in several tributaries, which included the North Fork Guadalupe River, Johnson Creek, and Blanco River. In the San Antonio River, *L. bracteata* occurred at its confluence with the Medina River, upstream to the City of San Antonio. Additionally, *L. bracteata* historically occurred in two tributaries, the Medina River and Cibolo Creek, though no recent survey efforts have documented live individuals in the San Antonio River basin (USFWS 2011).

Truncilla macrodon, Texas fawnsfoot, was known to occur in the Brazos River and Colorado River basins. In the Brazos River basin, *T. macrodon* occurred from Fort Bend County, upstream to the confluence of the Clear Fork Brazos River (Randklev et al. 2009; USFWS 2011). Furthermore, this species historically occurred in tributaries of the Brazos River, which includes the Clear Fork Brazos, Navasota, Leon, Little, San Gabriel Rivers, and Deer and Yegua Creeks (Randklev et al. 2013; Tsakiris & Randklev 2016; USFWS 2011). The most recent survey efforts have found *T. macrodon* live in multiple tributaries, which includes Yegua Creek, Navasota, Little, Leon, San Gabriel, and Clear Fork Brazos Rivers, among others (Randklev et al. 2013;

Tsakiris & Randklev 2016; Randklev et al. 2017). In the Colorado River basin, *T. macrodon* occurred in the majority of the mainstem, from Wharton County to the headwaters, as well as tributaries in the Edwards Plateau. *Truncilla macrodon* was thought to be extirpated from the Colorado River until 2009, when live individuals were found within the lower reaches of the mainstem (Burlakova & Karatayeu 2011).

Fusconaia mitchelli, False spike, is known to have occurred in the Brazos, Colorado, and Guadalupe River basins. In the Brazos River basin, historic records occurred in the Little River system, which includes the Leon and San Gabriel Rivers, as well at the mainstem Brazos. *Fusconaia mitchelli* was known to have occurred in the mainstem Colorado River in San Saba County, as well as in the Llano River. Furthermore, *F. mitchelli* occurred in the Guadalupe River from Victoria county to Kerr County. For over 30 years, *F. mitchelli* was thought to be extinct until live individuals were collected within the lower Guadalupe River in Gonzales County (Randklev et al. 2012). Recent survey efforts by Tsakiris and Randklev (2016) found the species persists in the Lower Guadalupe River, though in low abundance. Since *F. mitchelli* was rediscovered as extant, more recent survey efforts have also found live individuals in both the Brazos and Colorado River basins. In the Brazos basin, live individuals were collected within Brushy Creek, Leon, San Gabriel, and Little Rivers. Additionally, one live individual was found in the Llano River (Randklev et al. 2017).

Part I: Study Objectives

The goal of this study was to provide information to evaluate the freshwater mussel communities in portions of the Colorado, Brazos, and Guadalupe River basins of central Texas, with an emphasis on investigating five federal candidates and petitioned freshwater mussel

species (i.e., *C. houstonensis*, *C. petrina*, *L. bracteata*, *T. macrodon*, and *F. mitchelli*). Specific task objectives for the five candidate species were to assess distribution, catch per unit effort, and population size structure. We separated the basins of interest into 5 sub-basins, including the Lower Colorado River basin (from Longhorn Dam to Bay City Dam), the Middle Colorado River basin (from O.H. Ivie Reservoir to Lake Buchanan), the Upper Brazos River basin (upstream from Possum Kingdom Reservoir), the Little River drainage (from the confluence of the Lampasas and Leon Rivers to the confluence with the Brazos River), and the Upper Guadalupe River basin (from Canyon Lake upstream to the confluence of the North and South forks).

Part I Methods

Study Area

The Colorado River is the largest river in Texas that is completely confined within the states borders. The headwaters originate in Dawson County in the Great Plains of west Texas, and flows southeast through the Edwards Plateau, where is receives large contributions from several spring-fed rivers, such as the San Saba, Pedernales, and Llano Rivers. The river then transitions to a large alluvial system, as it flows through the Gulf Coastal Plain, eventually draining into Matagorda Bay (Dahm et al. 2005). Several mainstem reservoirs occur on the Colorado, most notably the Highland Lakes, a series of seven reservoirs (Lake Buchanan, Inks Lake, Lake LBJ, Lake Marble Falls, Lake Travis, and Lake Austin) which separate the Middle Colorado from the Lower Colorado. For this study, the Middle Colorado River is defined as the segment between O.H. Ivie Reservoir and Lake Buchanan. The Lower Colorado River is defined as the segment from Longhorn Dam (forming Lady Bird Lake) to Bay City Dam, which

is 32 miles above the river's mouth at Matagorda Bay. The Lower and Middle Colorado river mainstem were each broken up into 5 reaches each. The Lower Colorado mainstem reaches included Longhorn Dam to Bastrop (Reach I; river mile [RM] 292 – 237), Bastrop to La Grange (Reach II; RM 237 – 174), La Grange to Columbus (Reach III; RM 174 – 132), Columbus to Wharton (Reach IV; RM 132 – 64), and Wharton to Bay City Dam (Reach V; RM 64 – 32). In addition to the mainstem, we surveyed in Onion Creek, a tributary of the Lower Colorado River. The Middle Colorado mainstem reaches included O.H. Ivie to State Highway (SH) 377 (Reach I; RM 608 – 553), SH 377 to SH 45 (Reach II; RM 553 – 529), SH 45 to SH 16 (Reach III; RM 529 – 493), SH 16 to Bend (Reach IV; RM 493 – 447), and Bend to Lake Buchannan (Reach V; RM 447 – 422). In addition to the mainstem, we surveyed in two Middle Colorado tributaries, Cherokee Creek and Pecan Bayou.

The Brazos River is the third largest river in the state of Texas, and originates in Stonewall County at the confluence of the Salt Fork Brazos and Double Mountain Fork Brazos Rivers. It flows southeast through the Great Plains before reaching the Gulf Coastal Plain, where it drains into the Gulf of Mexico. The Brazos River is 1390 km long and drains an area of about 115600 km^2 (Dahm et al. 2005). For this study, the area of interest includes the Upper Brazos River basin, from the headwaters to Lake Possum Kingdom. This includes the mainstem portion of the river as well as the Clear Fork Brazos River. Furthermore, because candidate species have been documented in the area, another sub-basin of interest within the Brazos River watershed is the Little River. This tributary to the Brazos originates at the confluences of the Leon and Lampasas Rivers in Bell County. It flows east for approximately 258 km before it meets with the Brazos mainstem, draining an area of about 12,485 km^2 (Rose & Echelle 1981).

The Guadalupe River originates in Kerr County in Hunt, at the confluence of the North and South Fork Guadalupe Rivers, and flows through the Edwards Plateau until it reaches the coastal plain, and eventually drains into San Antonio Bay. The Guadalupe River is a part of the Guadalupe-San Antonio basin and is approximately 370 km long, with the entire watershed draining an area of about 26,231 km^2 (Dahm et al. 2005). For this study, the area of interest includes the upper portion of the Guadalupe River, from its headwaters, downstream to Canyon Lake.

Survey Design

To delineate survey sites within each basin, we utilized aerial imagery to target areas with heterogeneous habitats. We chose sites within sections of each river with a mosaic of habitat types to investigate habitat associations among mussel communities, as well as candidate species. Habitat types were divided into mesohabitats, which included riffles, runs, pools, and backwaters. Dividing habitat types with a mesohabitat scale is useful for investigating habitat associations because they can be easily identified (Frissell et al. 1986). Moreover, due to differences in mussel abundance between bank and mid-channel habitats observed in previous studies (Brown & Banks 2001; Brim Box et al. 2002), we partitioned runs and pools into sub-mesohabitats to increase resolution and identify potential differences within these mesohabitats. As a result, we separated each site by six potential mesohabitat types: run bank, run mid-channel, pool bank, pool mid-channel, riffle, and backwater.

Within each mesohabitat type, we utilized qualitative surveys via timed visual and tactile search methods. A qualitative survey approach is an efficient search method to establish a list of taxa, as well as increase the detection probability of rare species (Vaughn et al. 1997; Strayer &

Smith 2003). At each site, we surveyed one of each mesohabitat type. If a particular mesohabitat type was absent within a site, we surveyed additional mesohabitat types present until a total of 6 mesohabitats were searched at each site. For each mesohabitat, areas with a maximum of 300 m^2 were marked off and initially surveyed for one person-hour (p-h). If we found no live mussels, that mesohabitat was complete. If live mussels were collected, we conducted a second p-h. If we collected a new species within the second p-h, a third p-h was conducted. We conducted additional one p-h searches until no new species were collected (Metcalfe-Smith et al. 2000). Once sampling efforts were complete, all native freshwater mussels were identified, enumerated, measured to the nearest millimeter (mm) shell length, and sexed (if applicable), before being returned to the area of capture.

Habitat Measurements

At each mesohabitat type, we estimated percent substrate composition based on the standard Wentworth particle size scale (Wentworth 1922). We utilized a Hach flowmeter and top-set wading rod to measure average depth (ft), mean water column velocity (ft/s), and benthic velocity (ft/s) at one point near the center of each mesohabitat. We used FST Hemispheres (Statzner et al. 1991) to quantify shear stress at one point within each mesohabitat. To measure substrate compaction (kg/cm^2), we took three readings from random points within the mesohabitat using a Humboldt soil penetrometer (Johnson & Brown 2000). Additionally, we recorded the percent coverage of other habitat parameters such as large woody debris, aquatic vegetation, and undercut banks. We used a HydroTech multiprobe water quality sonde to measure water quality parameters including temperature (°C), dissolved oxygen (mg/L, %

saturation), pH, conductivity (µS/cm), and turbidity (NTU). Lastly, we collected a GPS waypoint near the center of each mesohabitat.

Data Analysis

We analyzed average mussel catch-per-unit effort (CPUE; mussels/p-h) for all mussels in aggregate by basin, reach, and mesohabitat type. We assessed differences among reaches and mesohabitats with a nonparametric Kruskal-Wallis Test. If we observed significant differences among groups, a nonparametric pairwise multiple comparison using Dunn's Test with a Bonferroni adjustment was applied to identify between which groups differences occurred. Lastly, we constructed CPUE by site vs. river mile scatterplots to investigate relationships between relative abundance and longitudinal stream position. Tributary sites were not included in relative abundance vs. stream position analysis.

For candidate species, we evaluated each species average CPUE among basins, reach, and mesohabitats. We assessed differences among reaches and mesohabitats for each candidate species with a nonparametric Kruskal-Wallis Test. If we observed significant differences among groups, a nonparametric pairwise multiple comparison using Dunn's Test with a Bonferroni adjustment was applied. Lastly, we analyzed size structures of each candidate species within each basin by constructing length frequency histograms.

Part I Results

Freshwater mussel assemblages by reach and basin

Lower Colorado River Basin

We collected 2,327 live mussels representing 14 species across 49 sites (Table 1). The unionids collected in the Lower Colorado River contributed to 73% of all the individuals collected during our survey efforts across all basins. We observed live mussels at 26 sites (54%) surveyed. The most common species in this basin were *Lampsilis teres* (occurring at 46% of sites sampled), *Leptodea fragilis* (33% occurrence), *Amblema plicata* (33% occurrence), and *C. houstonensis* (31% occurrence). The unionid assemblage of the Lower Colorado was numerically dominated by *A. plicata*, which accounted for 57% of all live mussels collected. The second and third most abundant species were *L. teres* and *C. houstonensis*, which accounted for 16.9% and 16.6% of all live mussels collected, respectively. The remaining 11 species found within the Lower Colorado only accounted for 9.5% of all live unionids collected. The rarest species collected was *Uniomerus tetralasmus*, with only one individual observed at one site. Other infrequently documented taxa included *Quadrula apiculata*, *Toxolasma parvum*, *Utterbackia imbecillis*, and *T. macrodon* (Table 1).

Overall CPUE of unionids in the Lower Colorado River was 4.15 ± 1.34 (SE) mussels/p-h. Mean CPUE among reaches ranged from 0.67 ± 0.33 (SE) mussels/p-h in Reach I to 12.09 ± 6.01 (SE) mussels/p-h in Reach IV (Figure 1). Comparison of CPUE by Kruskal-Wallis analysis detected significant differences in reaches ($p = 0.02$). Despite this, pairwise multiple comparison using Dunn's Test did not provide evidence for where differences occurred. Longitudinal changes in CPUE were observed with increasing distance downstream from Longhorn Dam. CPUE was relatively low from RM 292 (Longhorn Dam) to RM 392, and only reached a maximum CPUE of 3.00 mussels/p-h. CPUE from about RM 240 to RM 188 was 0.00 mussels/p-h, which was the longest distance where no live unionids were recorded within the Lower Colorado River. CPUE began to increase at about RM 178 within Reach II, peaking at

approximately RM 110 within Reach IV, at a maximum of 51.60 mussels/p-h. After this peak, CPUE decreased rapidly, never exceeding 11.00 mussels/p-h for the remaining 70 river miles surveyed (Figure 2).

CPUE within individual mesohabitats ranged from 0.00 mussels/p-h to 124.30 mussels/p-h. Mean CPUE was highest in pool mid-channel habitats at 8.58 ± 4.57 (SE) mussels/p-h (range: 0.00 – 114.00 mussels/p-h). The lowest CPUE was observed within riffle habitats at 0.47 ± 0.47 (SE) mussels/p-h (range: 0.00 – 15.00 mussels/p-h) (Figure 3). Comparison of CPUE by Kruskal-Wallis analysis detected significant differences among mesohabitat types ($p = 0.001$). Pairwise multiple comparison using Dunn's Test detected CPUE within pool edge habitats was significantly higher compared to riffle habitats ($p = 0.008$) and run mid-channel habitats ($p = 0.03$), and CPUE was significantly higher in run edge habitats compared to riffle habitats ($p = 0.02$)

We observed live individuals of three (*C. houstonensis*, *C. petrina*, *T. macrodon*) of the four state-listed species historically known to occur in the Lower Colorado River basin (i.e., *C. houstonensis*, *C. petrina*, *L. bracteata*, *T. macrodon*; Howells 2014; Table 1). *Cyclonaias houstonensis* collected in the Lower Colorado accounted for 73% of individuals collected from all basins surveyed. Within the Lower Colorado, they were the third most abundant species encountered and occurred at 31% of sites. *Cyclonaias petrina* made up 1.3% of the Lower Colorado unionid community and occurred at 13% of sites surveyed. *Truncilla macrodon* was the least abundant candidate species collected live, numerically comprising 0.39% of the community and occurring at 15% of sites surveyed (Table 1). We observed no live *L. bracteata* in Onion Creek, although only one site was sampled as part of this study. Lastly, we observed no live *F. mitchelli* within the Lower Colorado basin.

Mean CPUE of *C. houstonensis* by reach ranged from 0.00 mussels/p-h in Reach I to 1.72 mussels/p-h in Reach IV (Figure 4). Comparison of *C. houstonensis* CPUE by Kruskal-Wallis analysis detected significant differences in reaches ($p = 0.003$). Pairwise multiple comparison using Dunn's Test detected CPUE was significantly higher in Reach III compared to Reach I ($p = 0.01$) and Reach II ($p = 0.04$). CPUE of *C. petrina* by reach ranged from 0.00 mussels/p-h in Reach I, II, and III, to 0.2 mussels/p-h in Reach IV (Figure 4). Comparison of *C. petrina* CPUE by Kruskal-Wallis analysis detected significant differences in reaches ($p = 0.01$). Pairwise multiple comparison using Dunn's Test detected CPUE was significantly higher in Reach IV compared to Reach I ($p = 0.04$) and Reach II ($p = 0.04$). CPUE of *T. macrodon* by reach ranged from 0.00 mussels/p-h in Reach I and II, to 0.06 mussels/p-h in Reach IV (Figure 4). Comparison of *T. macrodon* CPUE by Kruskal-Wallis analysis detected significant differences in reaches ($p = 0.03$). Despite this, pairwise multiple comparison using Dunn's Test did not provide evidence of where differences occurred.

Middle Colorado River Basin

We collected 492 live mussels across 42 sites, representing 12 species (Table 2). The unionids collected in the Middle Colorado basin contributed to 15% of all the individuals collected during total survey efforts across all basins. We observed live mussels at 34 sites (83%), which was the highest occurrence among all basins. The most common species in the basin was *L. fragilis*, which occurred at 61% of sites sampled. *C. petrina* and *L. fragilis* were the most abundant species, accounting for 19.7% and 19.5% of live unionids, respectively (Table 2). The rarest species were *L. bracteata* and *A. plicata*, accounting for 0.41% and 1.02% of the unionids collected, respectively (Table 2).

Overall CPUE of unionids in the Middle Colorado was 1.08 ± 0.24 (SE), the second lowest among basins. Mean CPUE among reaches ranged from 0.48 ± 0.14 (SE) mussels/p-h to 1.77 ± 0.67 (SE) mussels/p-h. The highest CPUE occurred in Reach IV at 1.77 ± 2.22 (SD) mussels/p-h (range: 0.00 - 8.00 mussels/p-h; Figure 5). Comparison of CPUE by Kruskal-Wallis analysis failed to detect significant differences among reaches ($p = 0.22$). We observed no distinct longitudinal patterns between total mussel CPUE and longitudinal stream location (Figure 6). Mean CPUE was 0.53 ± 0.41 (SE) mussels/p-h among three sites in Pecan Bayou and 1.00 mussels/p-h at one site in Cherokee Creek.

CPUE within individual mesohabitats ranged from 0.00 mussels/p-h to 21.33 mussels/p-h. The highest mean CPUE among mesohabitats occurred within run edge habitats, averaging 1.69 ± 1.01 (SE) mussels/p-h (range: 0.00 – 21.33 mussels/p-h). The lowest CPUE among mesohabitats was within riffle habitats, averaging 0.16 ± 0.1 (SE) mussels/p-h (range: 0.00 – 0.50 mussels/p-h; Figure 7). Comparison of CPUE by Kruskal-Wallis analysis failed to detect significant differences among mesohabitats ($p = 0.06$).

We observed live specimens of three (*C. houstonensis*, *C. petrina*, *L. bracteata*) of the five state-listed species historically known to occur in the Middle Colorado River drainage (i.e., *C. houstonensis*, *C. petrina*, *L. bracteata*, *T. macrodon*, *F. mitchelli*; Howells 2014) (Table 2). Among all basins of occurrence, 71% of the *C. petrina* collected occurred within the Middle Colorado basin, most of which were found at one site in San Saba County. *Cyclonaias houstonensis* occurred at 12% of sites, and accounted for 2.6% of the community (Tables 2). No live *T. macrodon* or *F. mitchelli* were observed during field surveys, although a live *T. macrodon* was taken from the middle Colorado River during mark and recapture study.

Mean CPUE of *C. houstonensis* by reach ranged from 0 mussels/p-h in Reach III and V, to 0.07 mussels/p-h in Reach IV (Figure 8). CPUE by Kruskal-Wallis analysis failed to detect significant differences in reaches ($p = 0.32$). Mean CPUE of *C. petrina* by reach ranged from 0 mussels/p-h in Reach II and V, to 0.57 mussels/p-h in Reach IV (Figure 8). CPUE by Kruskal-Wallis analysis failed to detect significant differences in reaches ($p = 0.12$).

Upper Brazos River

We collected one mussel across 10 sites in the Upper Brazos River Basin. One single *U. tetralasmus* was collected in the Clear Fork Brazos River, at Fort Griffin, in Shackleford County. Although sites on the Clear Fork Brazos River contained a diverse community of dead shell material, we observed no live unionids and few shells in the mainstem portion of the Brazos River basin upstream from Possum Kingdom Reservoir.

Little River Basin

We collected 320 live mussels representing five species across 10 sites in the Little River (Table 3). The unionids collected in the Little River represented 10% of all the individuals collected during total survey efforts across all basins. Live mussels were observed at seven sites (70%), second highest occurrence among basins. The most common species in this basin was *Cyrtonaias tampicoensis*, which occurred live at 70% of the sites sampled. *C. houstonensis* was the most abundant species, which accounted for 42% of all live mussels collected and occurred at 40.00% of sites surveyed. The second and third most abundant species were *A. plicata* and *C. tampicoensis*, which accounted for 23% and 21% of all live mussels observed, respectively. The

rarest species found live within the Little River was *L. fragilis*, with only 6 individuals collected, accounting for 0.02% of the mussel community (Table 3).

Overall mean CPUE of unionids in the Little River was 3.06 ± 1.15 (SE) mussels/p-h and ranged from 0.00 mussels/p-h to 9.00 mussels/p-h among sites. We observed no longitudinal patterns between CPUE and longitudinal river location (Figure 9).

CPUE within individual mesohabitats ranged from 0.00 mussels/p-h to 37.50 mussels/p-h. Mean CPUE was highest within pool edge habitats at 4.03 ± 4.02 (SE) mussels/p-h (range: 0.00 – 37.50 mussels/p-h). CPUE was lowest within riffle habitats at 0.11 ± 0.11 (SE) mussels/p-h (range: 0.00 – 1.00 mussels/p-h). (Figure 10). Comparison of CPUE by Kruskal-Wallis analysis failed to detect significant differences among mesohabitats ($p = 0.56$).

We observed live individuals of one (*C. houstonensis*) of the three candidate species that are historically known to occur in the Little River Drainage (i.e., *C. houstonensis*, *T. macrodon*, *F. mitchelli*; Howells 2014; Table 3). *Cyclonaias houstonensis* occurred at 40% of sites surveyed and was the most numerically abundant species encountered. No live *T. macrodon* or *F. mitchelli* were collected in the Little River.

In addition to native unionids, it is important to note that live zebra mussels *Dreissena polymorpha* were documented at the two upstream-most sites (State Highway 95, Bell County; FM 437, Milam County) in the Little River. Thirty-eight live zebra mussels were documented from five mesohabitats at these two sites.

Upper Guadalupe River Basin

We collected 47 live mussels representing five species across 10 sites (Table 4). The unionids collected in the Upper Guadalupe River contributed only 1.5% of all the individuals

collected during total survey efforts across all basins, the second lowest among all drainages surveyed. Live mussels occurred at 4 sites (40%) surveyed. *L. bracteata* was the most common and abundant species present, which occurred at 40% of sites surveyed, and accounted for 34% of the mussel community. The second and third most abundant species were *Toxolasma texasiense* and *C. petrina*, which accounted for about 28% and 21% of live mussels collected, respectively. *Toxolasma parvum* was the rarest species observed, occurring at 10% of sites, and accounting for 2.13% of the mussel community (Table 4).

Overall CPUE for unionids in the Guadalupe River was 0.50 ± 0.30 (SE) mussels/p-h, and ranged from 0.00 mussels/p-h to 3.00 mussels/p-h among sites. Longitudinal patterns were observed with increasing distance from the North and South Fork Guadalupe Rivers confluence. The highest mean CPUE was observed at RM 417.5 (3.00 mussels/p-h.), with the last occurrence of live mussels at approximately RM 393. No live mussels were observed between RM 393 and Canyon Lake (Figure 11).

CPUE within individual mesohabitats ranged from 0.00 mussels/p-h to 6.67 mussels/p-h. Mean CPUE was highest within run edge habitats, averaging 1.24 ± 0.76 (SE) mussels/p-h (range: 0.00 – 6.67 mussels/p-h). Mean CPUE was lowest within pool-mid channel habitats, where no live mussels were detected (Figure 12). Comparison of CPUE by Kruskal-Wallis analysis failed to detect significant differences among mesohabitat types ($p = 0.33$).

We observed two (*C. petrina, L. bracteata*) of the three candidate species historically known to occur in the Upper Guadalupe River Drainage (i.e., *C. petrina, L. bracteata*, *F. mitchelli*; Howells 2014; Table 4). *L. bracteata* and *C. petrina* accounted for 55% of the unionid community combined. We observed no live *F. mitchelli* in the Upper Guadalupe basin.

Candidate Species

Cyclonaias houstonensis

We collected a total of 533 live *C. houstonensis* within the Little (40% of sites), Lower Colorado (31% of sites), and Middle Colorado (12%) rivers. In the Lower Colorado River, we observed *C. houstonensis* from Fayette County, upstream of La Grange, downstream to Wharton County, between Wharton and Bay City (Figure 13). In the Middle Colorado River, we observed *C. houstonensis* from Coleman County to San Saba County, though its distribution was patchy with large gaps between site occurrences (Figure 14). In the Little River, *C. houstonensis* was the numerically dominant species and was found live in Milam County, from near Val Verde, downstream to Cameron (Figure 15).

Among basins within its historical range, the highest mean CPUE for *C. houstonensis* occurred within the Little River, averaging 1.27 ±0.73 (SE) mussels/p-h (range: 0.00 – 5.75 mussels/p-h) (Figure 16). Comparison of CPUE by Kruskal-Wallis analysis detected significant differences among basins ($p = 0.01$). Despite this, pairwise multiple comparison using Dunn's Test did not provide evidence for where differences occurred.

CPUE of *C. houstonensis* within individual mesohabitats ranged from 0.00 mussels/p-h. to 52.00 mussels/p-h. Mean CPUE was highest within run mid-channel habitats at 0.87 ± 0.46 (SE) mussels/p-h (range: 0.00 – 52.00 mussels/p-h; Figure 17). Comparison of CPUE by Kruskal-Wallis analysis detected significant differences among mesohabitats ($p = 0.02$). Pairwise multiple comparison using Dunn's Test detected CPUE was significantly higher in run edge habitats compared to riffle habitats ($p = 0.006$).

The size structure of *C. houstonensis* in the Lower Colorado River was dominated by size classes of 50 - 60 mm, with a few smaller individuals from 20 – 30 mm. In the Middle Colorado

River, all the *C. houstonensis* we observed were large individuals greater than 60 mm. The size structure of the Little River was dominated by size classes of 50 - 60 mm, with a few smaller individuals (30 – 40 mm) present (Figure 18).

Cyclonaias petrina

We collected a total of 140 live *C. petrina* within the Lower Colorado (13% of sites), Middle Colorado (15% of sites), and Upper Guadalupe (20%) rivers. In the Lower Colorado River, we observed *C. petrina* from Colorado County, upstream of Columbus, downstream to Wharton County, near Wharton, Texas (Figure 19). In the Middle Colorado River, we observed *C. petrina* from Coleman County to San Saba County, with the majority of occurrences within San Saba County (Figure 20). In the Upper Guadalupe River, we observed live mussels within the upper reaches in Kerr County, from Hunt, downstream to Center Point *(*Figure 21).

Among basins, *C. petrina* mean CPUE was highest within the Middle Colorado River at 0.18 ± 0.12 (SE) mussels/p-h (range: 0.00 – 4.44 mussels/p-h). Mean CPUE was lowest within the Lower Colorado River at 0.04 ± 0.03 (SE) mussels/p-h (range: 0.00 – 1.46 mussels/p-h) (Figure 22). Comparison of CPUE by Kruskal-Wallis analysis failed to detect significant differences among basins ($p = 0.76$).

Among mesohabitats, mean CPUE of *C. petrina* was highest in run edge habitats at 0.27 ± 0.2 (SE) mussels/p-h (range: 0.00 – 18.00 mussels/p-h), and lowest in riffle habitats at 0.01 ± 0.01 (SE) mussels/p-h (range: 0.00 – 0.50 mussels/p-h) (Figure 23). Comparison of CPUE by Kruskal-Wallis analysis failed to detect significant differences among mesohabitats ($p = 0.6$).

The size structure of *C. petrina* collected within the Lower Colorado River was dominated by individuals from 65 – 80 mm, but included a few individuals from 40 – 60 mm.

Size structure within the Middle Colorado River represented was dominated by individuals from 70 – 80 mm, and contained no individuals less than 50 mm. Within the Upper Guadalupe River, individuals from 55 – 60 mm were most common, with one individual at approximately 20 mm collected (Figure 24).

Lampsilis bracteata

We collected a total of 18 live *L. bracteata* within the Middle Colorado (2.4% of sites) and Upper Guadalupe (40%) rivers. In Middle Colorado River, we found no live *L. bracteata* within the mainstem, though two live individuals were collected in Cherokee Creek (Figure 25). In the Upper Guadalupe River, we observed *L. bracteata* in Kerr County, from Hunt to just upstream of Comfort (Figure 26). No *L. bracteata* were observed in the Lower Colorado River basin.

Among basins, mean CPUE for *L. bracteata* was highest within the Upper Guadalupe River, averaging 0.18 ± 0.09 (SE) mussels/p-h (range: 0.00 – 0.80 mussels/p-h; Figure 27). Comparison of CPUE by Kruskal-Wallis analysis detected significant differences among basins ($p = 0.001$). Pairwise multiple comparison using Dunn's Test detected that CPUE was significantly higher in the Upper Guadalupe compared to the Middle Colorado basin ($p < 0.001$).

Among mesohabitats, the highest mean CPUE occurred within run edges at 0.17 ± 0.11 (SE) mussels/p-h (range: 0 – 1.67 mussels/p-h). Mean CPUE was lowest within backwater habitats, where no live individuals were collected (Figure 28). Comparison of CPUE by Kruskal-Wallis analysis failed to detect significant differences among mesohabitats ($p = 0.1$).

Size structure of *L. bracteata* in the Upper Guadalupe was dominated by individuals 50 - 60 mm, with only one small individual (25 mm) collected (Figure 29). The two individuals collected from Cherokee Creek in the Middle Colorado basin were 48 and 64 mm.

Truncilla macrodon

Nine live *T. macrodon* were collected within the Lower Colorado River (15% of sites). We observed *T. macrodon* from Colorado County to Matagorda County (Figure 30). No live *T. macrodon* were found in the Brazos River upstream of Possum Kingdom Lake, including three sites within the Clear Fork Brazos River. No live *T. macrodon* were collected from the Little River.

CPUE of *T. macrodon* within the Lower Colorado averaged 0.02 ± 0.007 (SE) mussels/p-h (range: 0.00 – 0.22 mussels/p-h). Mean CPUE was highest within run edge habitats at 0.02 ± 0.008 (SE) mussels/p-h (range: 0.00 – 0.50 mussels/p-h). No live *T. macrodon* were observed in pool mid-channel, riffle, or run mid-channel habitats (Figure 31). Comparison of CPUE by Kruskal-Wallis analysis detected significant differences among mesohabitats ($p = 0.01$). Pairwise multiple comparison using Dunn's Test detected that CPUE was significantly higher in run edge habitats compared to run mid-channel ($p = 0.03$) and pool mid-channel ($p = 0.02$) habitats.

T. macrodon ranged in size from 18 – 54 mm, with the most frequently encountered size class being approximately 50 mm (Figure 32).

Fusconaia mitchelli

During our survey efforts, no live *F. mitchelli* were collected, though recently dead shells with nacre still intact were collected in the Little River.

Part 1 Synthesis

Cyclonaias houstonensis

Cyclonaias houstonensis is historically known from four of the five basins surveyed (i.e., Lower Colorado River, Middle Colorado River, Little River, and Upper Brazos River), and was located in three of these basins. It occurred at 31% of sites in the Lower Colorado River and was found from Fayette County downstream to Wharton County. In the Middle Colorado River, *C. houstonensis* was found patchily distributed (12% of sites) from Coleman County to San Saba County. Our results indicate *C. houstonensis* currently persists in the Middle Colorado River within Coleman County, which was uncertain prior to these surveys (USFWS 2016). In the Little River, it was the numerically dominant species and occurred at 40% of sites. Live *C. houstonensis* were not encountered in our surveys within the Upper Brazos River system, including the Clear Fork Brazos River.

Although visual and tactile surveys are biased towards large and sculptured individuals (Strayer & Smith 2003; Haag 2012), the presence of smaller *C. houstonensis* (20 – 40 mm) supports that recruitment has successfully occurred recently in the Lower Colorado River and Little River, though we cannot quantify recruitment based on these surveys. Within the Middle Colorado River, only large adults over 60 mm were encountered.

Across all basins, *C. houstonensis* was found in a variety of mesohabitats, with the highest CPUE in run mid-channel and pool mid-channel mesohabitats. Analysis revealed significantly higher CPUE in run edge mesohabitats than in riffles. Others have suggested that *C. houstonensis* prefers bank, backwater, and front of point bar habitats (Randklev et al. 2014).

Cyclonaias petrina

Live *C. petrina* were found in all survey basins where they were historically documented (i.e., Lower Colorado, Middle Colorado, and Upper Guadalupe). In the Lower Colorado River, we observed *C. petrina* at 13% of sites and located them from Colorado County, upstream of Columbus, downstream to Wharton County, near Wharton, Texas. In the Middle Colorado River, *C. petrina* were found at 15% of sites and located from Coleman County to San Saba County, with the majority of occurrences within San Saba County below the San Saba River confluence. Individuals collected below O.H. Ivie in Coleman County fill a distributional gap in the currently occupied range of this species (Randklev et al. 2017). In the Upper Guadalupe River, we observed live *C. petrina* at 20% of sites sampled and located them within the upper reaches in Kerr County, from Hunt, downstream to Center Point, Texas.

Among all basins, the smallest *C. petrina* collected were > 40 mm, excepting one 18 mm individual from the Upper Guadalupe River, which suggests recent recruitment in this population. Among mesohabitats, *C. petrina* mean CPUE was highest in run edge habitats and lowest in riffles. This differs from previous studies (Tsakiris & Randklev 2016; Randklev et al. 2017) that suggested riffle habitats were optimal for *C. petrina* in the lower Guadalupe River.

Lampsilis bracteata

Live *L. bracteata* were found in two of the three survey basins where they were historically documented (i.e., Upper Guadalupe, Lower Colorado, Middle Colorado). We observed *L. bracteata* restricted to the upper reaches of the Upper Guadalupe River from the confluence of the North and South Forks downstream to approximately Comfort. This distribution may be a result of water permanency in recent drought years, as portions of the Upper Guadalupe River closer to Canyon Lake experienced extensive desiccation during recent

drought years of 2011 -2013 (BL personal observation), whereas the river never went dry in Kerr County (Tara Bushnoe, Upper Guadalupe River Authority, personal communication). Similarly, recent surveys in Colorado River tributaries (San Saba, Llano, Pedernales rivers) found *L. bracteata* restricted to the upper reaches of these systems (Randklev et al. 2017), suggesting that water permanency associated with Edwards Plateau spring systems may be influencing distribution of this species. In the Middle Colorado, no *L. bracteata* were observed in the mainstem, but two live individuals were collected in Cherokee Creek. Based on known historical records (USFWS 2011), this represents a previously undocumented population. To confidently identify the extent of this population, further surveys are warranted. In the Lower Colorado River basin, *L. bracteata* has been previously reported from Onion Creek (Randklev et al. 2017). We only sampled one site in Onion Creek and did not observe *L. bracteata*.

The Upper Guadalupe River *L. bracteata* population was dominated by large adults of 50 – 60 mm, although one small individual of 25 mm was noted. Based on only one small individual collected, it is difficult to infer the level of recruitment within this system. However, it should be noted that we observed female *L. bracteata* gills fully charged with glochidia, which supports that spawning has successfully occurred recently on the Upper Guadalupe. The two individuals collected in Cherokee Creek within the Middle Colorado River basin were both larger adults (48 mm and 64 mm).

L. bracteata were found in four of the five mesohabitat types sampled, but were not located in backwaters. The highest mean CPUE was in run edge habitats. This association with edge habitats corroborates the work of previous researchers who have found *L. bracteata* in bank and pool habitats, but not in backwaters, mid-channel habitats, or riffles (Randklev et al. 2017).

Truncilla macrodon

Live *T. macrodon* were observed in one of the four basins during qualitative surveys. *Truncilla macrodon* were historically documented in all four basins (i.e., Lower Colorado River, Middle Colorado River, Little River, Upper Brazos River). In the Lower Colorado River, we collected a total of nine live individuals from Colorado, Wharton, and Matagorda counties, and found them in 15% of the sites sampled. In the Middle Colorado River, no live individuals were documented during survey efforts, but we found fresh dead adult *T. macrodon* shells (e.g., adductor muscle intact) in San Saba County. In addition, one live juvenile *T. macrodon* was collected during a mark-recapture study in San Saba County. The presence of fresh dead adult shells and one live juvenile indicates that *T. macrodon* persists within the Middle Colorado River in San Saba County, though likely in low abundance. Although live *T. macrodon* have recently been documented in the Little River in low abundance (Randklev et al. 2017), no live individuals were found during our surveys there. Additionally, despite relatively recent records of *T. macrodon* from the Clear Fork Brazos River (Randklev et al. 2017), we sampled three sites and did not locate any live individuals. It should be noted that the Clear Fork Brazos River contained a diverse community of dead shell material suggesting a once diverse mussel community, but this system experienced extensive desiccation in recent drought years of 2011-2013 (BL personal observation) and only one live mussel was documented in the Clear Fork Brazos River during these surveys. Therefore, the status of the *T. macrodon* population in this system is uncertain, and additional surveys are needed.

Although a low number of individuals were collected, one 18 mm *T. macrodon* was observed in the Lower Colorado River which suggests that reproduction has recently occurred.

Additionally, one live juvenile collected in the Middle Colorado River during mark recapture work suggests a reproducing population exists, although evidently in low abundance.

T. macrodon were found in run edge, pool edge, and backwater habitats, with the highest mean CPUE observed in run edge habitats. This association with edge habitats corroborates previous work in the lower Brazos River that suggests *T. macrodon* prefer bank habitats (Randklev et al. 2014).

Fusconaia mitchelli

Given recent and historical records, *F. mitchelli* was previously documented in three of the five survey basins (i.e., Middle Colorado River basin, Upper Guadalupe River basin, and Little River basin; Randklev et al. 2017). Although *F. mitchelli* is known to occur in Colorado River tributaries mentioned above, it has never been documented in the Lower Colorado River basin. Despite surveying over 62 sites in the Upper Guadalupe, Middle Colorado, and Little River basins, we observed no live *F. mitchelli*. It should be noted that we observed relatively recently dead shells (nacre still present) at one site on the Little River, near where other surveyors have recently located live individuals (Randklev et al. 2017).

Part 1. Tables

Table 1. Occurrence of freshwater mussel species within the Lower Colorado River basin.

Species	Lower Colorado River Basin			
	Total Live	**Percentage Live**	**Occurrence (Number of Sites)**	**Percentage Occurrence**
Amblema plicata	1325	56.94	16	33.33
Cyclonaias houstonensis	387	16.63	15	31.25
Cyclonaias petrina	30	1.29	6	12.50
Cyrtonaias tampicoensis	37	1.59	10	20.83
Lampsilis teres	394	16.93	22	45.83
Leptodea fragilis	64	2.75	16	33.33
Potamilus purpuratus	15	0.64	5	10.42
Pyganodon grandis	16	0.69	3	6.25
Quadrula apiculata	5	0.21	4	8.33
Toxolasma parvum	3	0.13	3	6.25
Toxolasma texasiense	34	1.46	5	10.42
Truncilla macrodon	9	0.39	7	14.58
Uniomerus tetralasmus	1	0.04	1	2.08
Utterbackia imbecillis	7	0.30	4	8.33
Total	**2327**		**26**	**54.17**

Table 2. Occurrence of freshwater mussel species within the Middle Colorado River drainage.

Species	Middle Colorado River Basin			
	Total Live	**Percentage Live**	**Occurrence (Number of Sites)**	**Percentage Occurrence**
Amblema plicata	5	1.02	2	4.88
Cyclonaias houstonensis	13	2.64	5	12.20
Cyclonaias petrina	97	19.72	6	14.63
Cyrtonaias tampicoensis	49	9.96	10	24.39
Lampsilis bracteata	2	0.41	1	2.44
Lampsilis teres	27	5.49	8	19.51
Leptodea fragilis	96	19.51	25	60.98
Potamilus purpuratus	20	4.07	10	24.39
Pyganodon grandis	33	6.71	13	31.71
Quadrula appiculata	70	14.23	11	26.83
Tritogonia verruccosa	54	10.98	11	26.83
Utterbackia imbecillis	26	5.28	8	19.51
Total	**492**		**34**	**82.93**

Table 3. Occurrence of freshwater mussel species within the Little River drainage.

Species	Little River Basin			
	Total Live	**Percentage Live**	**Occurrence (Number of Sites)**	**Percentage Occurrence**
Amblema plicata	75	23.44	4	40.00
Cyclonaias houstonensis	133	41.56	4	40.00
Cyrtonaias tampicoensis	66	20.63	7	70.00
Lampsilis teres	40	12.50	4	40.00
Leptodea fragilis	6	0.02	2	20.00
Total	**320**		**7**	**70.00**

Table 4. Occurrence of freshwater mussel species within the Upper Guadalupe drainage.

Species	Upper Guadalupe River Basin			
	Total Live	**Percentage Live**	**Occurrence (Number of Sites)**	**Percentage Occurrence**
Cyclonaias petrina	10	21.28	2	20.00
Lampsilis bracteata	16	34.04	4	40.00
Toxolasma parvum	1	2.13	1	10.00
Toxolasma texasiense	13	27.66	2	20.00
Uniomerus tetralasmus	7	14.89	1	10.00
Total	**47**		**4**	**40.00**

Part 1 Figures

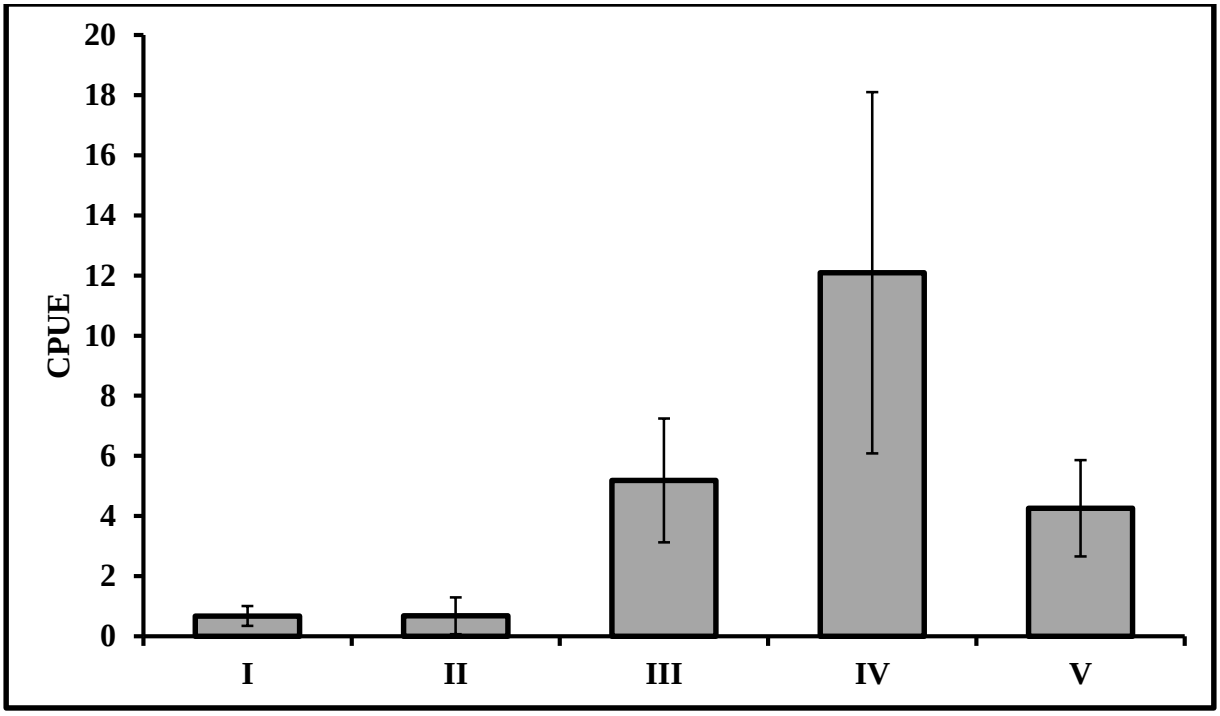


Figure 1. Catch-per-unit effort (CPUE; mussels/p-h) of unionids by reach within the Lower Colorado River drainage. Reach I - Longhorn Dam to Bastrop, Reach II - Bastrop to La Grange, Reach III - La Grange to Columbus, Reach IV - Columbus to Wharton, and Reach V - Wharton to Bay City.

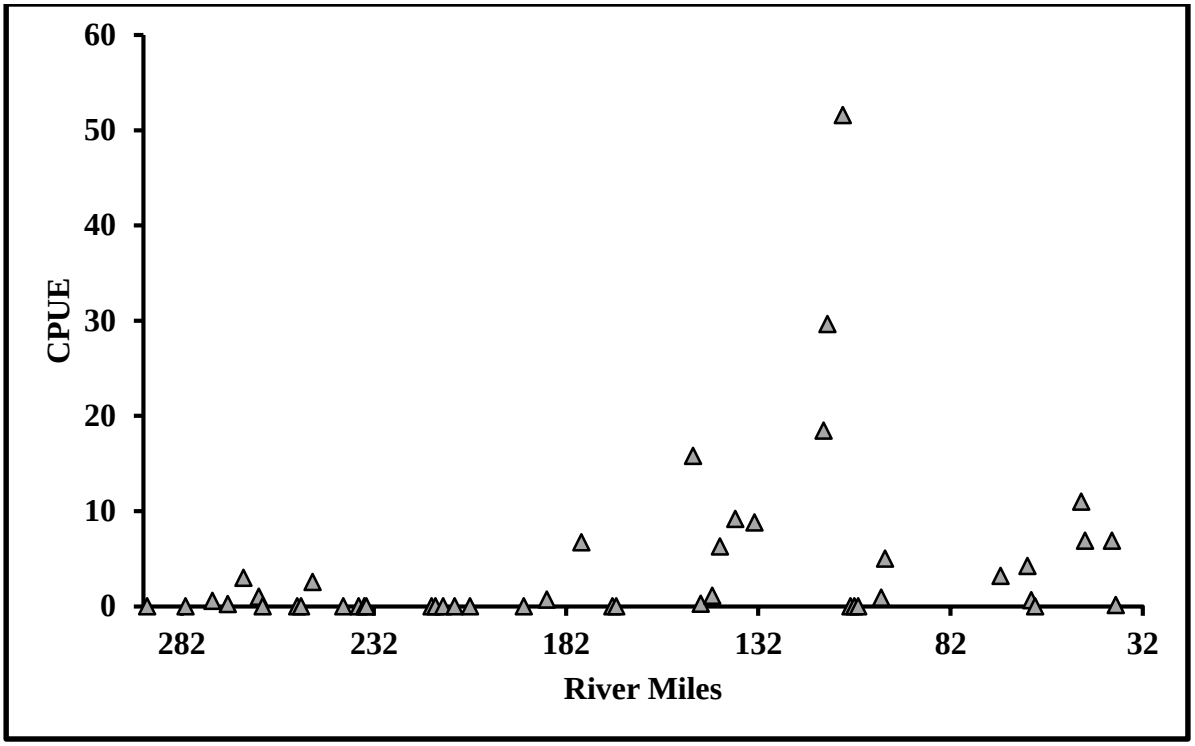


Figure 2. Catch-per-unit effort (CPUE; mussels/p-h) of unionids by river mile (RM) within the Lower Colorado River. For spatial reference, Longhorn Dam is at RM 292 and Bay City Dam is at RM 32.

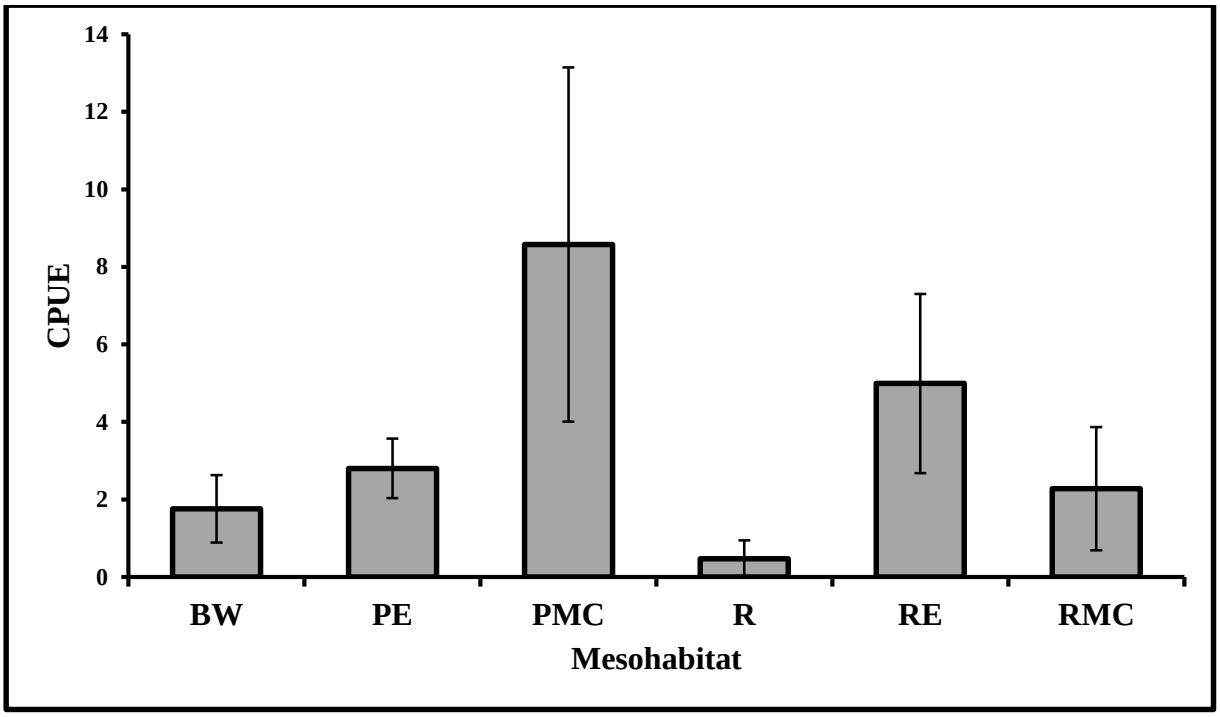


Figure 3. Catch-per-unit effort (CPUE; mussels/p-h) of unionids by mesohabitat within the Lower Colorado River drainage. BW = backwater, PE = pool edge, PMC = pool mid-channel, R = riffle, RE = run edge, RMC = run mid-channel.

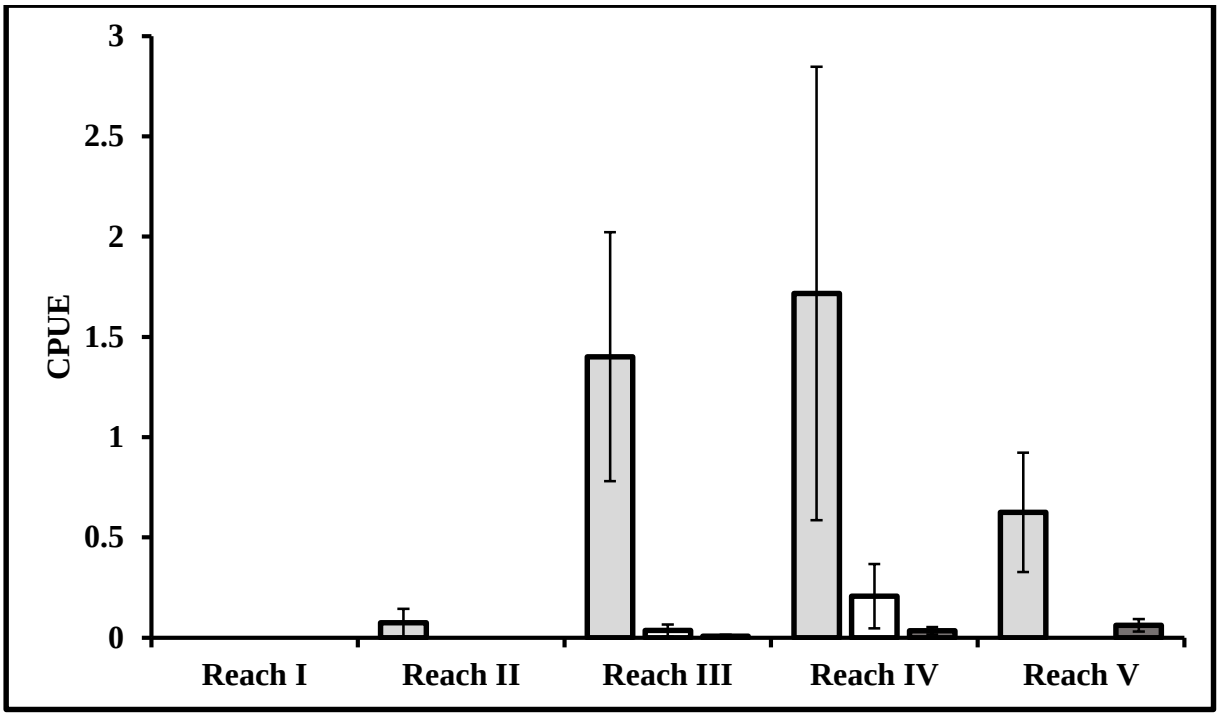


Figure 4. Catch-per-unit effort (CPUE; mussels/p-h) of *C. houstonensis* (light grey), *C. petrina* (white), and *T. macrodon* (dark grey) by reach within the Lower Colorado River drainage.

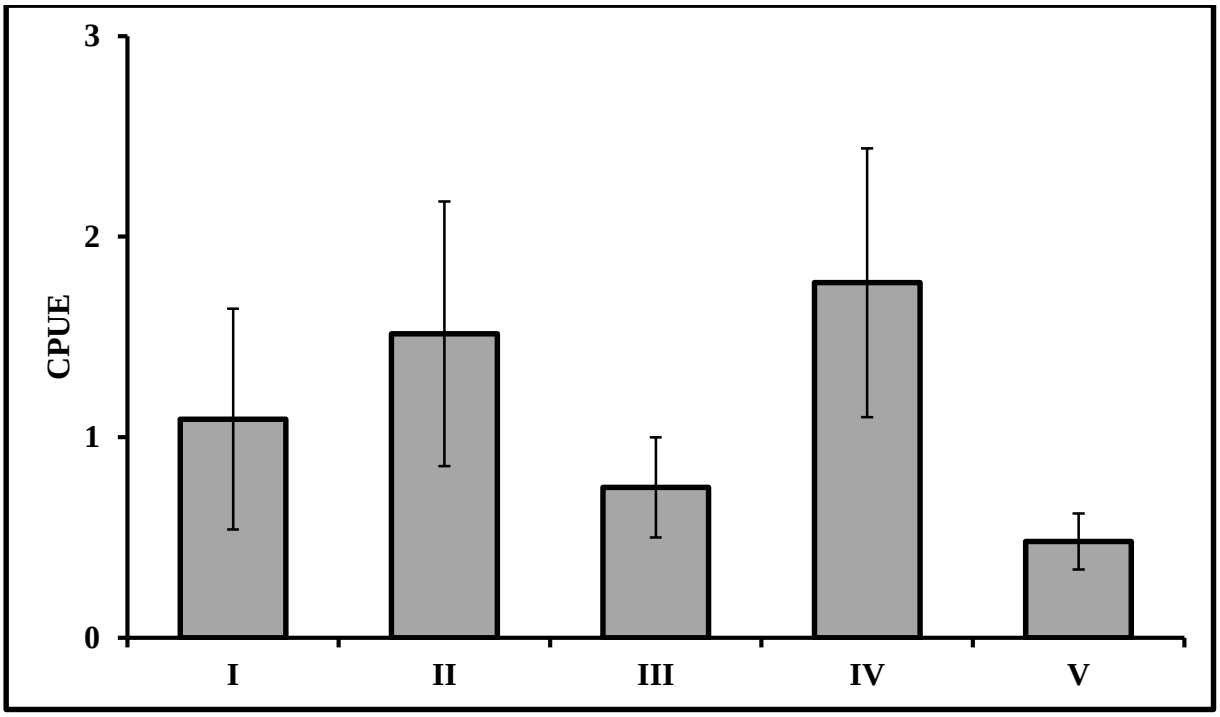


Figure 5. Catch-per-unit effort (CPUE; mussels/p-h) of unionids by reach within the Middle Colorado River drainage. Reach I - O.H. Ivie Reservoir to SH 377, Reach II - SH 377 to SH 45, Reach III - SH 45 to SH 16, Reach IV - SH 16 to Bend, and Reach V – Bend to Lake Buchannan.

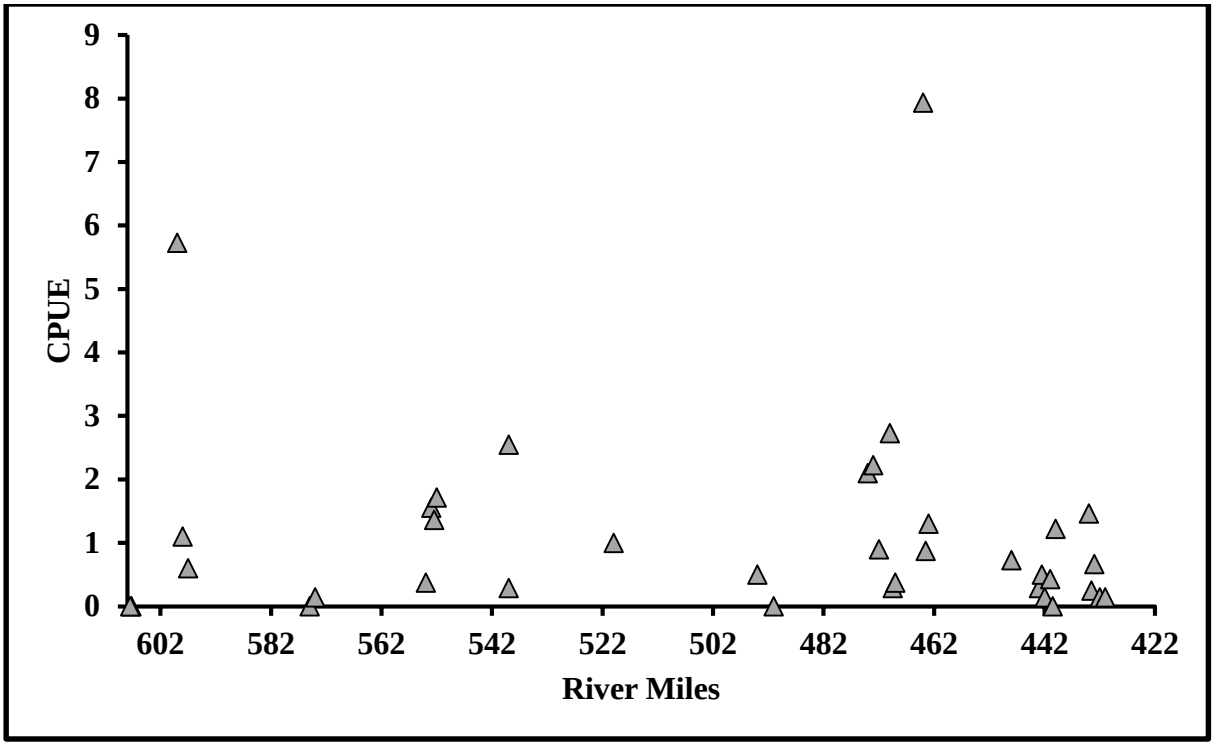


Figure 6. Catch-per-unit effort (CPUE; mussels/p-h) of unionids by river mile (RM) within the Middle Colorado River. O.H. Ivie Dam is located at approximately RM 608 and the headwaters of Lake Buchanan are located near RM 422.

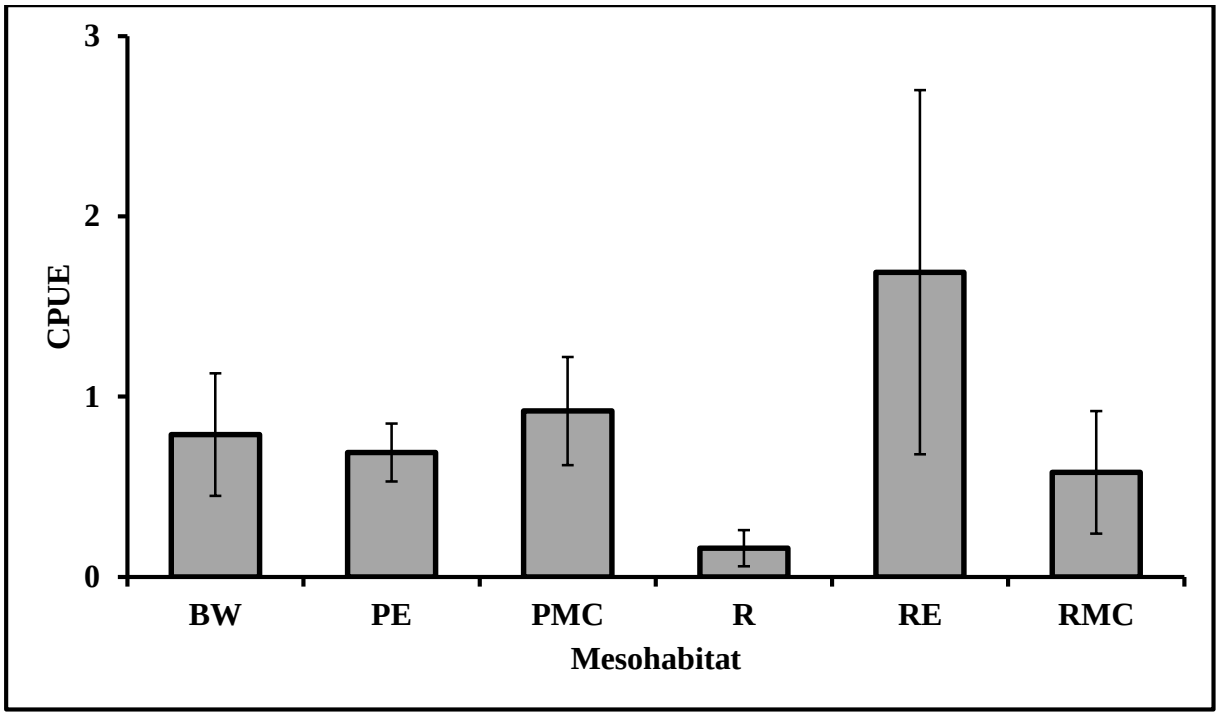


Figure 7. Catch-per-unit effort (CPUE; mussels/p-h.) of unionids by mesohabitat within the Middle Colorado River drainage. BW = backwater, PE = pool edge, PMC = pool mid-channel, R = riffle, RE = run edge, RMC = run mid-channel.

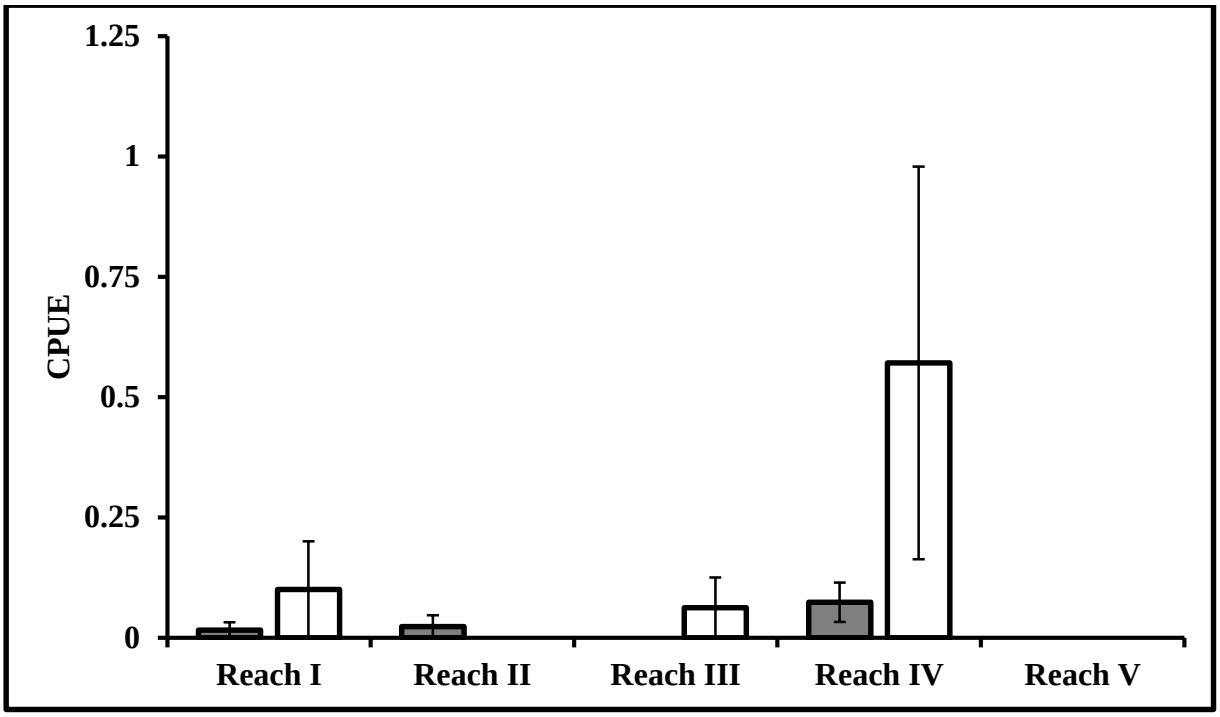


Figure 8. Catch-per-unit effort (CPUE; mussels/p-h) of *C. houstonensis* (dark grey) and *C. petrina* (white) by reach within the Middle Colorado River drainage.

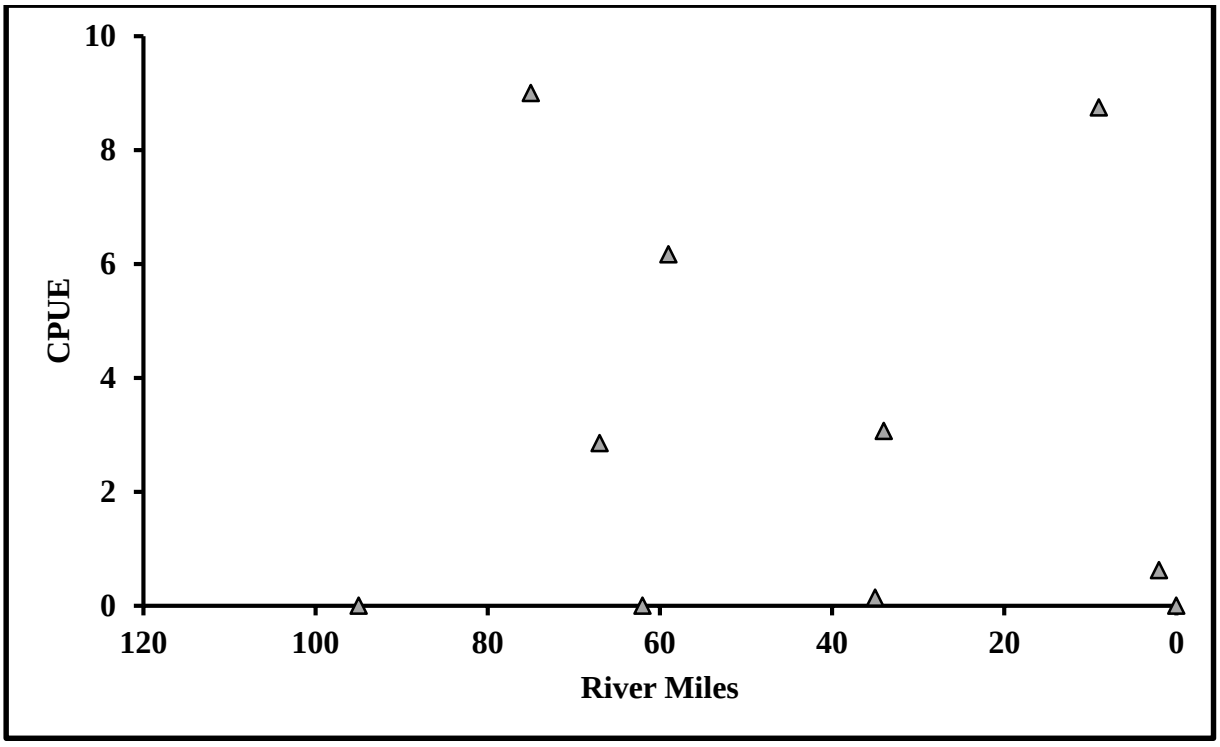


Figure 9. Catch-per-unit effort (CPUE; mussels/p-h) of unionids by river mile within the Little River. River mile 0 represents the confluence with the Brazos River.

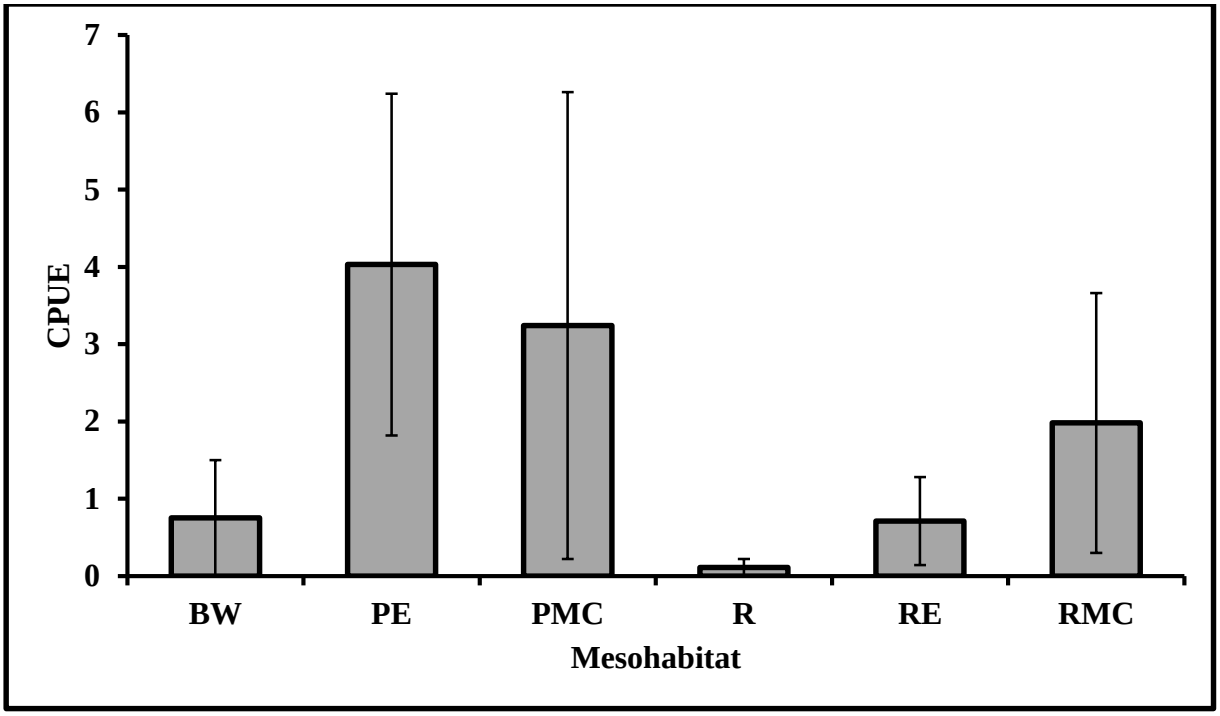


Figure 10. Catch-per-unit effort (CPUE; mussels/p-h.) of unionids by mesohabitat within the Little River drainage. BW = backwater, PE = pool edge, PMC = pool mid-channel, R = riffle, RE = run edge, RMC = run mid-channel.

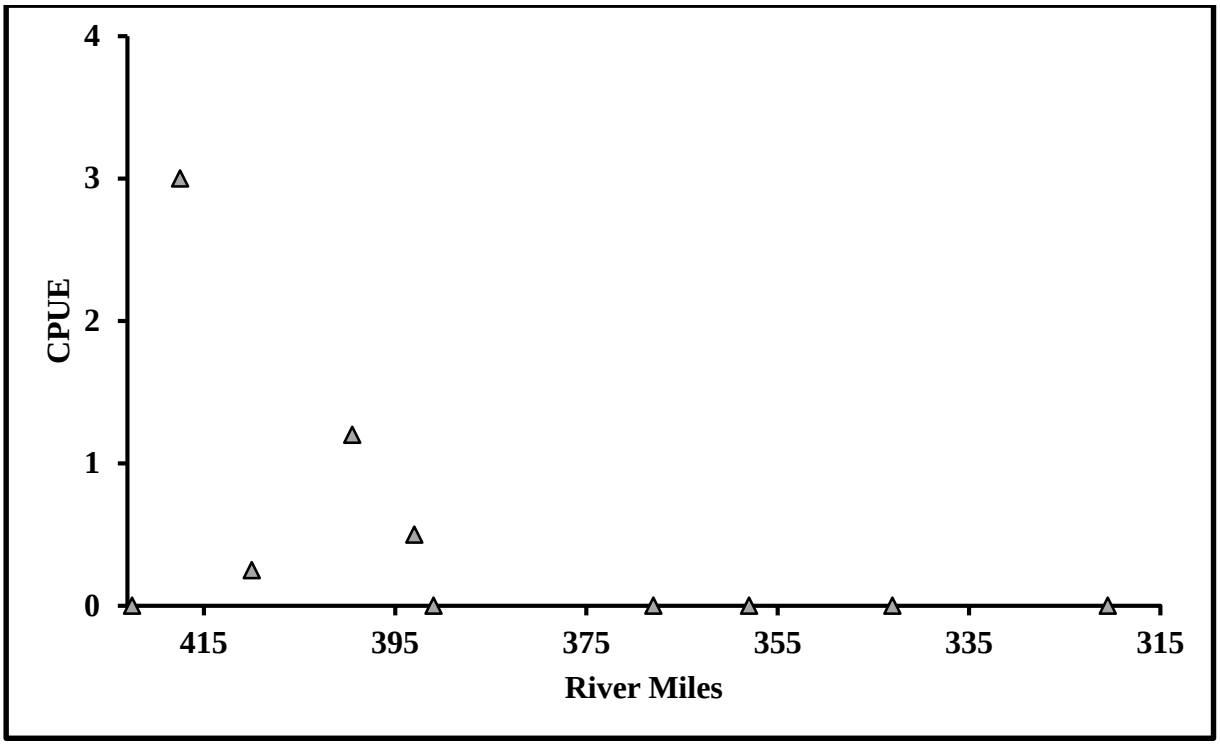


Figure 11. Catch-per-unit effort (CPUE; mussels/p-h.) of unionids by river mile within the Upper Guadalupe River. For spatial reference, the confluence of the North and South Fork Guadalupe River is at RM 423, and the headwaters of Canyon Lake are located at RM 315.

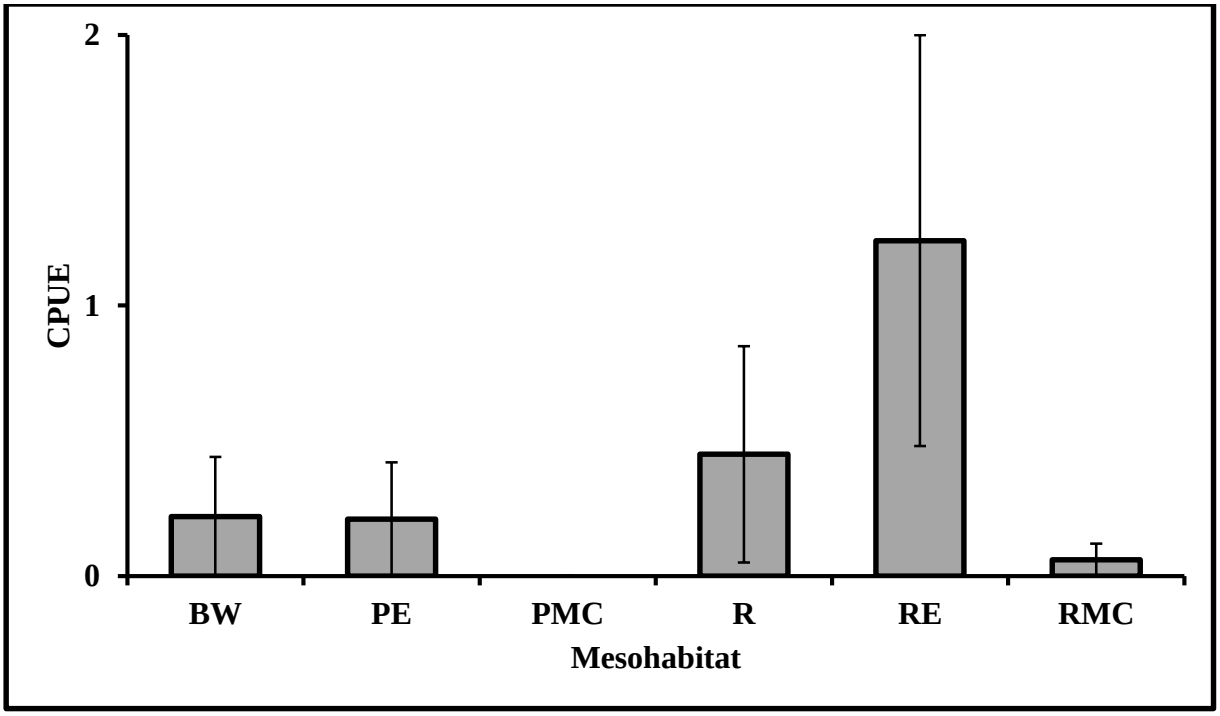


Figure 12. Catch-per-unit effort (CPUE; mussels/p-h.) of unionids by mesohabitat within the Upper Guadalupe drainage. BW = backwater, PE = pool edge, PMC = pool mid-channel, R = riffle, RE = run edge, RMC = run mid-channel.

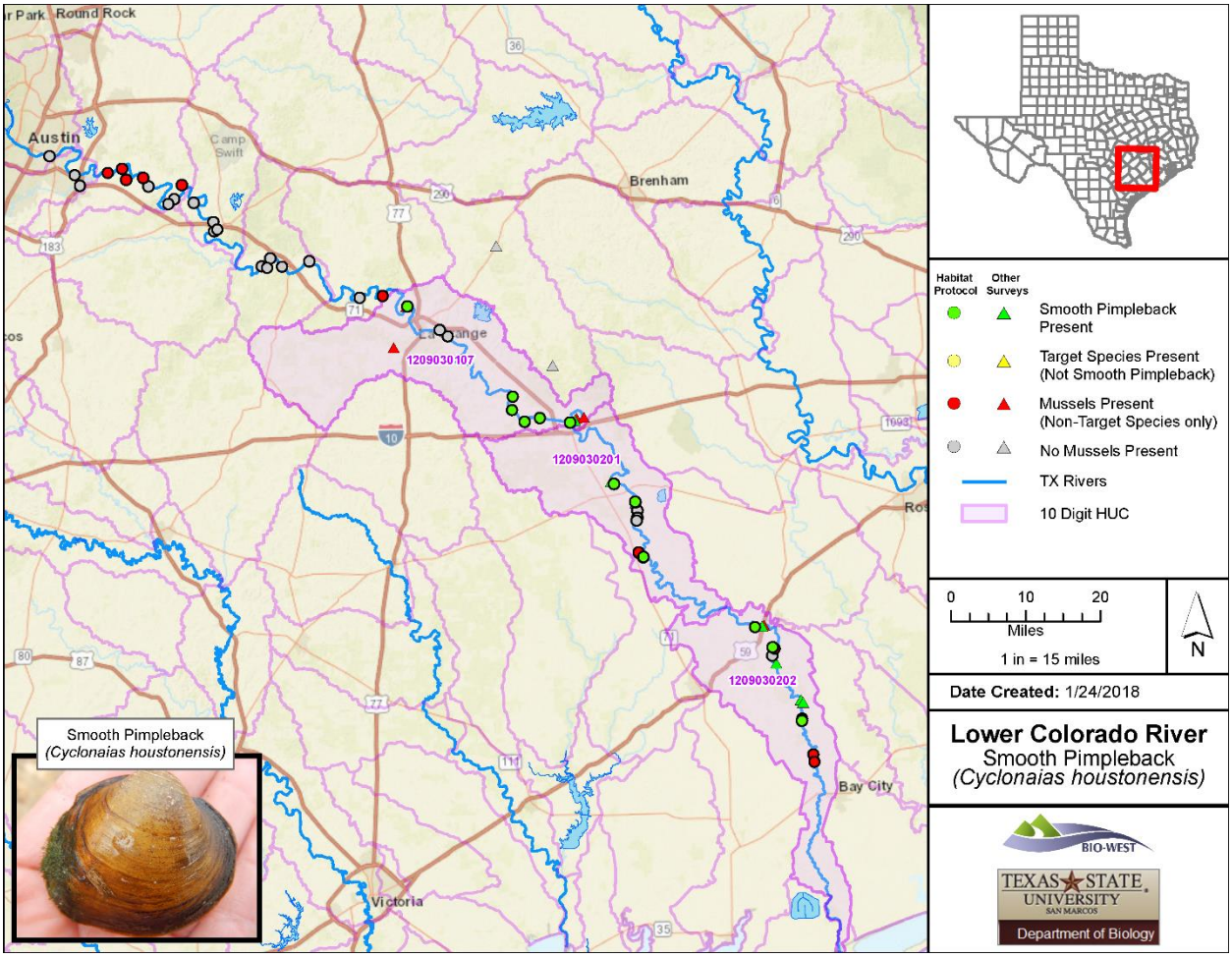


Figure 13. Map of *C. houstonensis* occurrence in the Lower Colorado River basin.

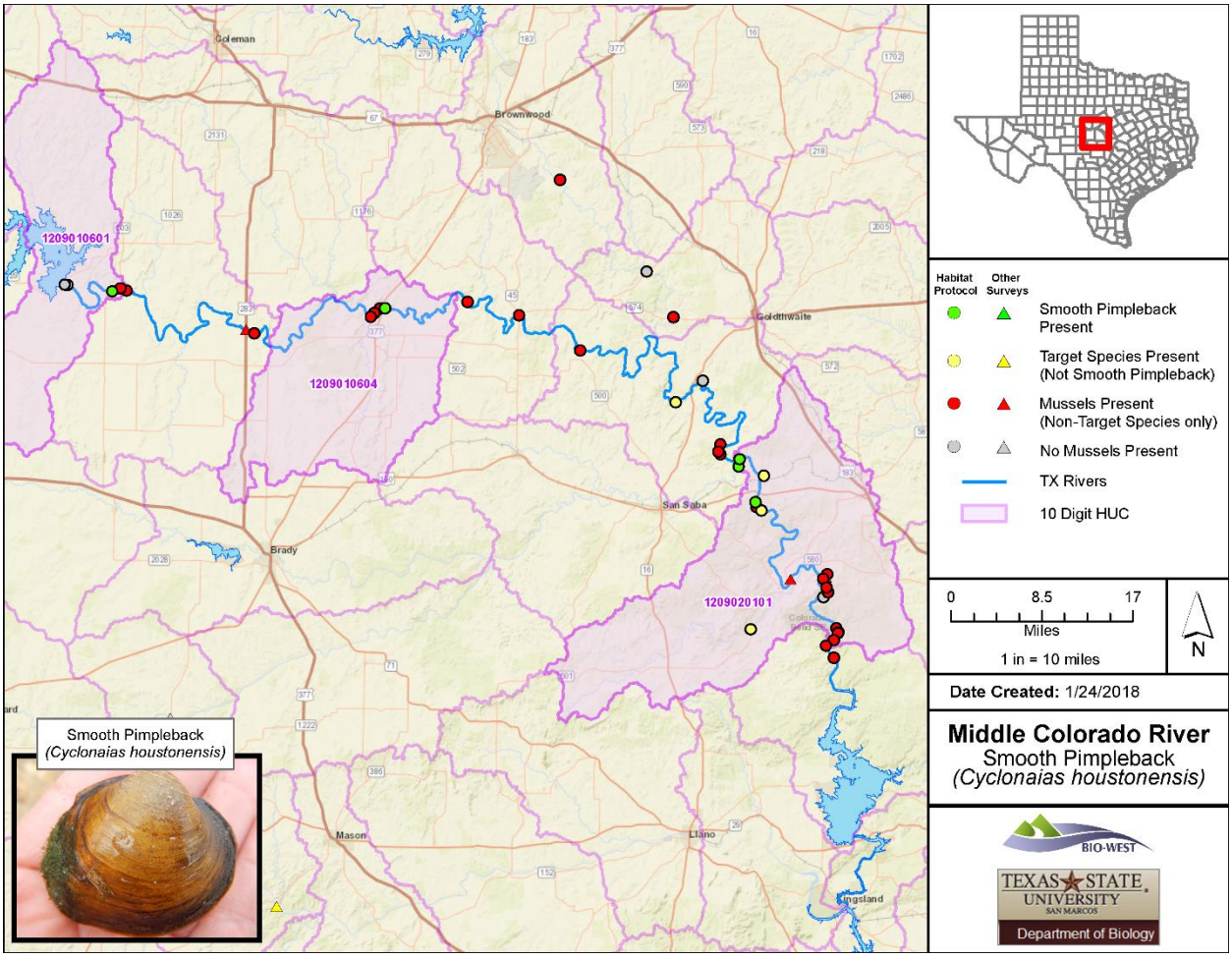


Figure 14. Map of *C. houstonensis* occurrence in the Middle Colorado River basin.

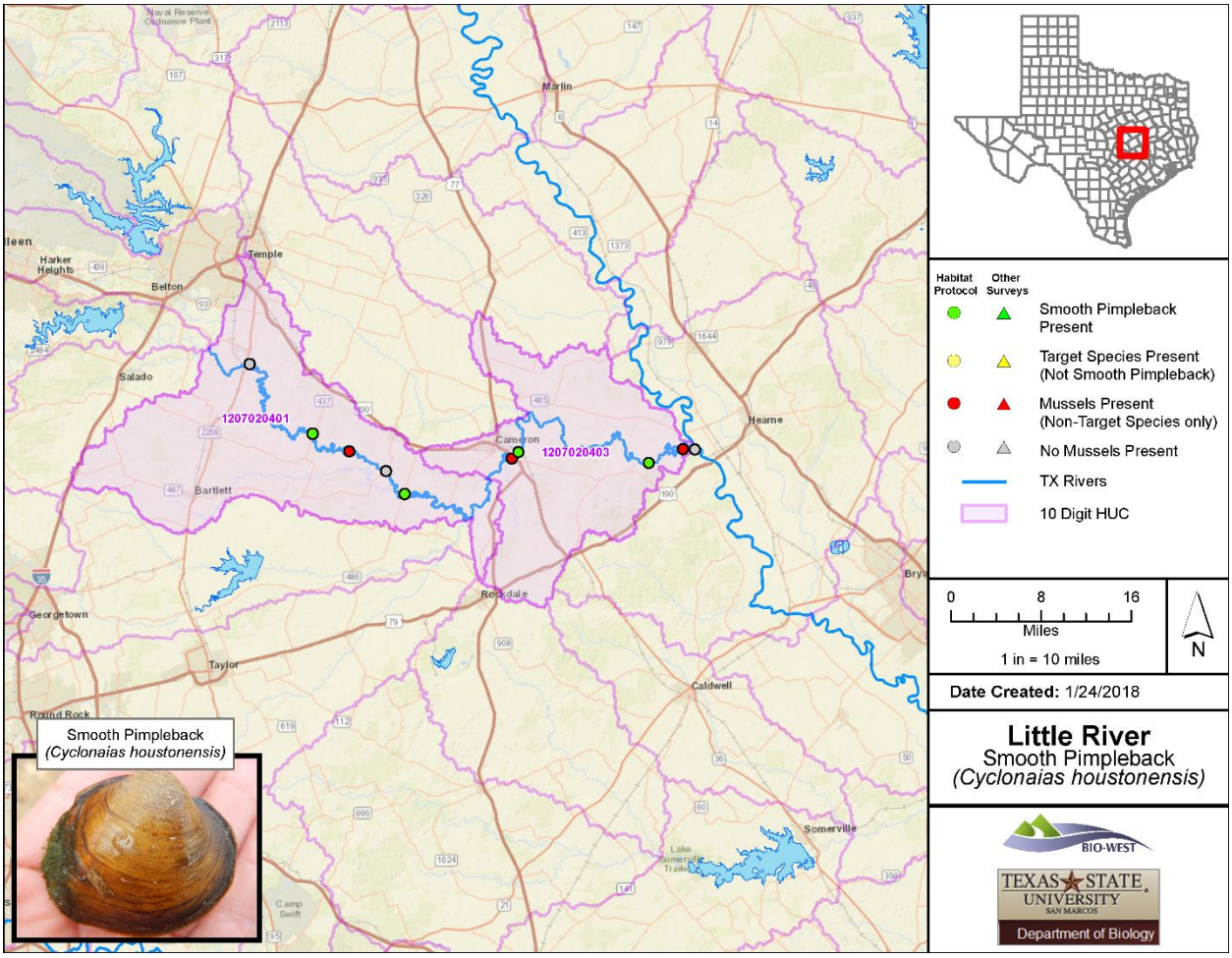


Figure 15. Map of *C. houstonensis* occurrence in the Little River basin.

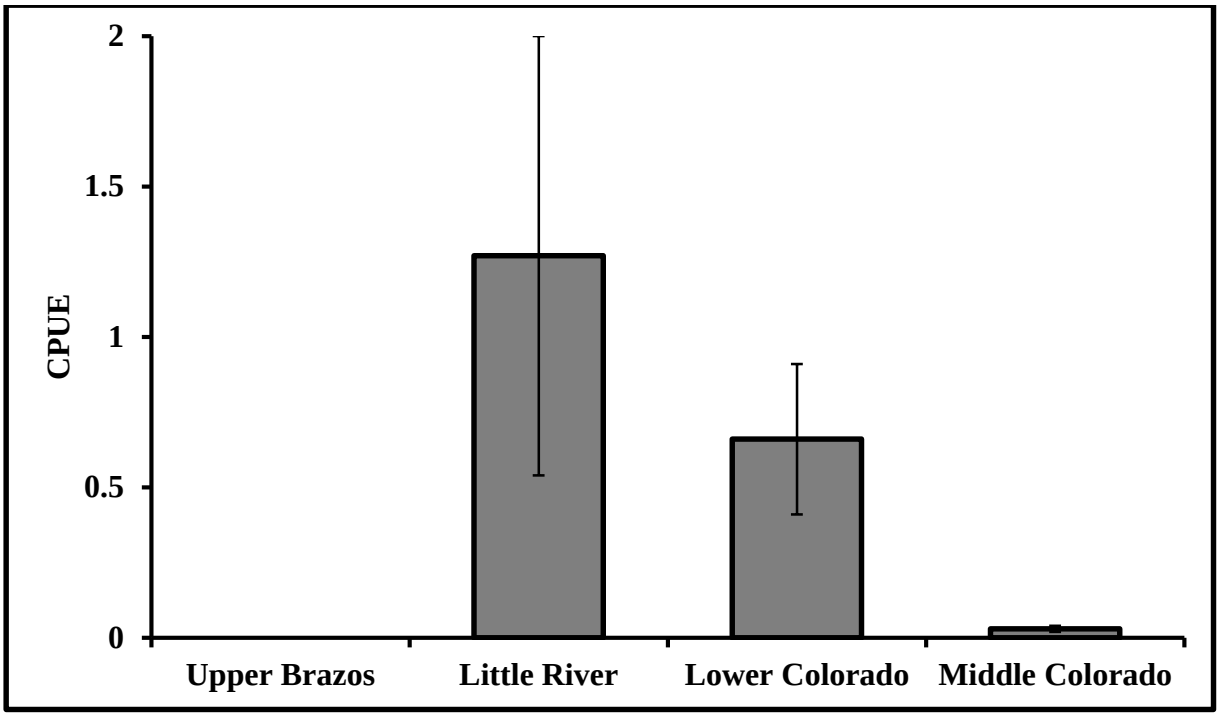


Figure 16. Catch per unit effort (CPUE; mussels/p-h.) of *C. houstonensis* among drainages.

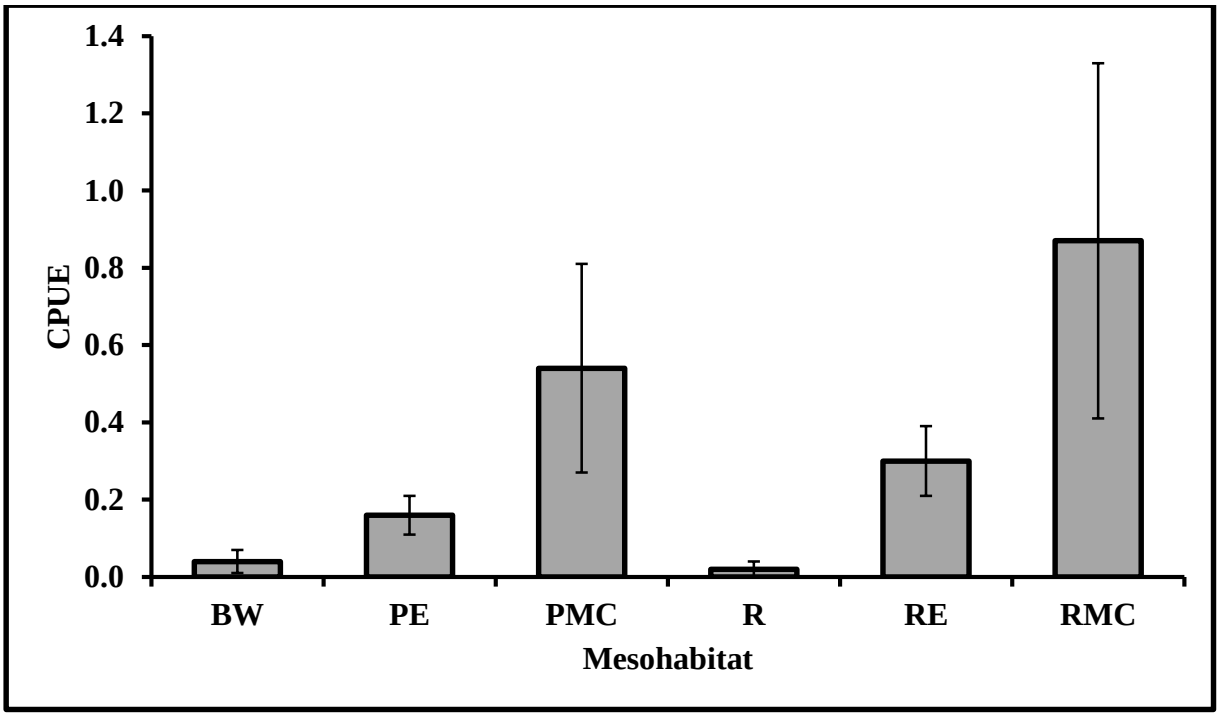


Figure 17. Catch-per-unit effort (CPUE; mussels/p-h.) of *C. houstonensis* by mesohabitat among all survey basins. BW = backwater, PE = pool edge, PMC = pool mid-channel, R = riffle, RE = run edge, RMC = run mid-channel.

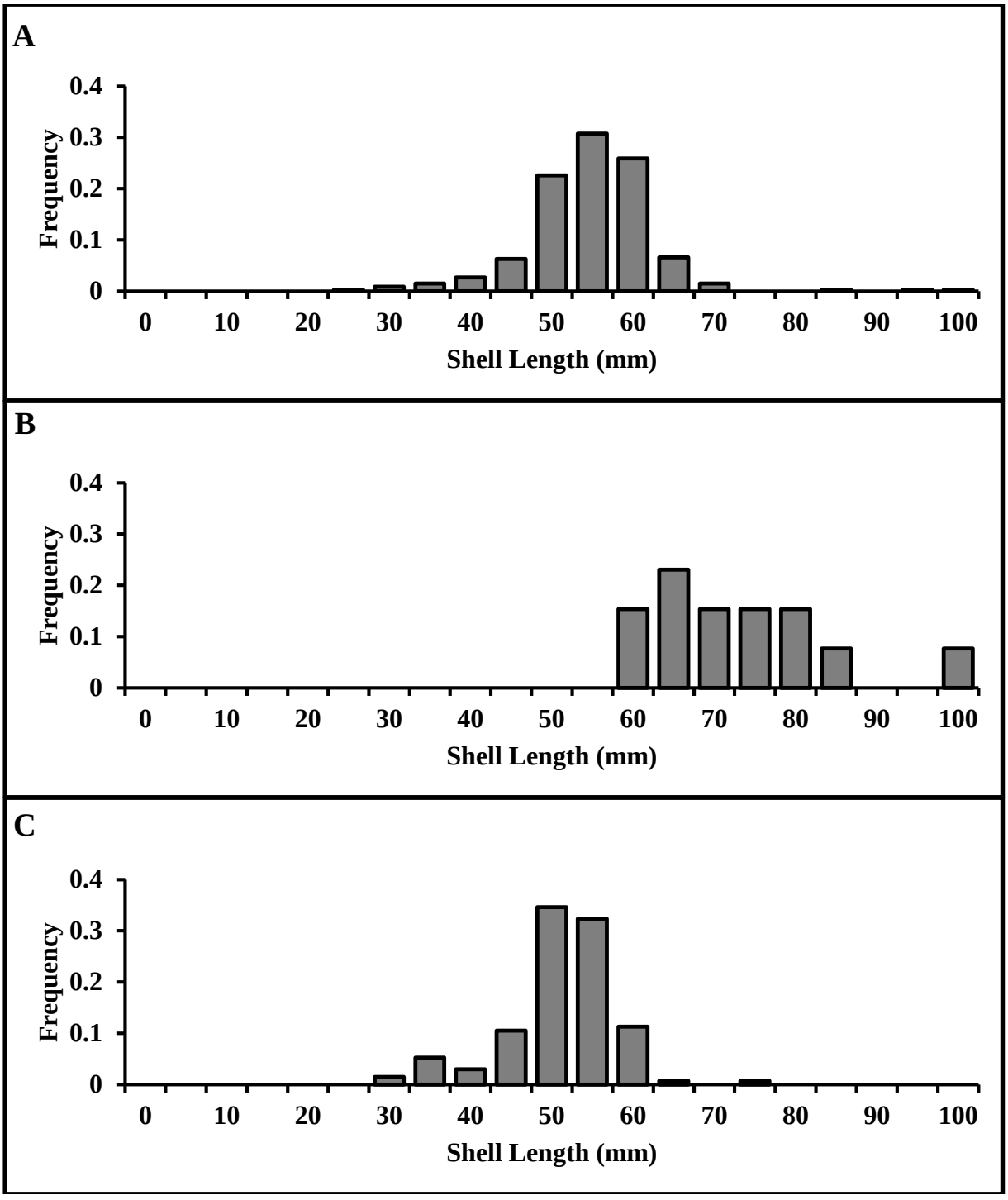


Figure 18. Proportional size structure of *C. houstonensis* within all basins of occurrence. A = Lower Colorado (N = 387), B = Middle Colorado (N = 13), C = Little River (N = 133).

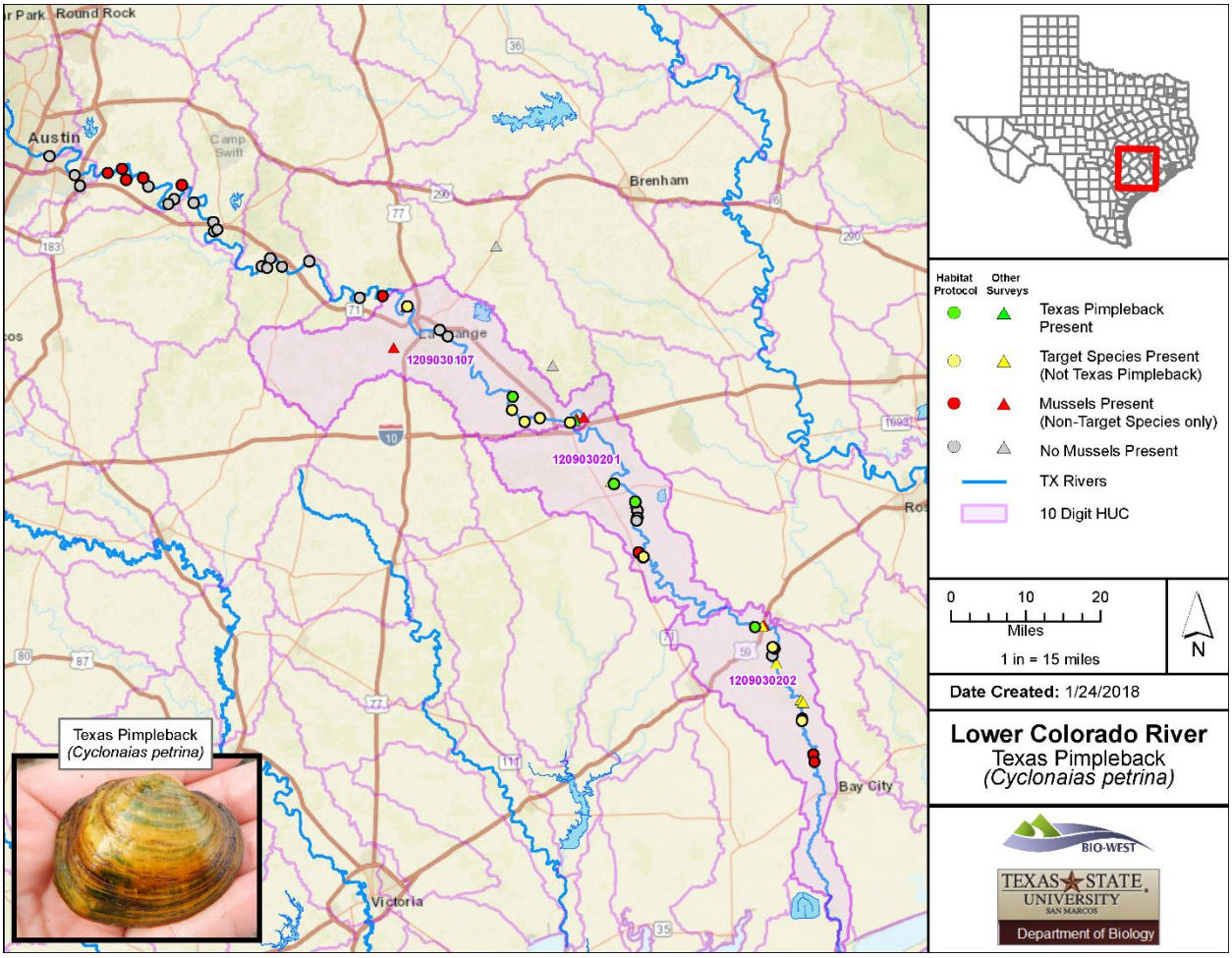


Figure 19. Map of *C. petrina* occurrence in the Lower Colorado River basin.

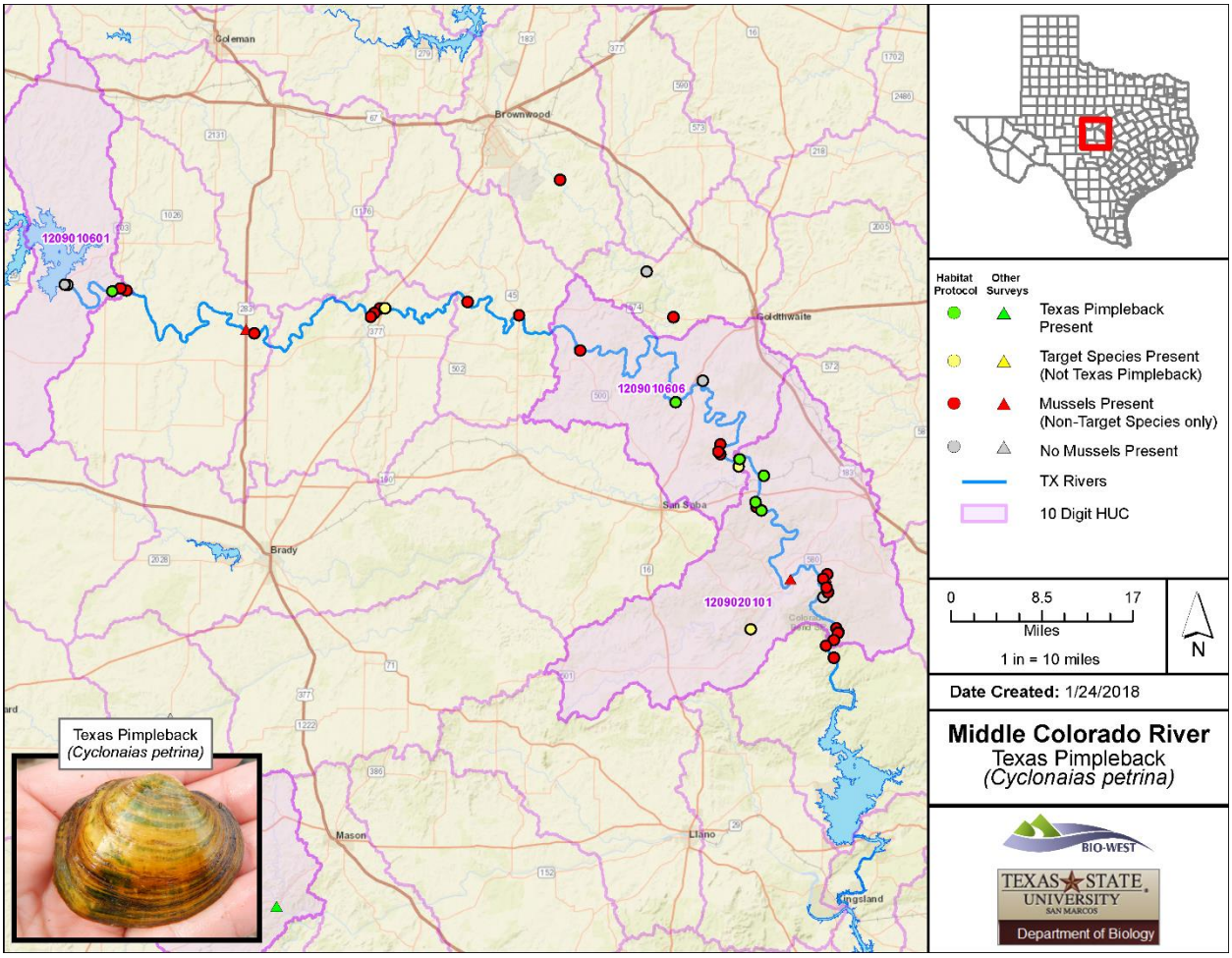


Figure 20. Map of *C. petrina* occurrence in the Middle Colorado River basin.

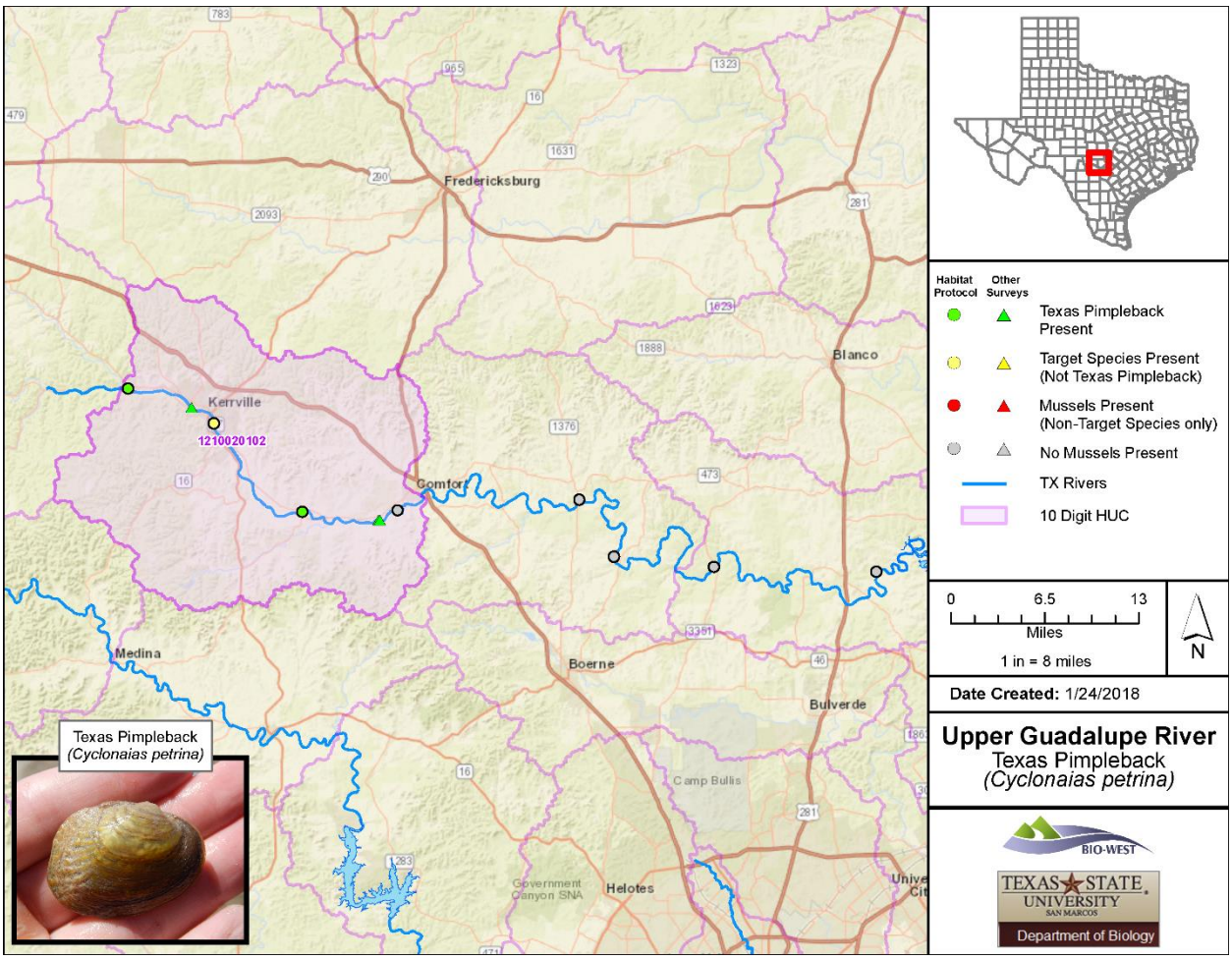


Figure 21. Map of *C. petrina* occurrence in Upper Guadalupe River basin.

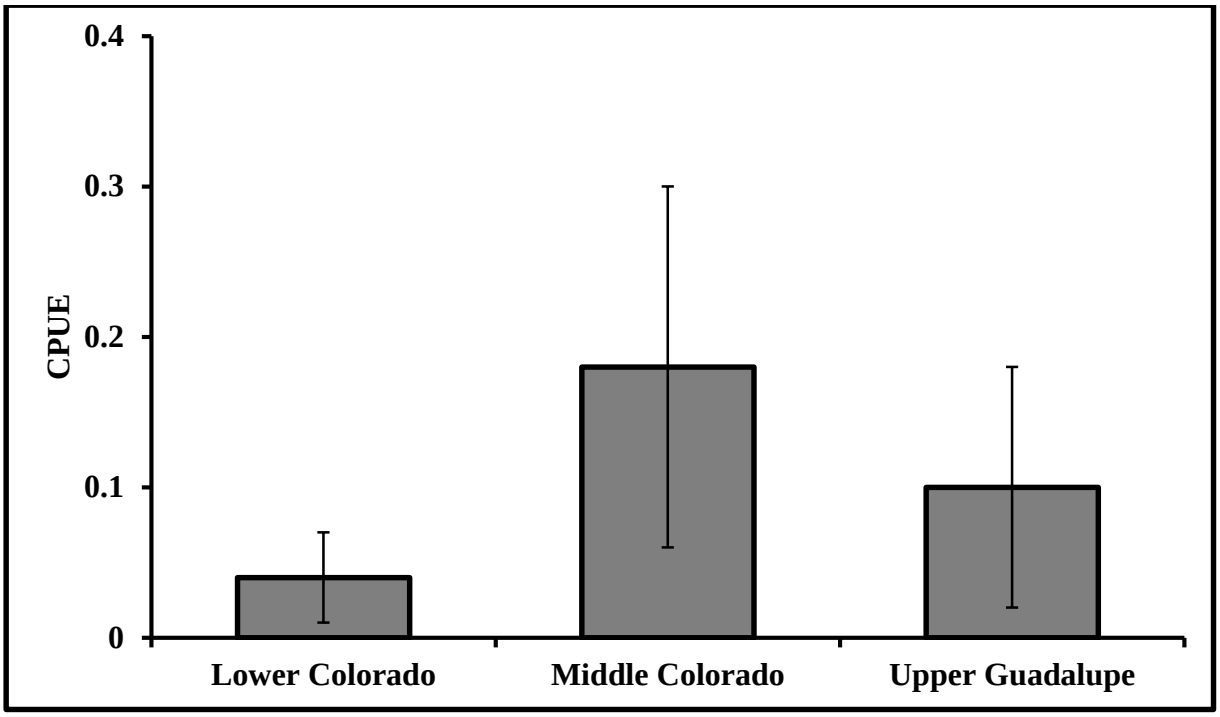


Figure 22. Catch-per-unit effort (CPUE; mussels/p-h.) of *C. petrina* among drainages.

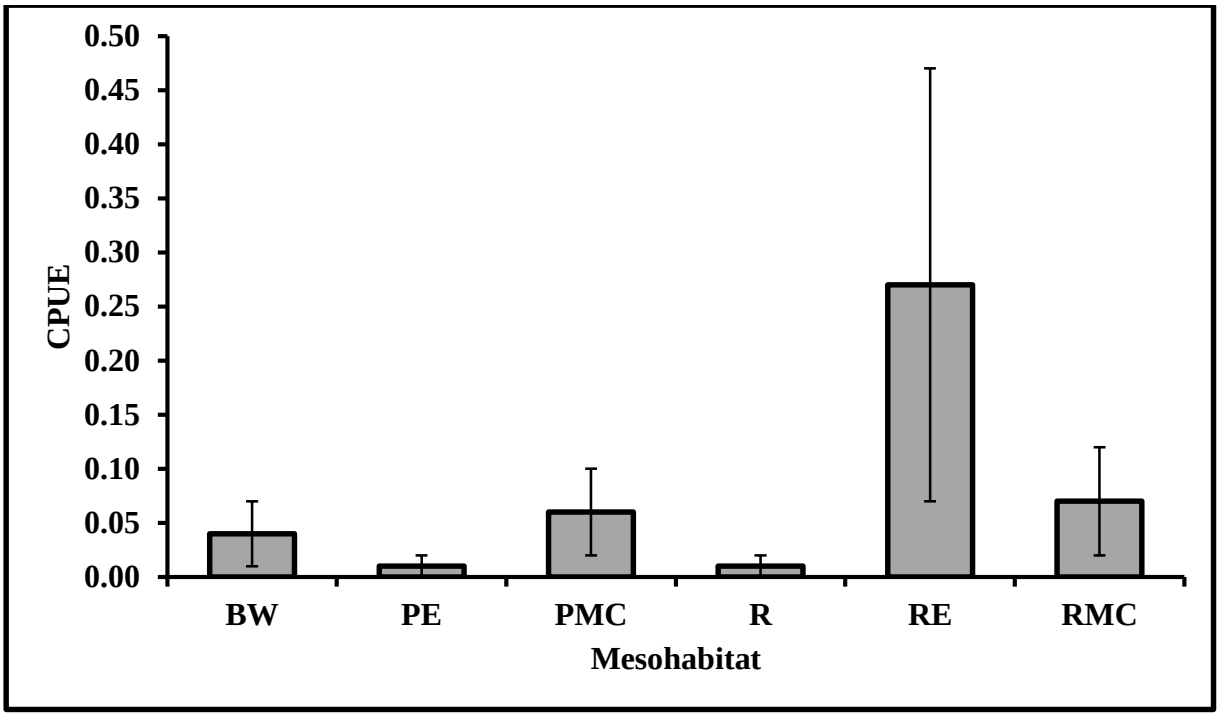


Figure 23. Catch-per-unit effort (CPUE; mussels/p-h.) of *C. petrina* by mesohabitat among all survey basins. BW = backwater, PE = pool edge, PMC = pool mid-channel, R = riffle, RE = run edge, RMC = run mid-channel

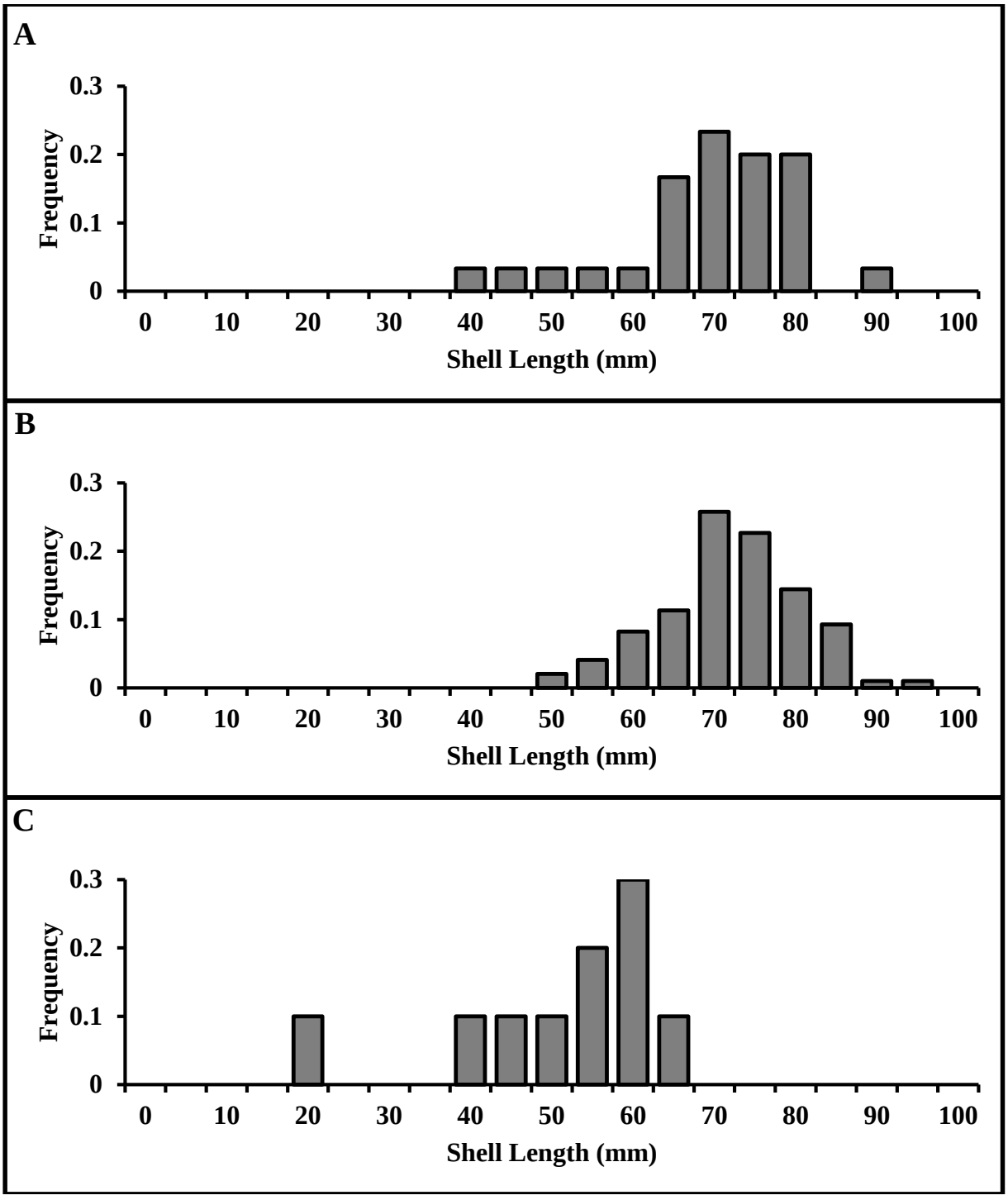


Figure 24. Proportional size structure of *C. petrina* within all basins of occurrence.
A = Lower Colorado (N = 30), B = Middle Colorado (N = 97), C = Upper Guadalupe (N = 10).

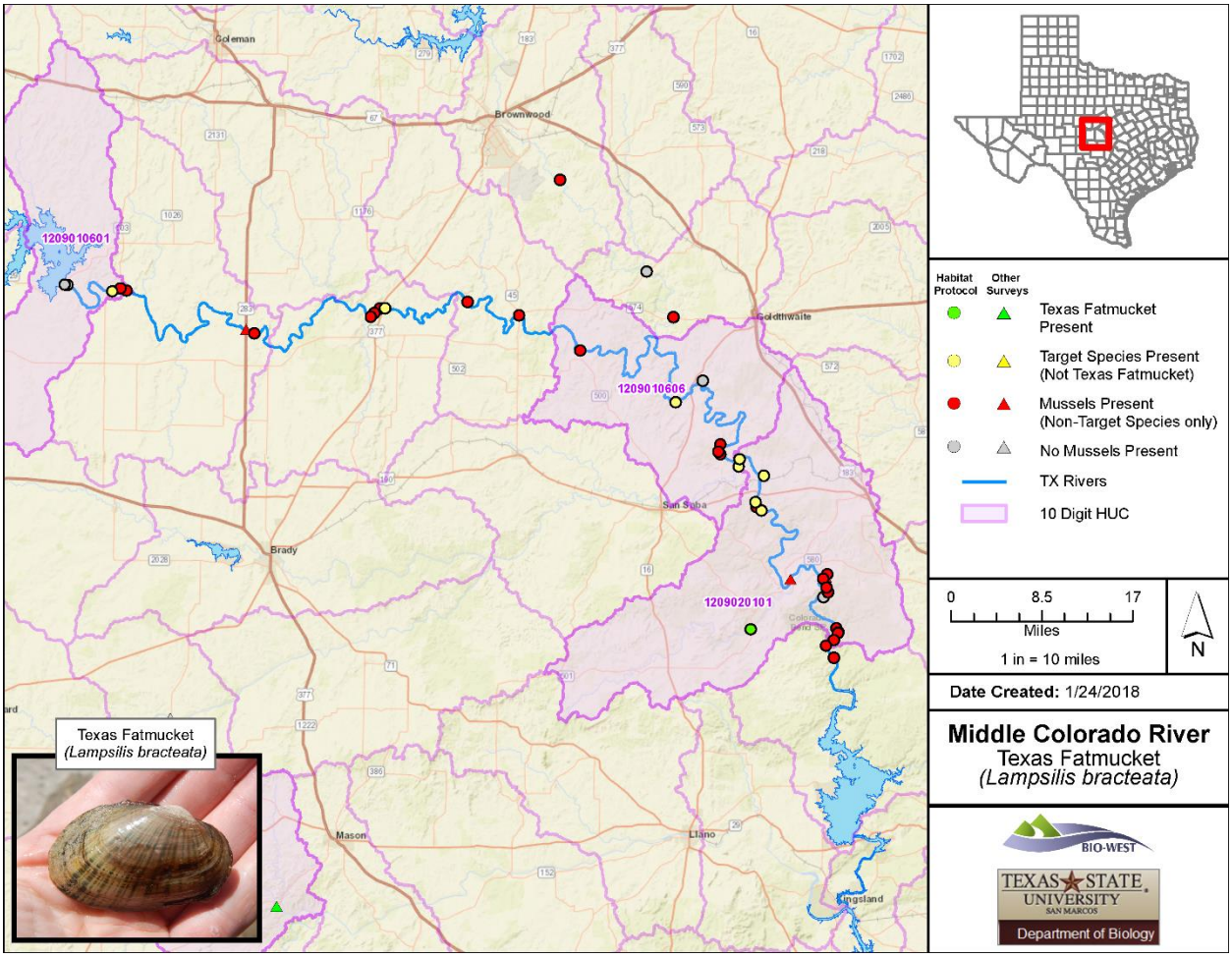


Figure 25. Map of *L. bracteata* occurrence in the Middle Colorado River basin.

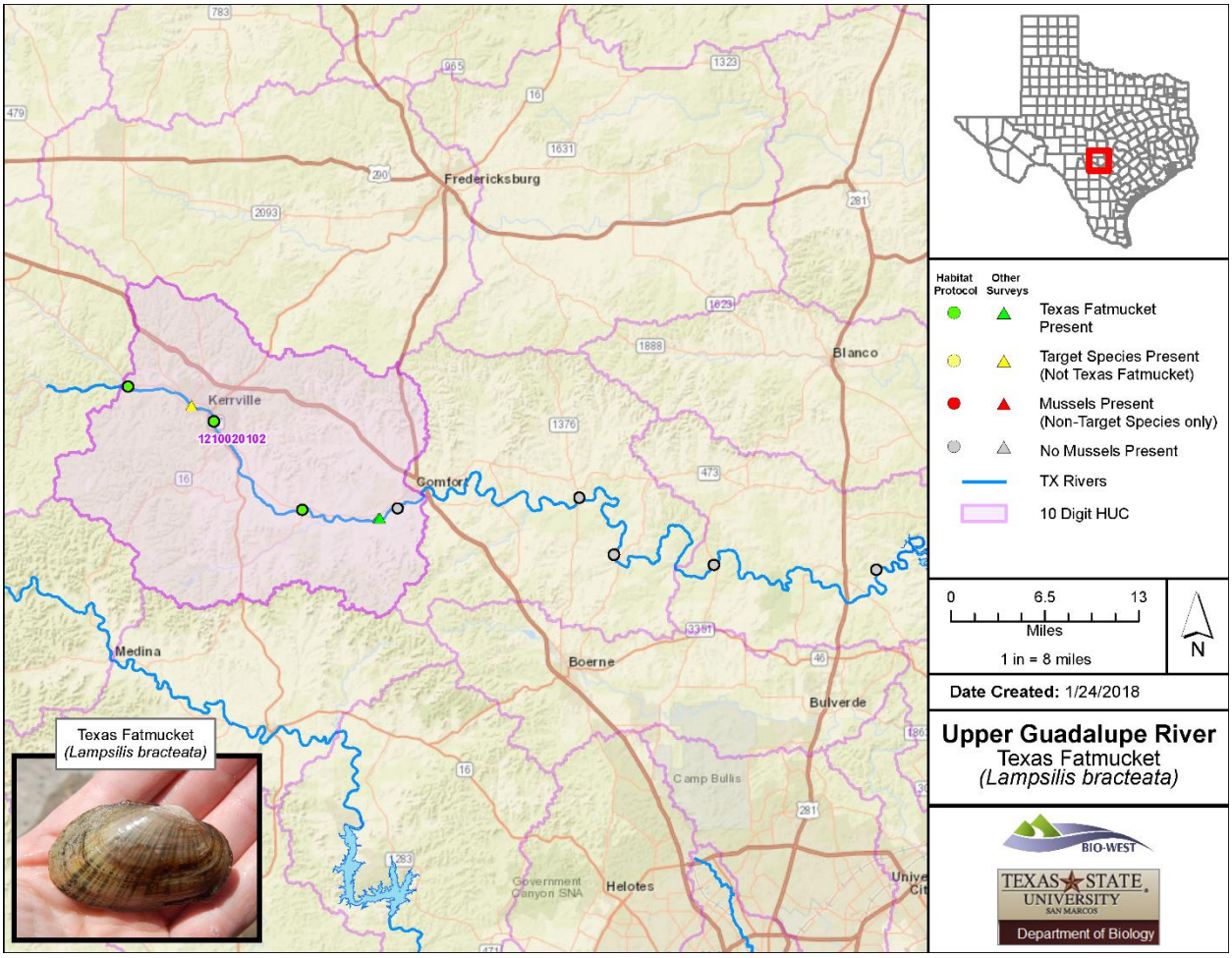


Figure 26. Map of *L. bracteata* occurrence in the Upper Guadalupe River basin.

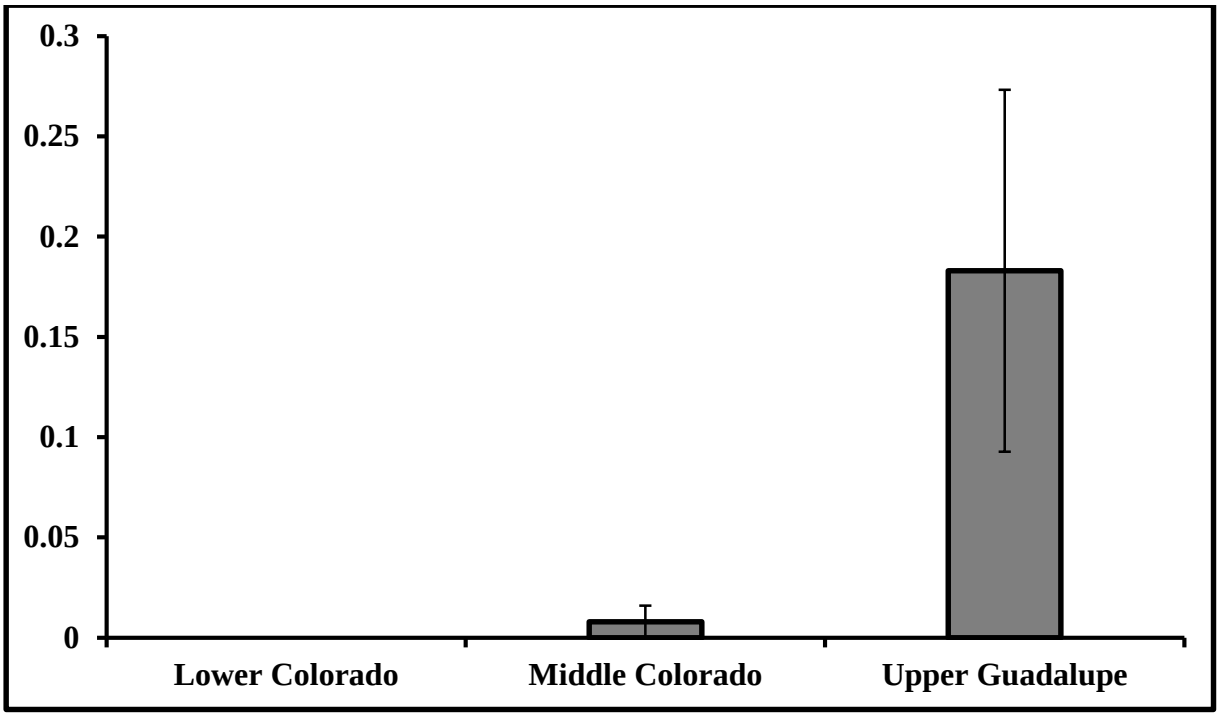


Figure 27. Catch-per-unit effort (CPUE; mussels/p-h.) of *L. bracteata* among drainages.

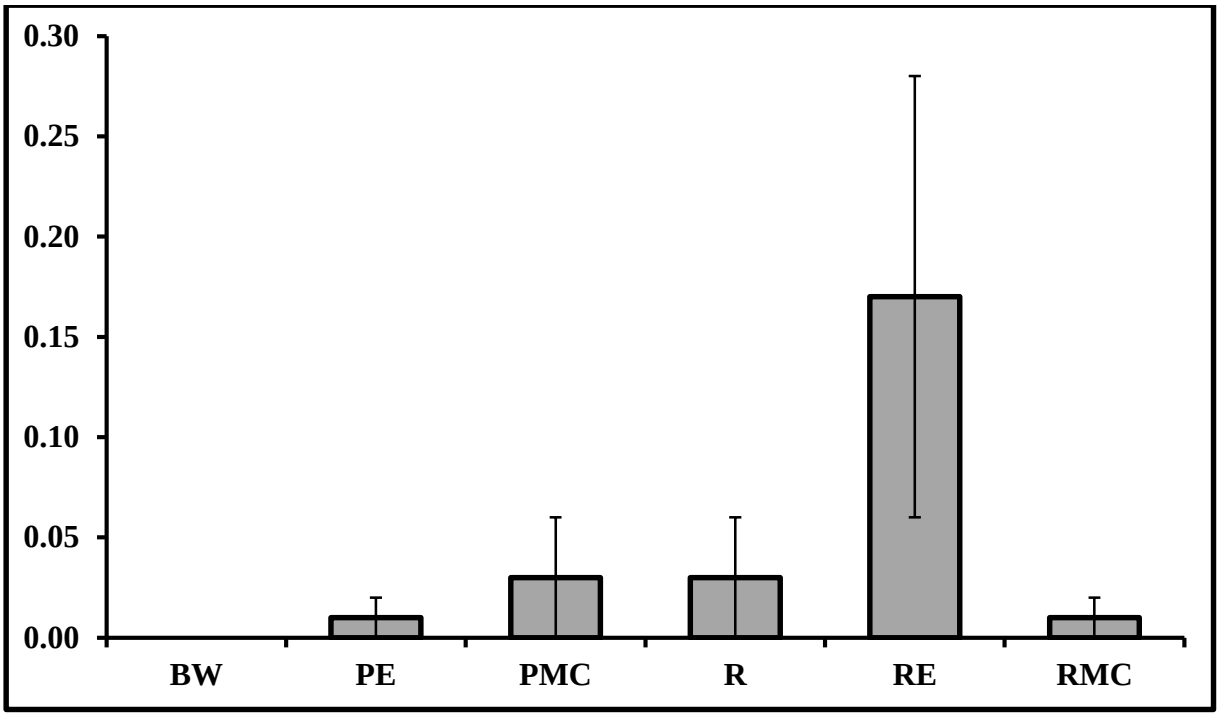


Figure 28. Catch-per-unit effort (CPUE; mussels/p-h.) of *L. bracteata* by mesohabitat among all survey basins. BW = backwater, PE = pool edge, PMC = pool mid-channel, R = riffle, RE = run edge, RMC = run mid-channel.

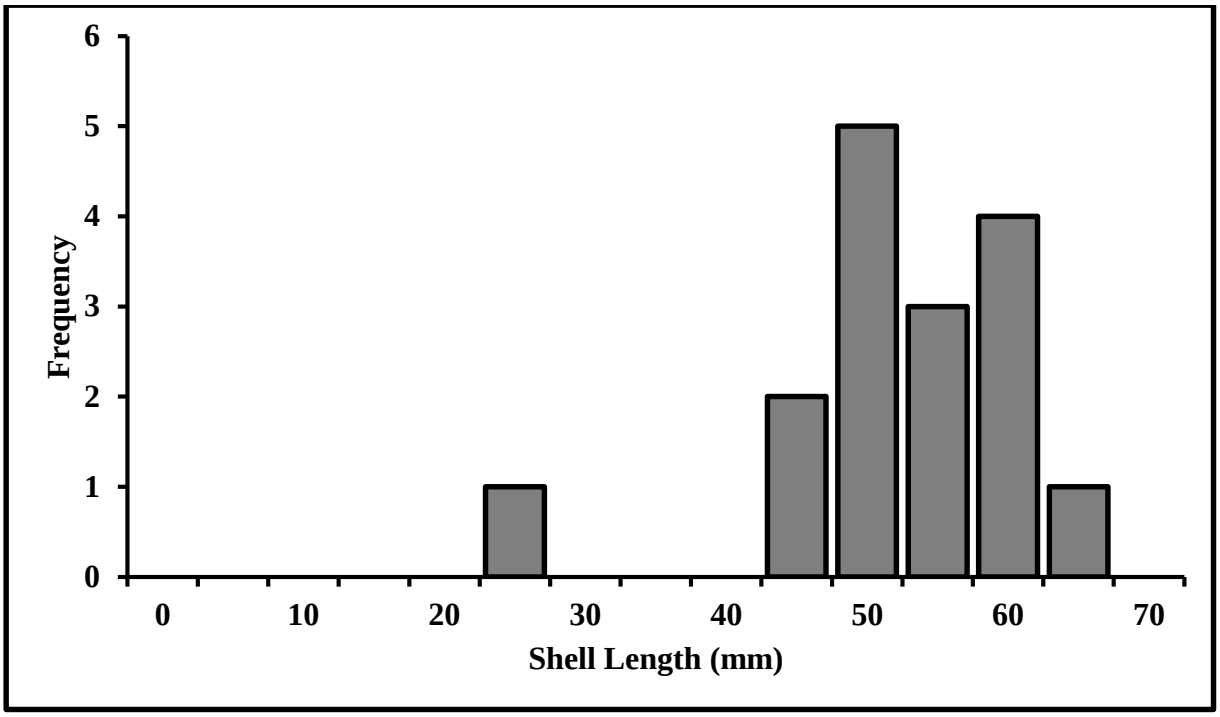


Figure 29. Size structure of *L. bracteata* within the Upper Guadalupe River.

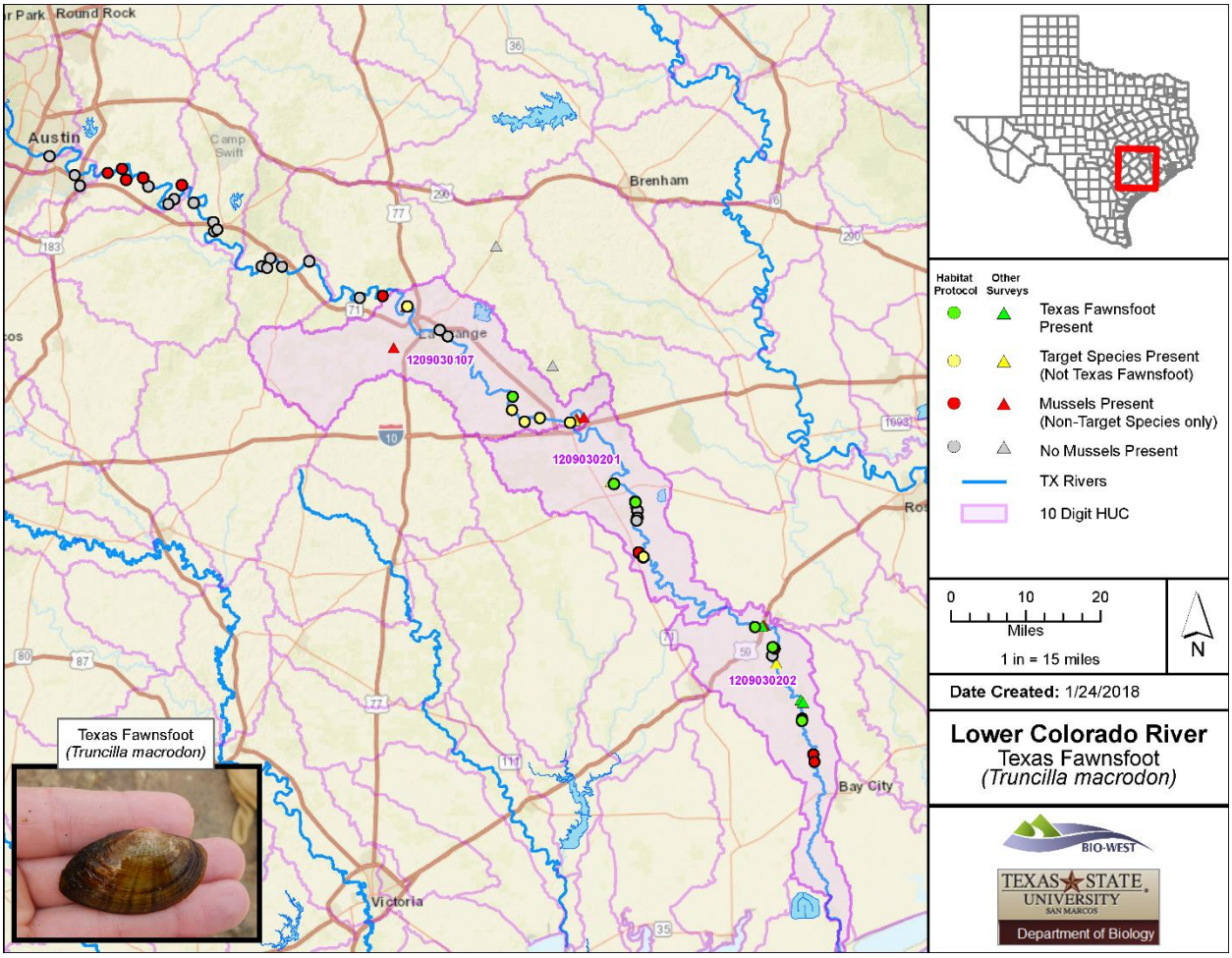


Figure 30. Map of *T. macrodon* occurrence within the Lower Colorado River basin.

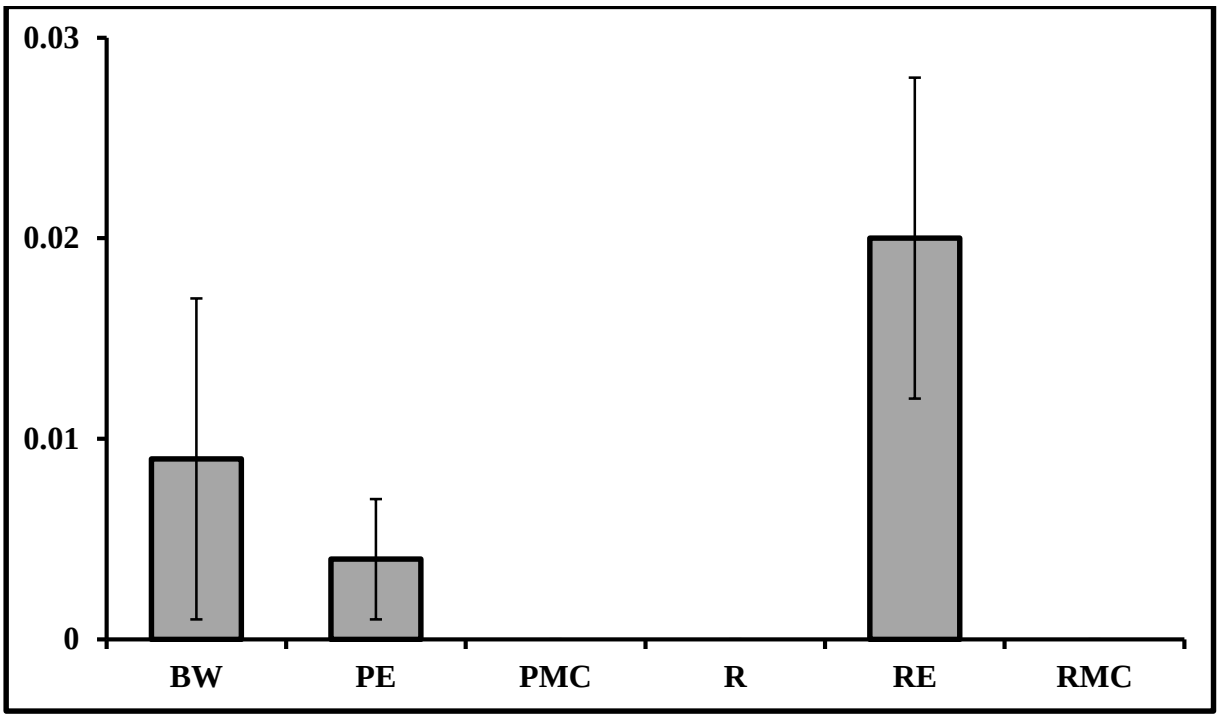


Figure 31. Catch-per-unit effort (CPUE; mussels/p-h.) of *T. macrodon* by mesohabitat among all survey basins. BW = backwater, PE = pool edge, PMC = pool mid-channel, R = riffle, RE = run edge, RMC = run mid-channel.

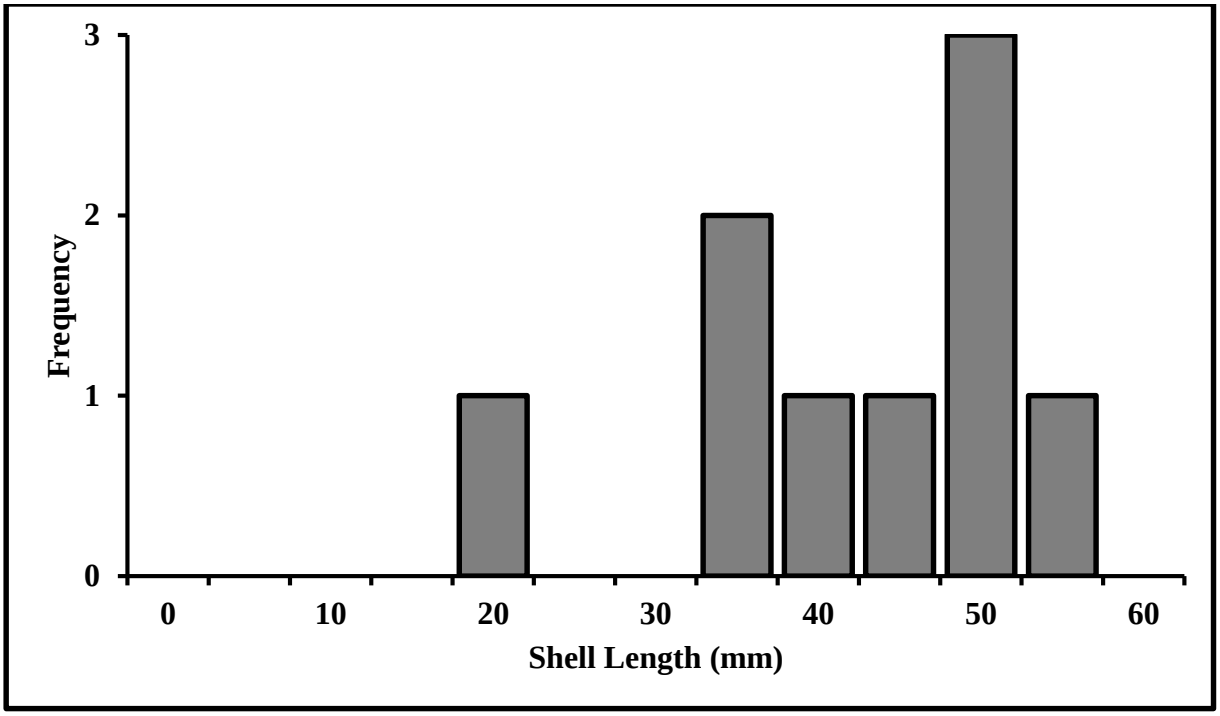


Figure 32. Size structure of *T. macrodon* within the Lower Colorado River.

Part II: Study Objectives

Objectives for Part II were to assess occurrences and abundances of mussels among habitat parameters (e.g., mesohabitat, current velocity, depth, substrates) within reaches and georegions of the Brazos River, Colorado River, and Guadalupe River basins. Univariate habitat associations were assessed only for candidate mussel species, when present, within a reach or basin.

Part II Methods

Information was taken from study areas and surveying techniques described in Part I methods. For Part II assessment, Colorado River basin was assessed by georegion instead of Middle Colorado River and Lower Colorado River. Middle Colorado River was divided among Georegion 1 (Prairie) and Georegion 3 (Llano Uplift). Lower Colorado River was divided among Georegion 4 (Balcones) and Georegion 5 (Lowland). Georegion 2 (Edwards Plateau) is described herein but was not sampled as part of this study. Georegions were developed for exploring correspondence between mussel communities and surface geology (Strayer 1983). Colorado River basin has a diverse geology from headwater reaches to terminus with Gulf of Mexico, along with diverse climate gradient that contains arid climate in the headwater reaches to a sub-humid climate near its terminus. Surface geology and climate interact to form unique georegional aquatic habitats with respect to groundwater quality and quantity, surface water quality and quantity, stream gradient, stream substrates, and stream morphology (Table 1).

Perennial flows of the Colorado River mainstem begin on the western edge of the Llano Estacado (deposits during Tertiary Period). In a general southeast direction, the perennial flowing portion of the Colorado River main stem and tributaries bisects Carboniferous, Permian,

and Triassic strata layers (Georegion 1), forming low gradient prairie streams with predominately silt substrates, although sandstones and limestones form a limited amount of rocky outcroppings and gravel to boulder substrates. Streams, common to this strata layer in nearby upper Brazos River and Red River, tend to be dominated by shallow runs, braided channels, and highly turbid, and of moderate to high salinity. The Colorado River then enters the Llano Uplift area with Precambrian and Cambrian strata (Georegion 3; Latitude: 31.090572, Longitude: -98.463806; upstream from Colorado Bend State Park, San Saba County) consisting primarily of granite but interspersed with some limestones, dolomites, and sandstones. The river and tributaries have less silt substrate and more gravel to boulder substrates and diversity in mesohabitats (e.g., run, riffle, pools), and are also characterized by their higher gradient, lower turbidity, and less saline waters. From the west, the Edwards Plateau with its Cretaceous limestone and karst aquifers (Georegion 2) contribute substantial spring flows to the Georegion 1 (via Concho River) and Georegion 3 (via San Saba River, Llano River, and Pedernales River). From the Llano Uplift, the Colorado River enters another section of the Edwards Plateau and the Balcones Escarpment (Georegion 4; Lake Travis area, Travis County) before bisecting the gulf coastal plains of Tertiary deposits (Georegion 5; Latitude: 30.200572, Longitude: -97.525748; upstream from Webberville Park, Travis County). Tertiary deposits and stream substrates of Georegion 5 are dominated by sands and silts, but various sandstone strata layers occur in the lower Colorado River main stem.

Principal component analysis (PCA; Canoco 4.5, Microcomputer Power 2002) was used to assess linear combinations of habitat parameters. Mesohabitats were coded as dummy variables and quantitative data (e.g., current velocity column, current velocity bottom, depth) were z-transformed. Parameters with diel fluctuations (e.g., water temperature) were omitted

from the analysis. The resulting PCA loadings were plotted and grouped to assess habitat variability within and among basins and georegions. Canonical correspondence analysis (CCA; Canoco 4.5) was used to assess patterns in habitat associations among Brazos River, Colorado River, and Colorado River mussel community. Initial plot was informative, but highlighted differences among basins (e.g., specific conductance, substrate types) and not mussel-habitat associations within basin. A subsequent CCA model was used to assess within basin mussel-habitat associations for the Colorado River basin. Brazos River and the Guadalupe River lacked sufficient samples to perform a CCA analyses with the same variables as used with the Colorado River basin. As such, CCA was not used on the Brazos River and Guadalupe River data. For univariate assessments, habitat observed was calculated for each habitat parameter and each candidate species using occurrence data. Habitat available was calculated for each habitat parameter by basin. A second habitat observed versus habitat available assessment was conducted on all mussels across basins using relative abundances (i.e., weighted occurrences) to compare similarities and differences in habitat variables among all mussels.

Part II Results

Qualitative surveys were conducted at 707 mesohabitats within 114 sites and among the three river drainages between March 15, 2017 and October 11, 2017. Exploratory surveys were conducted at an additional 12 sites in the Upper Colorado River (near Colorado City, Texas, one site, no mussels), tributary of the Middle Colorado River (upper reach of San Saba River, two sites, one Southern Mapleleaf), tributaries of the Lower Colorado River (Buckner's Creek, one site, one Giant Floater; Cummins Creek, two sites, no mussels), and Clear Fork Brazos River (6 sites, one Yellow Sandshell, two Fragile Papershell). The additional 12 sites were not sampled

with qualitative survey methodologies because lack of candidate mussel species observed during exploratory surveys.

Among the five basin or reach mesohabitats from qualitative surveys, principal component axis I explained 32% of the habitat variation and described a substrate and current velocity gradient (Figure 1). Principal component axis II explained 18% of the habitat variation and described primarily a substrate gradient. Central tendencies of mesohabitats within the Upper Brazos River and Little River were associated positively with PC I (i.e., greater amounts of silt, large woody debris, and detritus) and negatively associated with PC II (i.e., greater amounts of sand substrates). Central tendencies of mesohabitats within Colorado River Georegions 4 and 5 were associated negatively with PC I (i.e., greater amounts of gravel substrates, swifter water) and PC II. Upper Guadalupe River, and Colorado River Georegions 1 and 3 were positively associated with PC II (i.e., greater amounts of cobble substrates).

Within the Brazos River basin, 120 mesohabitats from 20 sites consisted of riffle, run, pool, and backwater habitats with predominately clay, silt, sand, and gravel substrates (Table 2). Habitats ranged from slack to swift waters in shallow depths and from fresh to brackish waters. Minimum water temperature was 16.6°C during surveys (Table 3). Only one mussel (Pondhorn) was taken from the Upper Brazos River (Table 4) occurring in 1.7% of total habitats sampled (Table 5). Six species, including non-native zebra mussel, and 358 individuals were taken from the Little River. Most abundant mussels were Smooth Pimpleback (37% in relative abundance) followed by Threeridge (21%) and Tampico Pearlymussel (18%). Most wide spread mussels were Tampico Pearlymussel (occurred in 25% of total habitats sampled) followed by Threeridge (18%) and Smooth Pimpleback (18%). For the one candidate species, Smooth Pimpleback occurrences were associated with slack and shallow waters and at low to moderate minimum

bottom shear stress and FST hemispheres (Figure 2). Smooth Pimpleback occurrences were in pool edges (45%), pool main channel (18%), run edge (18%), and run main channel (18%) and habitats having predominantly silt (57%), sand (21%), and gravel (13%) substrates. Smooth Pimpleback had a positive association with pool edge habitats and silt substrates.

Within the upper Guadalupe River, 60 mesohabitats from 10 sites consisted of riffle, run, pool, and backwater habitats with predominately silt, gravel, cobble, and bedrock substrates. Minimum water temperature was 17.7°C during surveys. Five species and 47 individuals were taken from the upper Guadalupe River. Most abundant mussels were Texas Fatmucket (34% in relative abundance) followed by Texas Lilliput (28%) and Texas Pimpleback (21%). Most wide spread mussels were Texas Fatmucket (occurred in 10% of total habitats sampled) followed by Texas Pimpleback (6.7%) and Texas Lilliput (6.7%). Texas Fatmucket and Texas Pimpleback occurrences were associated with flowing waters at shallower than average depths and low to moderate minimum bottom shear stress and FST hemispheres (Figures 3, 4, 5). Texas Fatmucket occurrences were in run edges (50%), riffles (17%), run main channel (17%), and pool edges (17%) and habitats having predominately gravel (31%) and bedrock (29%) substrates. Texas Fatmucket had a positive association with run edge habitats and bedrock substrates (Figure 6). Texas Pimpleback occurrences were in run edges (50%), riffle (25%), and pool edges (25%) and habitats having predominately bedrock (35%), gravel (25%), and cobble (13%) substrates. Texas Pimpleback had a positive association with run edge habitats and bedrock substrates.

Within the Colorado River basin, 527 mesohabitats from 84 sites consisted of riffle, run, pool, and backwater habitats with predominately silt, sand, gravel, cobble, boulder, and bedrock substrates (Table 6). Minimum water temperature was 22.3°C during surveys (Table 7). Sixteen species and 2,819 individuals were taken from the Colorado River (Table 8) with mussels

occurring in 25 to 87% of the habitats sampled by georegion (Table 9). Habitats explained 31% ($P < 0.01$) of the mussel community variation (Figure 7). Physical parameters and mesohabitats with strong loadings were specific conductance (bi-plot score: 0.80), pool channel (0.39), gravel (0.38), bottom current velocity (-0.36), run edge (-0.44), and sand (-0.50) on CCA axis I and gravel (0.61), cobble (0.53), minimum bottom shear stress (0.50), pool edge (-0.44), and silt (-0.65) on CCA axis II. Among candidate mussels associated with CCA I and II, Smooth Pimpleback (N = 400) was associated with lower specific conductance and run habitats with sandy substrates and swifter current velocities. Texas Pimpleback (N = 127) was associated with run habitats, swifter current velocities, and gravel and cobble substrates. Texas Fawnsfoot (N = 9) was associated with run edges with clay, silt, and sandy substrates.

Abundances and occurrences of mussels differed among georegions. Georegion 1 consisted of 10 species with Fragile Papershell (24% in relative abundance), Southern Mapleleaf (22%), and Tampico Pearlymussel (16%) being most abundant and Southern Mapleleaf (9.8% occurrence among habitats sampled), Yellow Sandshell (9.0%), Bleufer (6.8%), and Paper Pondshell (6.8%) being most widespread. Relative abundances of candidate mussels were 2.4% for Texas Pimpleback (ranked 8th most abundant) and 1.2% for Smooth Pimpleback (ranked 9th). Both were taken in 1.5% of total habitats sampled. Georegion 3 consisted of 11 species with Texas Pimpleback (37% in relative abundance), Pistolgrip (21%), and Southern Mapleleaf (6.5%) being most abundant and Texas Pimpleback (7.1% occurrence among habitats sampled), Tampico Pearlymussel (6.2%), and Southern Mapleleaf (6.2%) being most widespread. Relative abundances of other candidate mussels were 2.7% for Smooth Pimpleback (ranked 6th) and 0.9% for Texas Fatmucket (ranked 11th). Georegion 4 consisted of seven species with Yellow Sandshell (33% in relative abundance), Giant Floater (33%), and Paper Pondshell (15%) being

most abundant and Tampico Pearlymussel (5.6% occurrence among habitats sampled) and Yellow Sandshell (5.6%) being more widespread. Candidate mussels were not taken from Georegion 4. Georegion 5 consisted of 14 species with Threeridge (58% in relative abundance), Smooth Pimpleback (17%), and Yellow Sandshell (17%) being most abundant and Threeridge (18% occurrence among habitats sampled), Yellow Sandshell (18%), and Smooth Pimpleback (15%) being most widespread. Relative abundances of candidate mussels were 1.3% for Texas Pimpleback (ranked 7^{th}) and 0.4% for Texas Fawnsfoot (ranked 9^{th}).

Univariate associations were assessed for candidate mussels across Colorado River basin georegions. Smooth Pimpleback, Texas Pimpleback, and Texas Fawnsfoot were taken from column and bottom current velocities at about the same current velocities available (Figure 8). Smooth Pimpleback, Texas Pimpleback, and Texas Fawnsfoot occurred proportionally less at shallowest depths (<0.5 m) than available and occurred at the lower range of substrate compaction (Figure 9). Smooth Pimpleback and Texas Pimpleback generally occurred in proportion to available minimum bottom shear stress and FST hemispheres, although low occurrence were observed at the greater ends of minimum bottom shear stress (>5 dyn/cm^2) and FST hemispheres (>10) (Figure 10). Smooth Pimpleback occurrences were in run edges (37%), pool edges (22%), pool main channel (17%), run main channel (15%), backwater (7.3%), and riffle (2.4%) mesohabitats. Smooth Pimpleback occurred more in run edge mesohabitats than expected (Figure 11), although the largest number of individuals taken (N = 104) was from a run main channel mesohabitat. Texas Pimpleback occurrences were in pool main channel (31%), run main channel (23%), run edge (18%), pool edge (14%), backwater (9.0%), and riffle (4.5%) mesohabitats. Texas Pimpleback occurred more in pool main channel, run main channel, and run edge than expected, with the largest number of individuals taken (N = 54) from a run edge

mesohabitat. Texas Fawnsfoot occurrences were in run edges (63%), pool edge (25%), and backwater (13%) mesohabitats, occurring in more run edge habitats than expected. Dominant substrates (mean percent composition) of habitats were sand (33%), silt (24%), and boulder (12%) boulder for Smooth Pimpleback, gravel (21%), cobble (17%), and boulder (16%) for Texas Pimpleback, and silt (34%), sand (32%), clay (16%), and boulder (9%) for Texas Fawnsfoot. Generally, Smooth Pimpleback occurred in habitats with more sand and boulder than expected, Texas Pimpleback occurred in more boulder and less silt than expected, and Texas Fawnsfoot occurred in more clay, silt, sand, and boulder and in less gravel and cobble than expected.

Univariate estimates across regions and basins

Summaries of habitat parameters across regions and basins are provided for Smooth Pimpleback (Table 10), Texas Pimpleback (Table 11), Texas Fatmucket (Table 12), and Texas Fawnsfoot (Table 13). In addition, weighted mean (i.e., using relative abundances) habitat summaries were calculated for other species within mussel community across regions and basin. Texas Fatmucket, Pistolgrip, and Texas Pimpleback had the swiftest mean current velocities (column and bottom), whereas Tampico Pearlymussel, Bleufer, and Giant Floater had the lowest mean current velocities (Figure 12). Smooth Pimpleback, Threeridge, and Tampico Pearlymussel had the deepest mean depths, whereas Texas Fatmucket, Texas Pimpleback, and Pistolgrip had the shallowest mean depths (Figure 13). Fragile Papershell, Pistolgrip, Paper Pondshell, Bleufer, and Giant Floater had the greater substrate compaction variability, whereas the remaining species had lesser substrate compaction variability. Relative abundances of mussels ranged from absent to abundant among all mesohabitats (Table 14). Most species (S =

15) were taken pool-edge mesohabitats (S=15), ranging in relative abundance scale (Stiers et al. 2011) from occasional to abundant, and fewest species (S=10) were taken from run-channel, ranging in relative abundant scale from rare to frequent. Majority of species (75%) were taken from habitats consisting of all substrate types (Table 15). Mean percent silt was occasional to common for all (S = 16) species. Mean percent sand, gravel, and cobble were rare to frequent for all species. Mean percent boulder and bedrock were rare to frequent for 15 species. Mean percent clay was rare to occasional for 14 species. Mean percent detritus was rare to occasional for 13 species.

Georegion 5 Pre-hurricane community compared to post hurricane community

In August 2017, Hurricane Harvey caused widespread flooding and high flows (>140,000 cfs) in the lower Colorado River. Previous to high flows, 179 mesohabitats were sampled in Georegion 5 (Figure 14). Pre-hurricane community consisted 14 species with Threeridge (59% in relative abundance), Yellow Sandshell (17%), and Smooth Pimpleback (16%) being most abundant. After high flows subsided, 66 mesohabitats were sampled in Georegion 5. Post hurricane community consisted of 11 species with Threeridge (53%), Smooth Pimpleback (21%), and Yellow Sandshell (17%) being most abundant. Changes in relative abundances between pre- and post-hurricane ranged from -5.9% for Threeridge to 4.7% for Smooth Pimpleback.

Part II. Tables

Table 1. Surface geology, groundwater sources, stream gradient, and water quality and quantity estimates among georegions of the Colorado River basin.

	Georegions				
	1	2	3	4	5
Descriptive name	Prairie	Edwards Plateau	Llano Uplift	Balcones	Lowland
Period[1]	Carboniferous, Permian, Triassic	Cretaceous	Pre-Cambrian, Cambrian	Cretaceous	Tertiary
Surface strata[1]	clay, sand, shale, sandstone, siltstone, mudstone, limestone, dolomite, gypsum, colluvium	limestone, colluvium, dolomite, chalk, marl, mudstone	Granite, gneiss, schist, limestone, colluvium, dolomite	limestone, chalk, marl, dolomite, mudstone, clay, alluvial shale	clay, silt, sand, sandstone
Ecoregions[2]	Southwest Tablelands, Central Great Plains, Cross Timbers	Semiarid Edwards Plateau, Edwards Plateau Woodland	Llano Uplift, Edwards Plateau Woodland	Balcones Canyonlands, Texas Blackland Prairie	Texas Blackland Prairie, Central Texas Plains, Western Gulf Coast Plains
Aquifer type[3]	alluvium	karst	karst	karst	alluvium
Aquifers[3]	Ogallala, Dockum, Lipan	Edwards-Trinity	Marble Falls, Ellenburger-San Saba	Edwards-Trinity	Carrizo-Wilcox, Gulf Coast
Groundwater type[3]	fresh to saline	fresh, some brackish	fresh, some brackish	fresh	fresh
Dominant substrates	sand and silt	gravel to bedrock	silt to boulder	silt to gravel in mainstem, limestone in tributaries	silt and sand with some boulders and bedrock
Mean stream gradient (m/km)	0.56	1.70	1.60	1.00	0.27

Table 1. Continued

	Georegions				
	1	2	3	4	5
Descriptive name	Prairie	Edwards Plateau	Llano Uplift	Balcones	Lowland
Water quantity[4]:					
N of stations	9	8	3	5	4
Average flow (cfs)	103	59	274	366	2,297
Coefficient of variation	5.6	7.5	5.5	4.6	2.1
% zero flow days	4.7	15.9	1.4	19.9	0.0
% of stations with zero flow days	56	44	33	20	0
Base flow index	0.08	0.23	0.16	0.04	0.23
Water quality[5]:					
Mean dissolved oxygen (mg/l)	9.0	7.9	8.3	8.7	8.5
1 SD	3.55	1.98	2.33	2.27	1.90
Median pH	8.0	8.0	8.2	8.0	8.1
range of pH	3.89	2.40	3.25	2.78	2.90
Mean specific conductance (µS/cm)	4,030	741	653	556	588
1 SD	4,369.8	390.8	348.9	111.4	199.7
Mean water temperature (°C)	19.5	19.9	20.3	21.1	21.9
1 SD	7.95	6.78	6.90	6.33	6.68
Mean turbidity (NTU)	55.5	19.4	22.1	10.9	50.5
1 SD	49.00	75.43	28.71	22.15	73.55

[1] Source: USGS Texas Geology https://txpub.usgs.gov/dss/texasgeology/
[2] Source: Griffith et al. 2007. Ecoregions of Texas.
[3] Source: Texas Aquifers; https://www.twdb.texas.gov/groundwater/aquifer/
[4] Source: USGS Stations-Colorado River basin; https://waterdata.usgs.gov/tx/nwis/current/?type=flow
[5] Source: LCRA Water Quality Data; waterquality.lcra.org; period of record: 1980 – 2016.

Table 2. Total number, percent, and physical characterizations of habitats sampled in the Brazos River and Guadalupe River basins from March through October 2017.

	Brazos River				Guadalupe River	
	Upper		Little River		Upper	
N of habitats	60		60		60	
Habitat types (%)						
Riffle	5		17		17	
Run-channel	16		23		15	
Run-edge	21		13		17	
Pool-channel	18		12		17	
Pool-edge	34		32		22	
Backwater	5		3		13	
	Mean	1 SD	Mean	1 SD	Mean	1 SD
Depth (m)	0.94	0.59	0.76	0.52	0.83	0.55
Current velocity column (m/s)	0.14	0.19	0.25	0.32	0.32	0.36
Current velocity bottom (m/s)	0.07	0.14	0.12	0.17	0.13	0.17
Penetrometer (kg/cm^2)	0.24	0.22	0.27	0.98	0.43	1.12
FST hemispheres	1.67	2.26	3.37	4.54	4.68	5.26
Minimum bottom shear stress (dyn/cm^2)	1.14	0.91	3.29	6.64	6.57	15.04
Specific conductance (uS/cm)	2,747	1,461	631	143	479	56
Substrate (%)						
Clay	15	21	14	28	1	5
Silt	24	32	32	37	22	23
Sand	34	33	25	23	7	12
Gravel	15	20	23	26	24	20
Cobble	6	15	5	11	22	24
Boulder	4	15	0.3	1	4	9
Bedrock	2	7			18	25
Detritus	1	3	1	3	1	3
Large woody debris (%)	5	12	8	15	3	9
Undercut bank (%)	0.2	1	5	15	3	11
Root wad (%)	0.2	1			11	23

Table 2 continued

	Brazos River		Guadalupe River	
	Upper	Little River	Upper	
Vegetation (%)	0	0	4	14
Chara				
Ceratophyllum			0.2	1
Filamentous Algae			2	10
Hydrilla				
Justicia			1	2
Nuphar			0.2	1
Potamogeton			1	6
Heteranthera				

Table 3. Water quality parameters (mean for temperature, dissolved oxygen, and turbidity, median for pH) for mesohabitats sampled in the Brazos River and Guadalupe River basins from March through October 2017.

		Temperature (°C)	Dissolved oxygen (mg/L)	pH	Turbidity (NTU)
Brazos River	Central tendency	29.7	8.6	8.2	44.7
Upper	1 SD	1.63	1.96		25.54
	Minimum	25.8	4.8	7.2	3.8
	Maximum	32.2	13.4	9.6	110.4
Little River	Central tendency	29.0	9.2	8.1	54.7
	1 SD	5.21	1.90		49.83
	Minimum	16.6	2.2	7.5	24.7
	Maximum	32.8	12.3	9.4	200.2
Guadalupe River	Central tendency	23.0	8.5	8.3	
Upper	1 SD	4.06	0.91		
	Minimum	17.7	5.0	7.2	
	Maximum	30.2	10.1	11.0	

Table 4. Mussel species and relative abundances (% of total N) taken from the Brazos River and Guadalupe River basins from March through October 2017.

		Abundance (%)		
		Brazos River		Guadalupe River
Scientific Name	Common Name	Upper	Little River	Upper
Amblema plicata	Threeridge		21	
Cyclonaias houstonensis	Smooth Pimpleback		37	
Cyclonaias petrina	Texas Pimpleback			21
Cyrtonaias tampicoensis	Tampico Pearlymussel		18	
Dreissena polymorpha	Zebra Mussel		11	
Lampsilis bracteata	Texas Fatmucket			34
Lampsilis teres	Yellow Sandshell		11	
Leptodea fragilis	Fragile Papershell		1.7	
Toxolasma parvum	Lilliput			2.1
Toxolasma texasiense	Texas Lilliput			28
Uniomerus tetralasmus	Pondhorn	100		15
	Total N	1	358	47

Table 5. Mussel species and occurrences (% of total habitats sampled) taken from the Brazos River and Guadalupe River basins from March through October 2017.

		Occurrence (%)		
		Brazos River		Guadalupe River
Scientific Name	Common Name	Upper	Little River	Upper
Amblema plicata	Threeridge		18	
Cyclonaias houstonensis	Smooth Pimpleback		18	
Cyclonaias petrina	Texas Pimpleback			6.7
Cyrtonaias tampicoensis	Tampico Pearlymussel		25	
Dreissena polymorpha	Zebra Mussel		6.7	
Lampsilis bracteata	Texas Fatmucket			10
Lampsilis teres	Yellow Sandshell		12	
Leptodea fragilis	Fragile Papershell		5.0	
Toxolasma parvum	Lilliput			1.7
Toxolasma texasiense	Texas Lilliput			6.7
Uniomerus tetralasmus	Pondhorn	1.7		3.3
	Total N of habitats	60	60	60
	Total % of habitats	1.7	78	28

Table 6. Total number, percent, and physical characterizations of habitats sampled within georegions of the Colorado River basin March through October 2017.

	Colorado River							
	Georegion 1		Georegion 3		Georegion 4		Georegion 5	
N of habitats	132		114		36		245	
Habitat types (%)								
Riffle	11		15		19		11	
Run-channel	12		12		28		20	
Run-edge	6		12		19		22	
Pool-channel	25		26		6		15	
Pool-edge	37		27		22		22	
Backwater	10		8		6		11	
	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD
Depth (m)	0.47	0.30	0.48	0.37	0.47	0.25	0.64	0.46
Current velocity column (m/s)	0.10	0.18	0.14	0.25	0.33	0.32	0.23	0.27
Current velocity bottom (m/s)	0.04	0.08	0.07	0.12	0.19	0.21	0.12	0.15
Penetrometer (kg/cm^2)	0.36	1.07	0.63	1.42	0.14	0.15	0.12	0.33
FST hemispheres	1.54	2.62	1.98	3.22	4.25	4.05	2.86	3.33
Minimum bottom shear stress (dyn/cm^2)	1.24	1.47	1.62	2.64	2.65	2.68	1.82	2.77
Specific conductance (uS/cm)	992	365	600	101	561	64	597	45
Substrate (%)								
Clay	3	10	7	17	3	12	5	15
Silt	26	28	23	27	16	30	21	28
Sand	9	15	6	11	30	23	38	30
Gravel	23	24	16	20	37	28	19	22
Cobble	26	25	23	27	14	25	9	18
Boulder	7	20	5	14			3	14
Bedrock	3	14	15	28			3	12
Detritus	3	9	5	13			1	5
Large woody debris (%)	3	10	4	11	1	2	3	7
Undercut bank (%)	1	5	1	4			1	5
Root wad (%)	<0.1	0.1	<0.1	0.2			0.4	2

Table 6 continued

	Georegion 1		Georegion 3		Georegion 4		Georegion 5	
Vegetation (%)	5	19	1	5	3	9	1	4
Chara	2	12						
Ceratophyllum								
Filamentous Algae	2	11	0.5	5				3
Hydrilla						1	0.1	1
Justicia								
Nuphar								
Potamogeton								
Heteranthera	1	10			3	9	0.4	3

Table 7. Water quality parameters (mean for temperature, dissolved oxygen, and turbidity, median for pH) for mesohabitats sampled within georegions of the Colorado River basin March through October 2017.

		Temperature (°C)	Dissolved oxygen (mg/L)	pH	Turbidity (NTU)
Georegion 1	Central tendency	28.7	8.0	8.3	96.2
	1 SD	2.52	2.01		51.67
	Minimum	24.3	3.6	7.2	19.5
	Maximum	34.5	14.1	10.9	152.2
Georegion 3	Central tendency	28.0	8.5	8.3	76.4
	1 SD	2.52	1.57		44.56
	Minimum	22.3	5.4	6.9	20.6
	Maximum	32.5	12.8	10.2	152.2
Georegion 4	Central tendency	26.9	8.8	7.8	11.7*
	1 SD	2.65	11.04		25.78
	Minimum	23.4	5.2	7.6	0.49
	Maximum	34.4	73.1	7.9	339
Georegion 5	Central tendency	28.8	9.0	8.1	33.9
	1 SD	2.75	3.14		40.60
	Minimum	22.7	5.7	6.9	1.3
	Maximum	35.1	41.0	11.4	309.3

*Estimates obtained from waterquality.lcra.org, Site 12474 and Site 12466 (downstream of Lady Bird Lake to Webberville; period of record: 1998 – 2016).

Table 8. Mussel species and relative abundances (% of total N) taken from georegions within the Colorado River basin from March through October 2017.

		Abundance (%)			
Scientific Name	Common Name	Georegion 1	Georegion 3	Georegion 4	Georegion 5
Amblema plicata	Threeridge		2.0		58
Cyclonaias houstonensis	Smooth Pimpleback	1.2	4.0		17
Cyclonaias petrina	Texas Pimpleback	2.4	37		1.3
Cyrtonaias tampicoensis	Tampico Pearlymussel	16	4.5	7.4	1.5
Lampsilis bracteata	Texas Fatmucket		0.8		
Lampsilis teres	Yellow Sandshell	11		33	17
Leptodea fragilis	Fragile Papershell	24	15	3.7	2.7
Potamilus purpuratus	Bleufer	4.5	3.6		0.7
Pyganodon grandis	Giant Floater	11	2.8	33	0.3
Quadrula apiculata	Southern Mapleleaf	22	6.5	3.7	0.2
Toxolasma parvum	Lilliput			3.7	0.1
Toxolasma texasiense	Texas Lilliput				1.5
Tritogonia verrucosa	Pistolgrip	0.8	21		
Truncilla macrodon	Texas Fawnsfoot				0.4
Uniomerus tetralasmus	Pondhorn				0.04
Utterbackia imbecillis	Paper Pondshell	7.8	2.8	15	0.1
	Total N	245	247	27	2,300

Table 9. Mussel species and occurrences (% of total habitats sampled) taken from georegions within the Colorado River basin from March through October 2017.

		Occurrence (%)			
Scientific Name	Common Name	Georegion 1	Georegion 3	Georegion 4	Georegion 5
Amblema plicata	Threeridge		1.8		18
Cyclonaias houstonensis	Smooth Pimpleback	1.5	2.7		15
Cyclonaias petrina	Texas Pimpleback	1.5	7.1		4.9
Cyrtonaias tampicoensis	Tampico Pearlymussel	6.0	6.2	5.6	4.9
Lampsilis bracteata	Texas Fatmucket		0.9		
Lampsilis teres	Yellow Sandshell	9.0		5.6	18
Leptodea fragilis	Fragile Papershell	13	21	2.8	11
Potamilus purpuratus	Bleufer	6.8	4.4		2.4
Pyganodon grandis	Giant Floater	12	4.4	2.8	1.6
Quadrula apiculata	Southern Mapleleaf	9.8	6.2	2.8	1.6
Toxolasma parvum	Lilliput			2.8	0.8
Toxolasma texasiense	Texas Lilliput				2.9
Tritogonia verrucosa	Pistolgrip	1.5	15		
Truncilla macrodon	Texas Fawnsfoot				3.3
Uniomerus tetralasmus	Pondhorn				0.4
Utterbackia imbecillis	Paper Pondshell	6.8	5.3	2.8	1.2
	Total N of habitats	132	114	36	245
	Total % of habitats	68	75	25	87

Table 10. Summary of parameters in habitats with Smooth Pimpleback.

		Little River	Colorado River			
			Overall	Georegion 1	Georegion 3	Georegion 5
N of habitats		11	41	2	3	36
N of individuals		133	400	3	10	387
Current velocity column (m/s)	Mean	0.15	0.13	0.02	0.26	0.13
	SD	0.30	0.17	0.03	0.06	0.18
Current velocity bottom (m/s)	Mean	0.09	0.08	0.01	0.15	0.08
	SD	0.19	0.11	0.01	0.09	0.12
Depth (m)	Mean	0.74	0.73	0.99	0.74	0.71
	SD	0.70	0.67	0.75	0.94	0.66
Penetrometer (kg/cm^2)	Mean	0.02	0.06	0.07	0.29	0.04
	SD	0.01	0.11	0.01	0.31	0.06
Shear stress (dyn/cm^2)	Mean	1.15	1.28	0.77	2.35	1.22
	SD	0.95	0.82	0.00	1.50	0.73
FST hemispheres	Mean	1.40	2.27	0.00	5.33	2.14
	SD	2.60	2.61	0.00	3.06	2.47
Substrate (%)	Clay	4	7	5		7
	Silt	57	24	30		26
	Sand	21	33	10	13	36
	Gravel	13	10	35	20	8
	Cobble	2	8	15	60	3
	Boulder	1	12		7	13
	Bedrock		5			6
	Detritus	2	1			1
Habitat types (%)	Riffle		2			3
	Run-channel	18	15		67	11
	Run-edge	18	37		33	39
	Pool-channel	18	17	100		14
	Pool-edge	45	22			25
	Backwater		7			8

Table 11. Summary of parameters in habitats with Texas Pimpleback.

		Guadalupe River	Colorado River			
			Overall	Georegion 1	Georegion 3	Georegion 5
N of habitats		4	22	2	8	12
N of individuals		10	127	6	91	30
Current velocity column (m/s)	Mean	0.17	0.17	0.04	0.19	0.17
	SD	0.07	0.20	0.01	0.14	0.25
Current velocity bottom (m/s)	Mean	0.09	0.09	0.01	0.12	0.09
	SD	0.07	0.13	0.01	0.09	0.15
Depth (m)	Mean	0.40	0.76	0.91	0.34	1.02
	SD	0.15	0.82	0.65	0.16	1.01
Penetrometer (kg/cm^2)	Mean	0.08	0.08	0.16	0.11	0.04
	SD	0.04	0.07	0.12	0.08	0.04
Shear stress (dyn/cm^2)	Mean	1.49	1.36	0.77	1.56	1.32
	SD	0.91	0.97	0.00	1.08	0.98
FST hemispheres	Mean	3.30	2.45	0.00	3.25	2.33
	SD	3.30	2.76		2.96	2.71
Substrate (%)	Clay		3			5
	Silt	15	12	20	9	13
	Sand	10	25	20	19	30
	Gravel	25	22	40	33	12
	Cobble	13	18	15	38	6
	Boulder		15	5		28
	Bedrock	35	5		1	9
	Detritus	3				
Habitat types (%)	Riffle	25	5			8
	Run-channel		23		38	17
	Run-edge	50	18		25	17
	Pool-channel		32	100	13	33
	Pool-edge	25	14			25
	Backwater		9		25	

Table 12. Summary of parameters in habitats with Texas Fatmucket.

		Guadalupe River	Colorado River Georegion 3
N of habitats		6	1
N of individuals		16	2
Current velocity column (m/s)	Mean	0.28	0.00
	SD	0.18	
Current velocity bottom (m/s)	Mean	0.15	0.00
	SD	0.14	
Depth (m)	Mean	0.43	0.40
	SD	0.26	
Penetrometer (kg/cm^2)	Mean	0.05	0.00
	SD	0.04	
Shear stress (dyn/cm^2)	Mean	1.65	0.77
	SD	0.89	
FST hemispheres	Mean	3.83	0.00
	SD	2.99	
Substrate (%)	Clay		
	Silt	13	50
	Sand	7	
	Gravel	31	
	Cobble	16	
	Boulder	3	50
	Bedrock	29	
	Detritus	2	
Habitat types (%)	Riffle	17	
	Run-channel	17	
	Run-edge	50	
	Pool-channel		100
	Pool-edge	17	
	Backwater	0	

Table 13. Summary of parameters in habitats with Texas Fawnsfoot.

		Colorado River Georegion 3
N of habitats		8
N of individuals		9
Current velocity column (m/s)	Mean	0.10
	SD	0.15
Current velocity bottom (m/s)	Mean	0.06
	SD	0.11
Depth (m)	Mean	0.52
	SD	0.17
Penetrometer (kg/cm^2)	Mean	0.05
	SD	0.07
Shear stress (dyn/cm^2)	Mean	0.97
	SD	0.49
FST hemispheres	Mean	1.13
	SD	2.03
Substrate (%)	Clay	16
	Silt	34
	Sand	32
	Gravel	8
	Cobble	1
	Boulder	9
	Bedrock	0
	Detritus	
Habitat types (%)	Riffle	0
	Run-channel	0
	Run-edge	63
	Pool-channel	0
	Pool-edge	25
	Backwater	13

Table 14. Mesohabitat associations by species using ACFOR scale (Stiers et al. 2011): Abundant (75 – 100% in a species relative abundance), Common (50 – 74%), Frequent (25 – 49%), Occasional (5 – 24%), and Rare (>0 – 4%). For example, Threeridge were taken rarely from riffle, occasionally from run-channel, pool-edge, and backwater, and frequently taken from run-edge and pool-channel habitats. Blank represents a species was not found in the mesohabitat.

Species	N	Riffle	Run-channel	Run-edge	Pool-channel	Pool-edge	Backwater
Threeridge	1405	rare	occasional	frequent	frequent	occasional	occasional
Smooth Pimpleback	533	rare	frequent	occasional	frequent	occasional	rare
Yellow Sandshell	461	rare	rare	frequent	occasional	frequent	occasional
Fragile Papershell	166	rare	rare	frequent	occasional	frequent	occasional
Tampico Pearlymussel	152	rare	rare	rare	frequent	frequent	occasional
Texas Pimpleback	137	rare	occasional	common	occasional	occasional	occasional
Southern Mapleleaf	75	occasional	occasional	rare	abundant	occasional	rare
Pistolgrip	54	occasional	occasional	frequent	occasional	occasional	occasional
Giant Floater	49			rare	occasional	common	occasional
Texas Lilliput	47	occasional		occasional	occasional	common	occasional
Bleufer	35				frequent	common	occasional
Paper Pondshell	33		occasional	occasional	occasional	frequent	occasional
Texas Fatmucket	18	occasional	occasional	common	occasional	occasional	
Texas Fawnsfoot	9			common		frequent	occasional
Pondhorn	9			abundant			occasional
Lilliput	4	frequent				abundant	

Table 15. Species-substrate associations using ACFOR scale (Stiers et al. 2011): Abundant (75 – 100% mean percent substrate), Common (50 – 74%), Frequent (25 – 49%), Occasional (5 – 24%), and Rare (>0 – 4%). For example, Threeridge were taken from substrates comprised, on average, rarely of cobble and detritus and occasionally of clay, silt, sand, gravel, boulder, and bedrock. Blank represents a substrate type where a species was not found.

Species	N	Clay	Silt	Sand	Gravel	Cobble	Boulder	Bedrock	Detritus
Threeridge	1405	occasional	occasional	occasional	occasional	rare	occasional	occasional	rare
Smooth Pimpleback	533	rare	frequent	frequent	occasional	occasional	occasional	rare	rare
Yellow Sandshell	461	occasional	frequent	frequent	rare	rare	occasional	rare	rare
Fragile Papershell	166	occasional	occasional	occasional	occasional	occasional	occasional	rare	rare
Tampico Pearlymussel	152	rare	frequent	occasional	occasional	occasional	occasional	rare	rare
Texas Pimpleback	137	rare	occasional	frequent	occasional	occasional	occasional	rare	rare
Southern Mapleleaf	75	rare	occasional	occasional	frequent	occasional	occasional	rare	rare
Pistolgrip	54	rare	occasional	occasional	occasional	frequent	occasional	occasional	rare
Giant Floater	49	occasional	common	occasional	occasional	occasional	occasional	rare	occasional
Texas Lilliput	47	rare	frequent	rare	occasional	rare	occasional	occasional	occasional
Bleufer	35	rare	frequent	frequent	occasional	occasional	occasional	rare	rare
Paper Pondshell	33	rare	frequent	rare	occasional	occasional	rare	occasional	occasional
Texas Fatmucket	18		occasional	rare	frequent	occasional	rare	frequent	rare
Texas Fawnsfoot	9	occasional	frequent	frequent	occasional	rare	occasional		
Pondhorn	9	rare	occasional	occasional	frequent	occasional		occasional	
Lilliput	4		common	rare	occasional	occasional	occasional	occasional	

Part II. Figures

Figure 1. Plot of principal components axes I and II for physical characters of mesohabitats taken from the Brazos, Guadalupe, and Colorado River basins taken from March through October 2017. Black circles represent mean and whiskers represent 1 SD of mesohabitat scores grouped by basin, reach, or georegion.

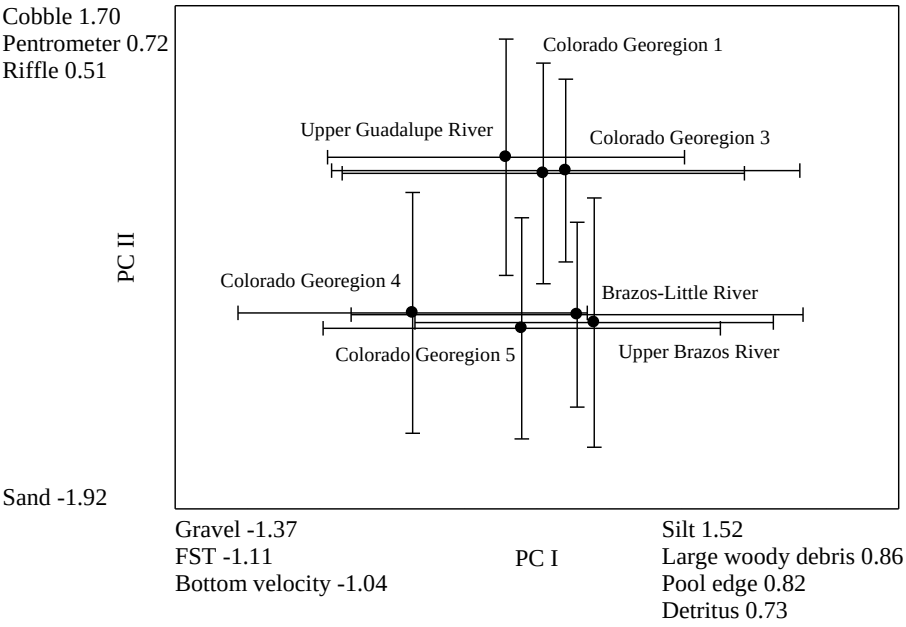


Figure 2. Percent occurrences of Smooth Pimpleback (gray bars) among multiple physical parameters taken from the Little River (Brazos River basin) from March through October 2017. Black line in each graph represents the percent of available parameter. Mesohabitat and substrate graphs plot percent observed minus percent expected to infer a positive or negative association.

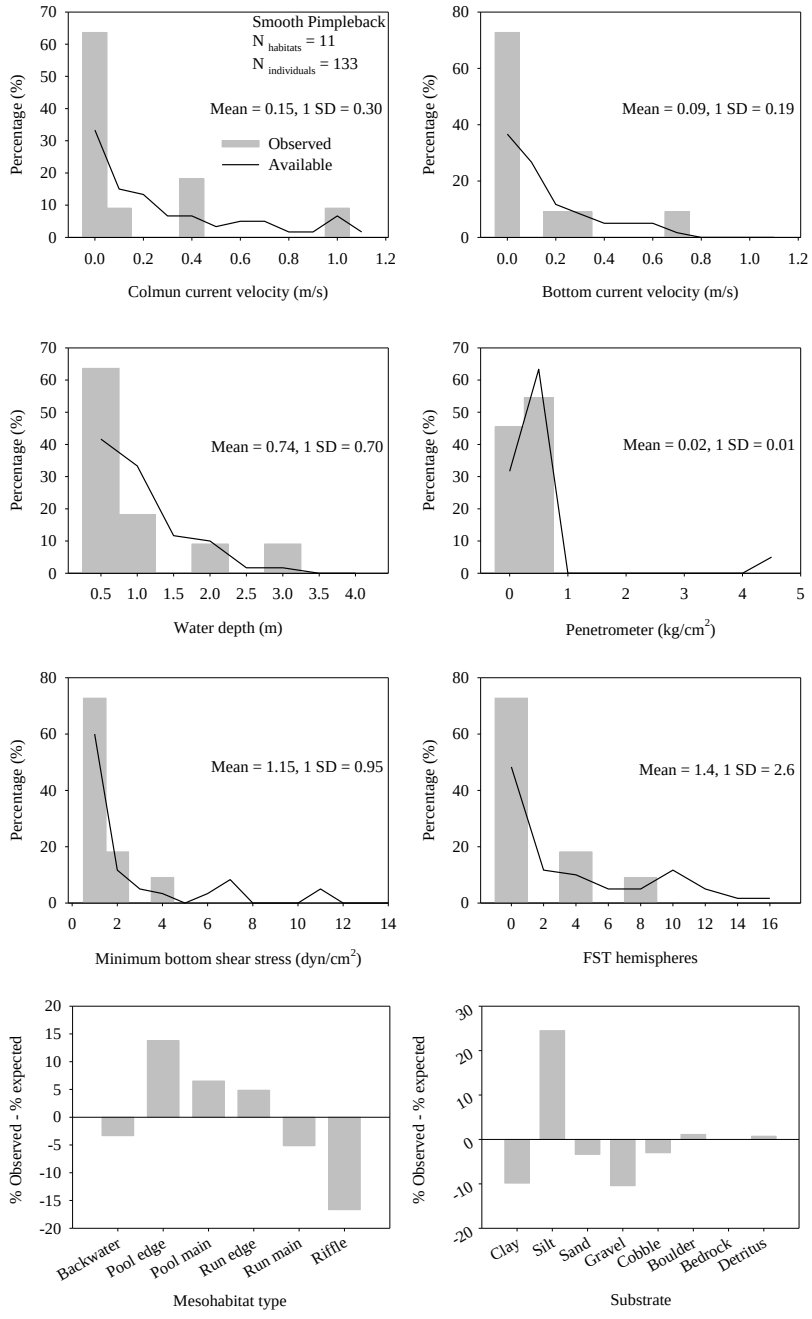


Figure 3. Percent occurrences of Texas Fatmucket and Texas Pimpleback (gray bars) by column current velocity and bottom current velocity taken from upper Guadalupe River from March through October 2017. Black line represents the percent of current velocities available. Bars above line suggest positive association and bars below line (or absent) suggest negative association.

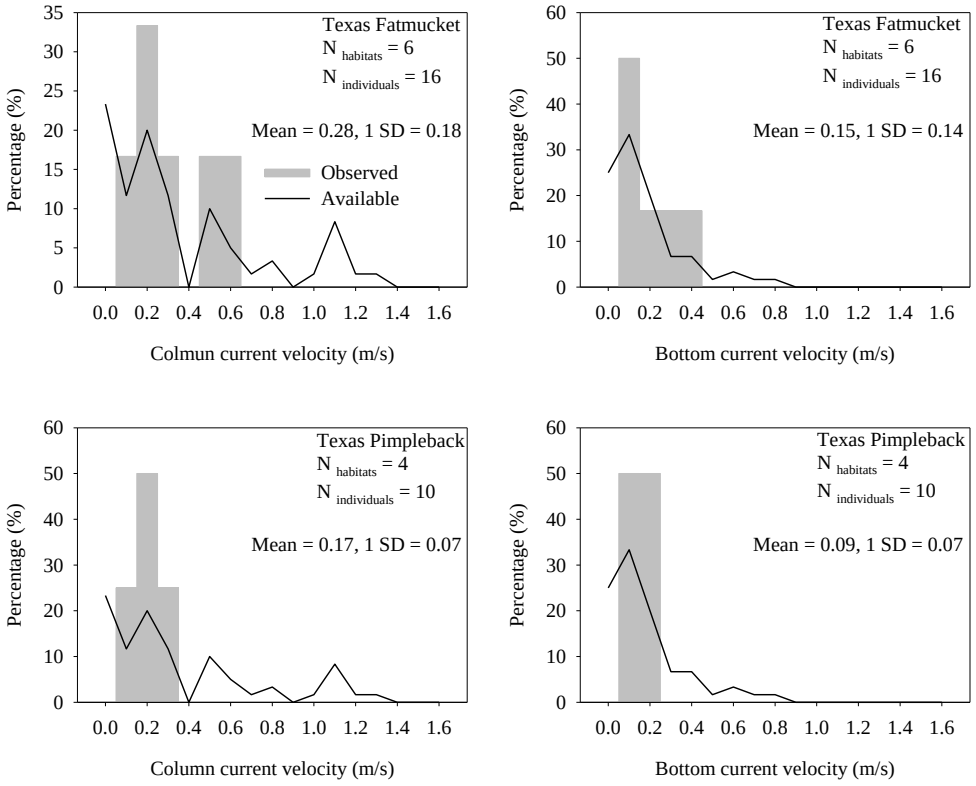


Figure 4. Percent occurrences of Texas Fatmucket and Texas Pimpleback (gray bars) by water depth and substrate penetrometer measurement taken from upper Guadalupe River from March through October 2017. Black line represents the percent of current velocities available. Bars above line suggest positive association and bars below line (or absent) suggest negative association.

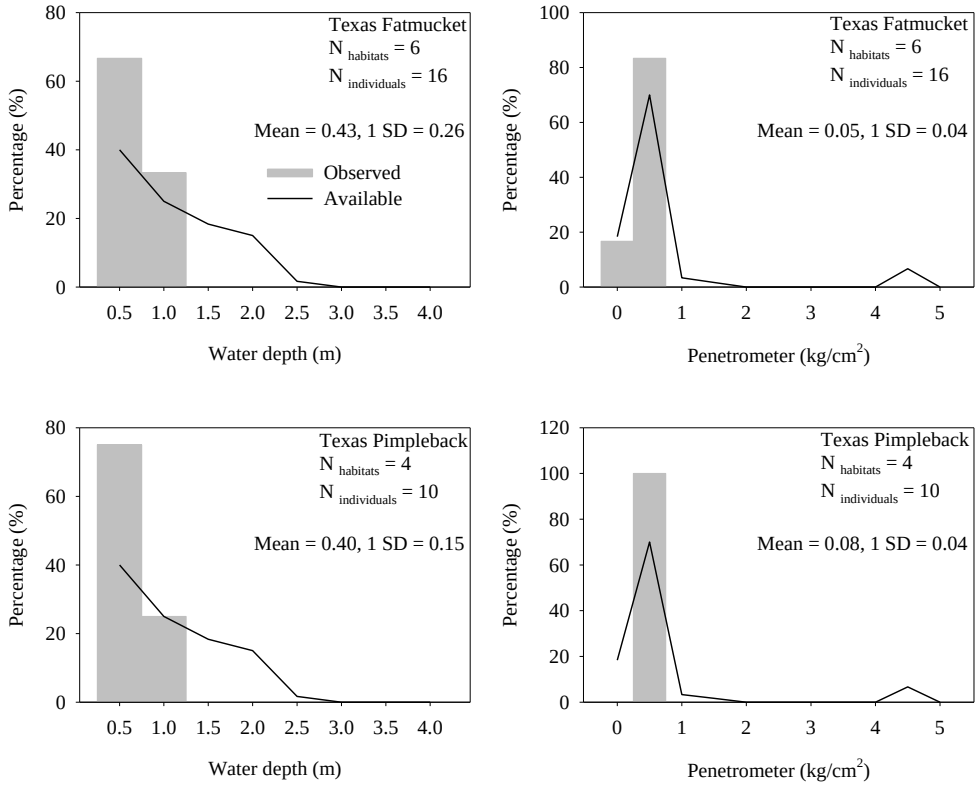


Figure 5. Percent occurrences of Texas Fatmucket and Texas Pimpleback (gray bars) by minimum bottom shear stress and FST hemispheres taken from upper Guadalupe River from March through October 2017. Black line represents the percent of current velocities available. Bars above line suggest positive association and bars below line (or absent) suggest negative association.

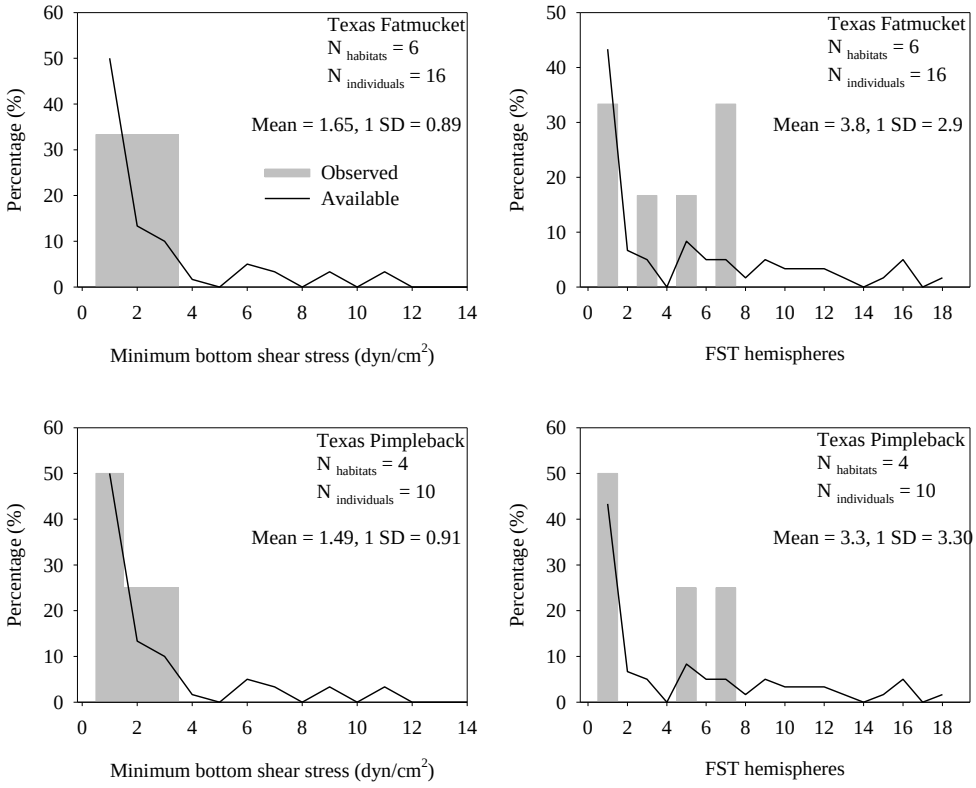


Figure 6. Differences in percent observed occurrences and percent available (i.e., expected) among mesohabitats and substrates for Texas Fatmucket and Texas Pimpleback from upper Guadalupe River from March through October 2017. Positive differences suggest positive association with a mesohabitat or substrate. Negative differences suggest negative association with a mesohabitat or substrate.

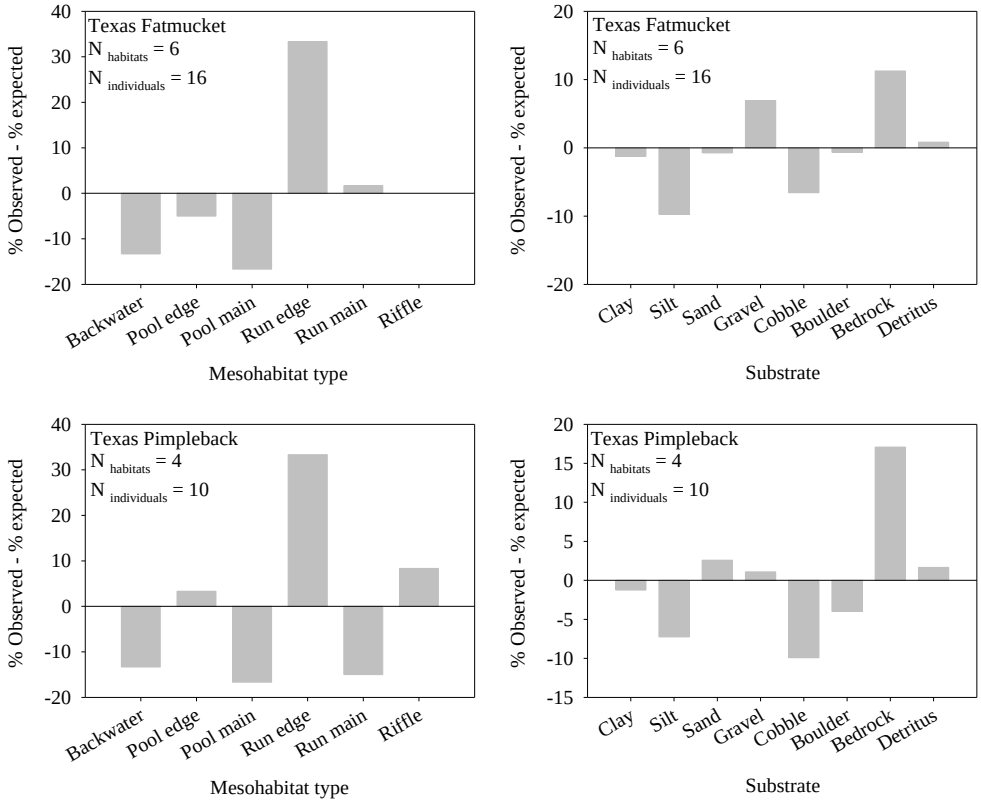


Figure 7. Plot of conical correspondence axes I and II for mesohabitats and their physical characters taken from the Colorado River basin from March through October 2017 (top panel). Arrow lengths indicate weight of mesohabitat and physical parameters along axes I and II. Centroid of species scores are represented by the first three letters of a species generic and specific epithets.

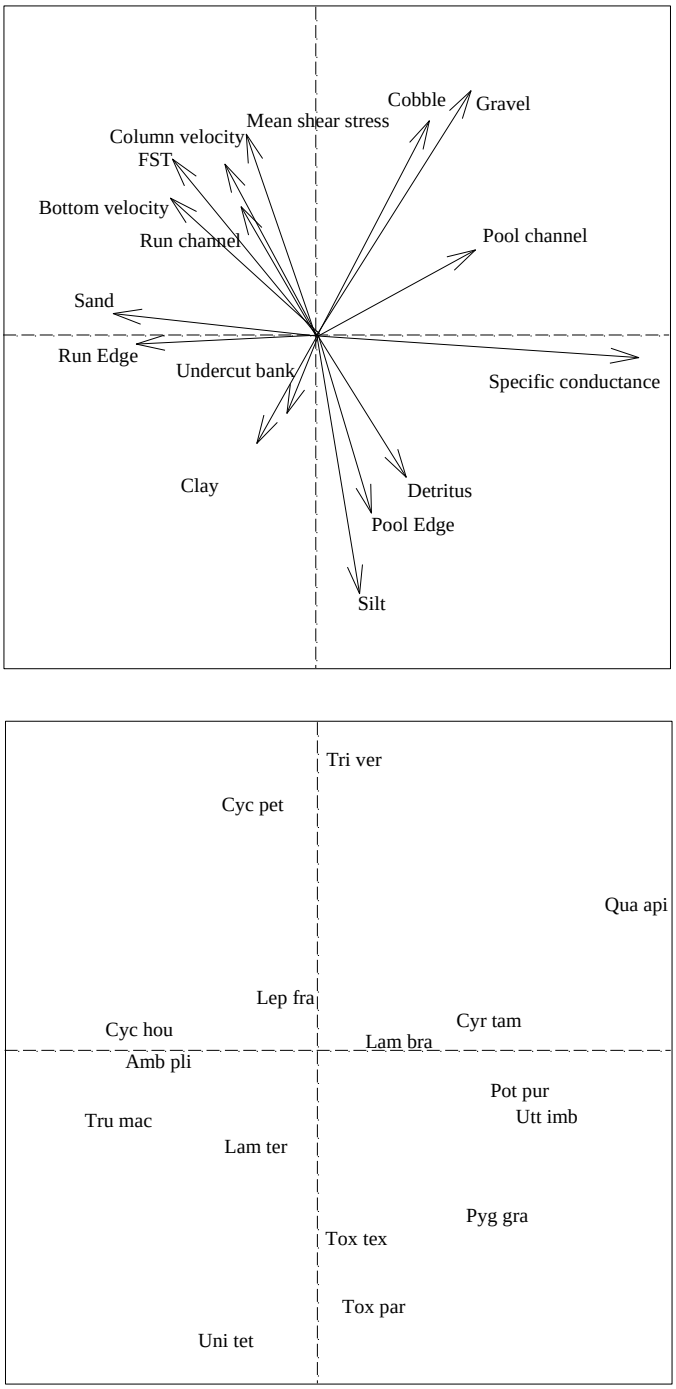


Figure 8. Percent occurrences of Smooth Pimpleback, Texas Pimpleback, and Texas Fawnsfoot (gray bars) by column current velocity and bottom current velocity taken from Colorado River from March through October 2017. Black line represents the percent of current velocities available. Bars above line suggest positive association and bars below line (or absent) suggest negative association.

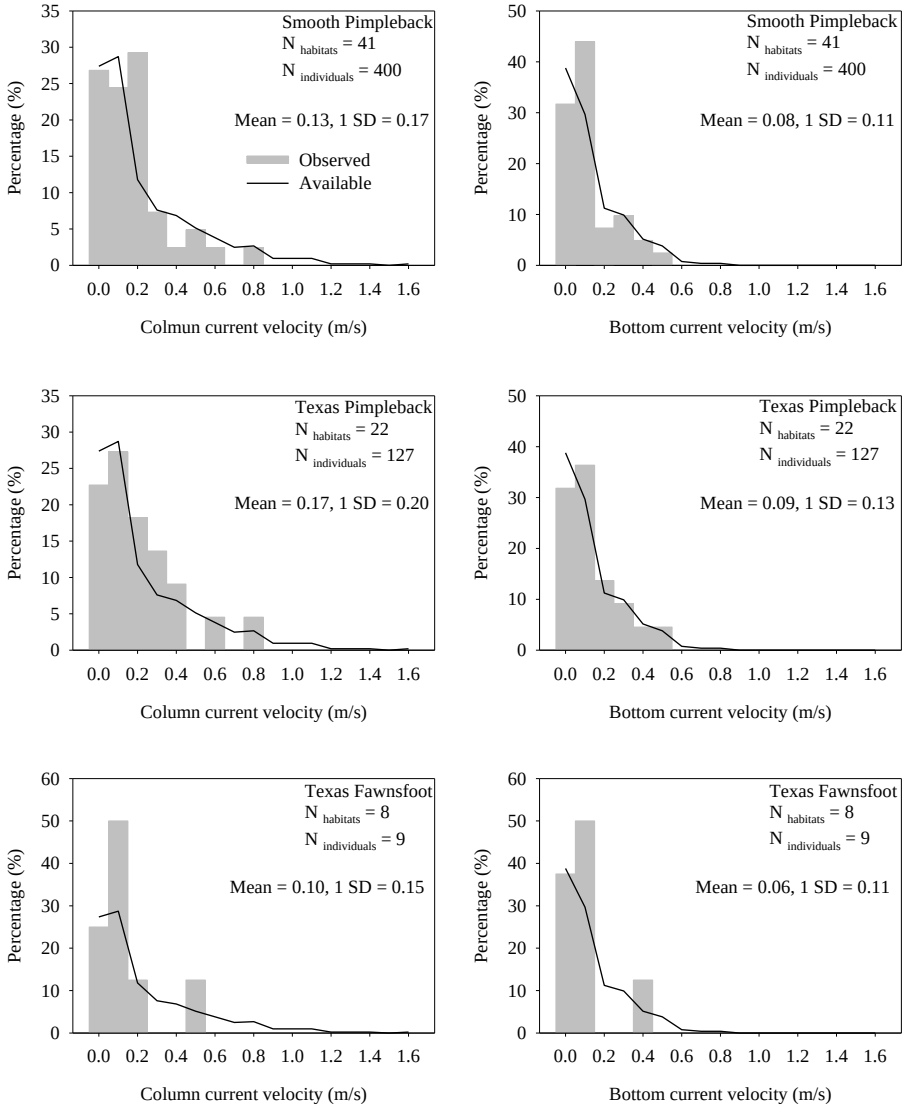


Figure 9. Percent occurrences of Smooth Pimpleback, Texas Pimpleback, and Texas Fawnsfoot (gray bars) by water depth and substrate penetrometer measurement taken from the Colorado River from March through October 2017. Black line represents the percent of current velocities available. Bars above line suggest positive association and bars below line (or absent) suggest negative association.

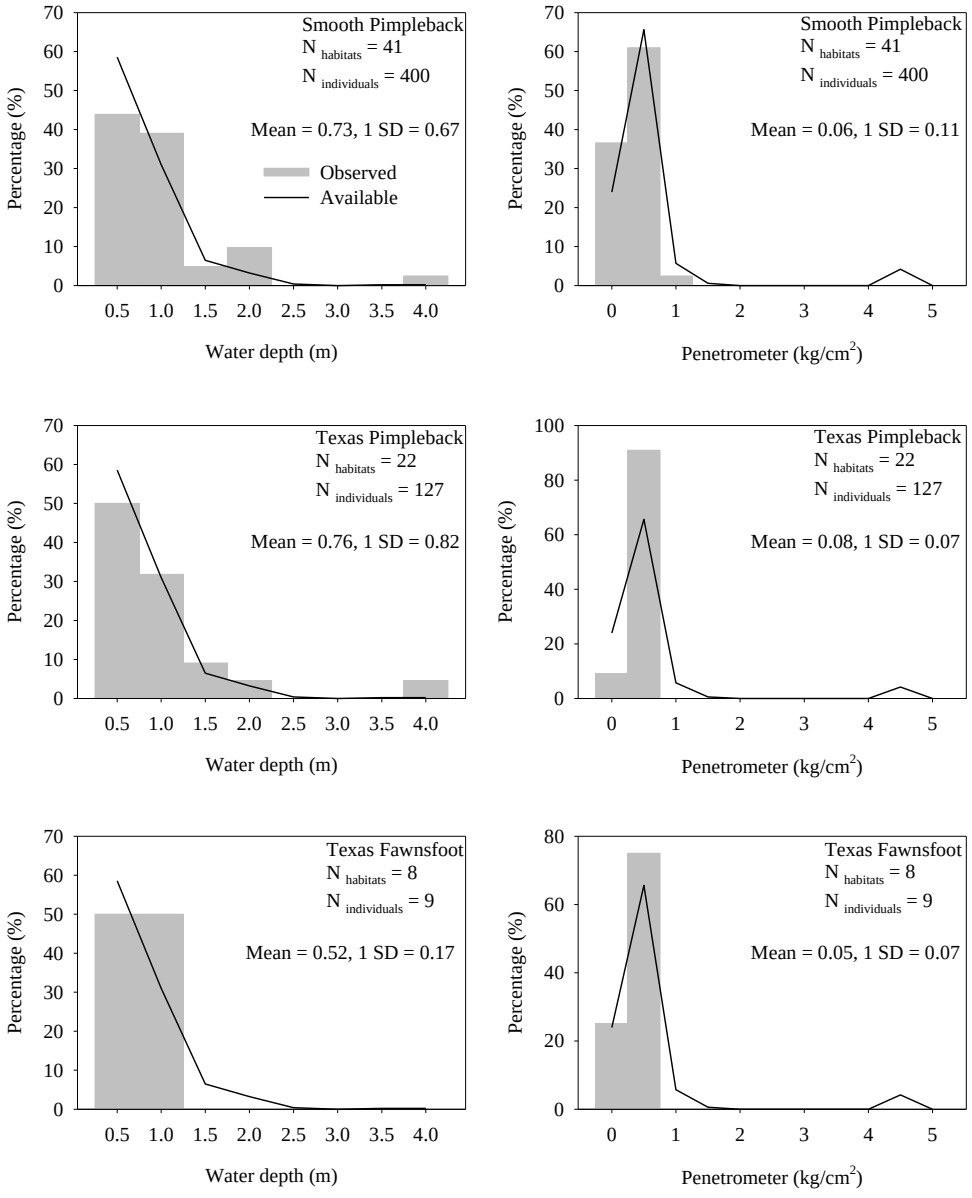


Figure 10. Percent occurrences of Smooth Pimpleback, Texas Pimpleback, and Texas Fawnsfoot (gray bars) by minimum bottom shear stress and FST hemispheres taken from Colorado River from March through October 2017. Black line represents the percent of current velocities available. Bars above line suggest positive association and bars below line (or absent) suggest negative association.

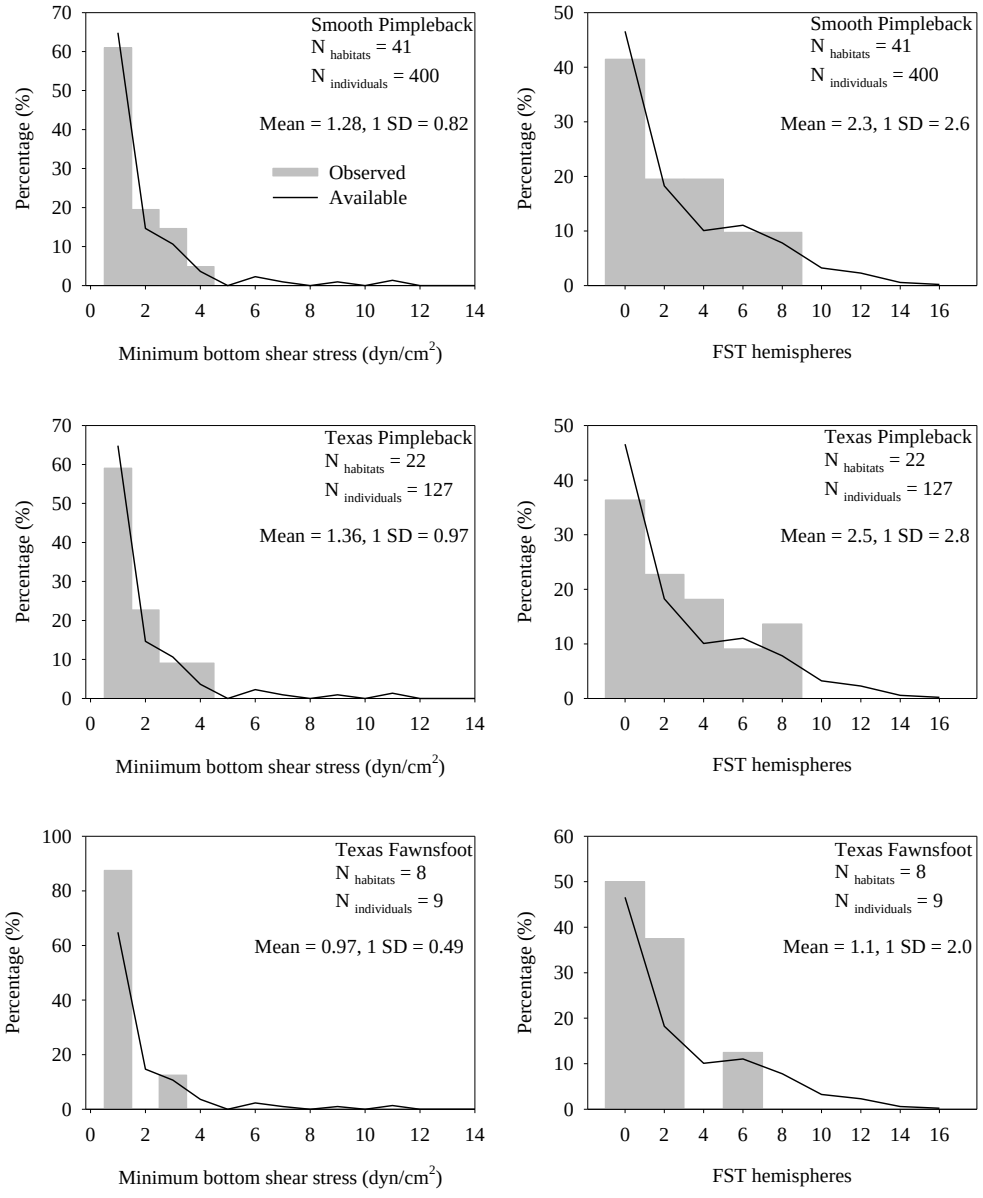


Figure 11. Differences in percent observed occurrences and percent available (i.e., expected) among mesohabitats and substrates for Smooth Pimpleback, Texas Pimpleback, and Texas Fawnsfoot from the Colorado River from March through October 2017. Positive differences suggest positive association with a mesohabitat or substrate. Negative differences suggest negative association with a mesohabitat or substrate.

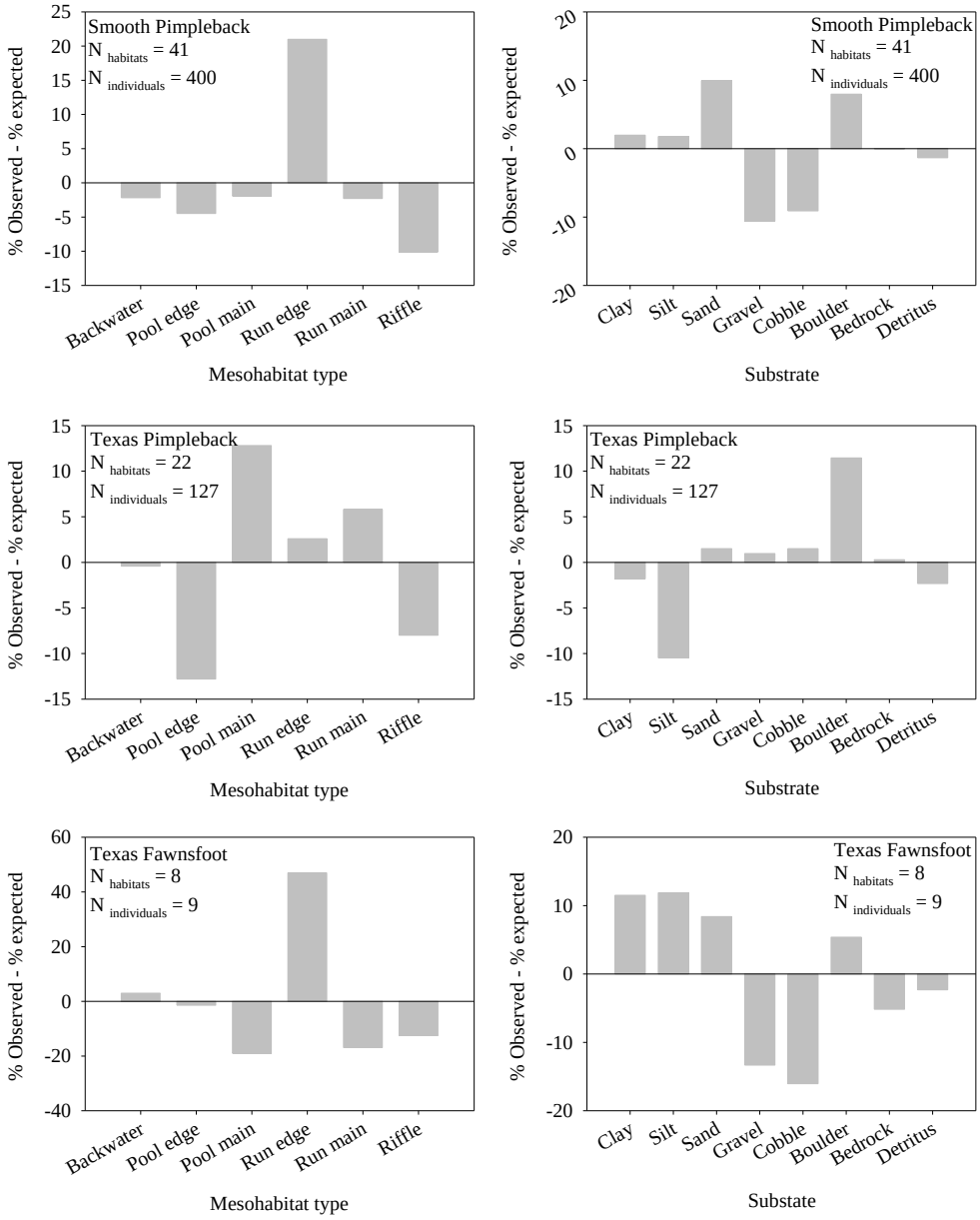
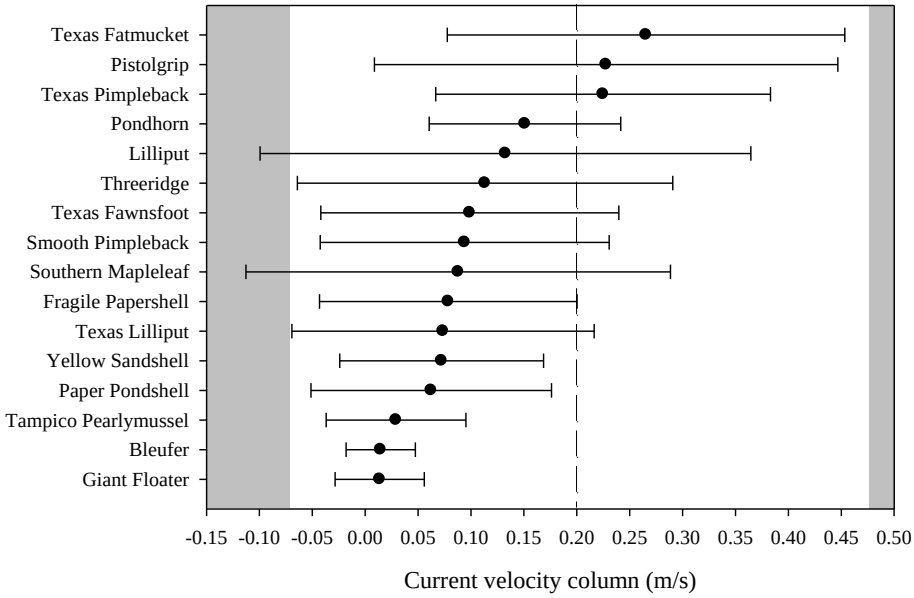


Figure 12. Weighted mean (black circle) and one SD (whiskers) of current velocities (column, top panel; bottom, bottom panel) for mussels taken from Brazos River, Colorado River, and Guadalupe Rivers from March through October 2017. Dashed vertical line represents mean of all available habitats, white area represents within 1 SD of all available habitats, and gray represents >1 SD of all habitats available. Total N for each species is provided in Table 14.



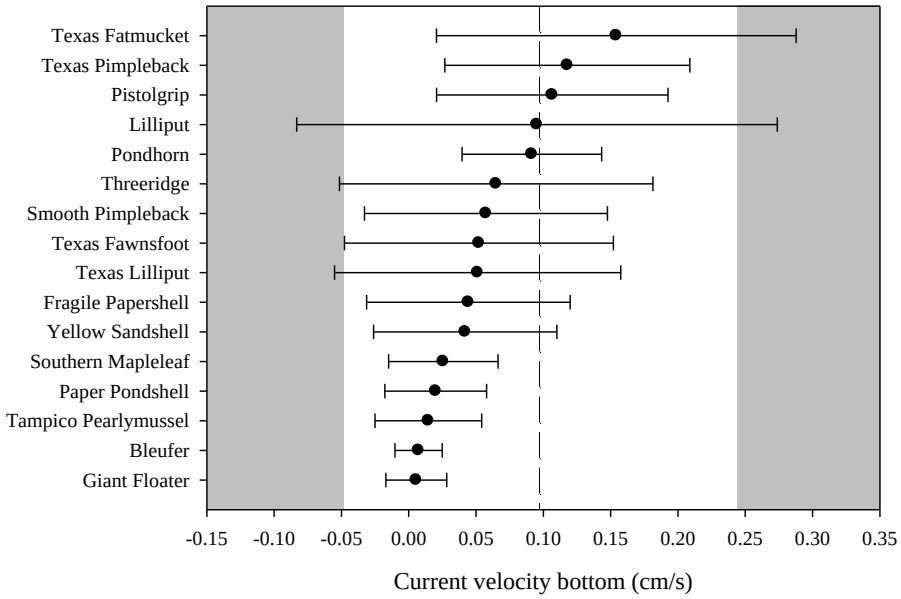
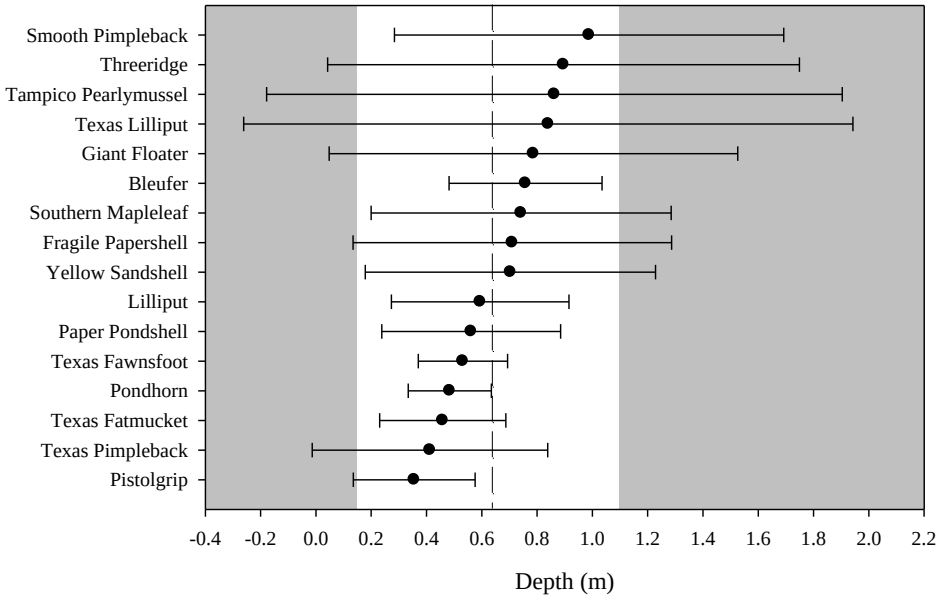


Figure 13. Weighted mean (black circle) and one SD (whiskers) of depth (top panel) and penetrometer (bottom panel) for mussels taken from Brazos River, Colorado River, and Guadalupe Rivers from March through October 2017. Dashed vertical line represents mean of all available habitats, white area represents within 1 SD of all available habitats, and gray represents >1 SD of all habitats available. Total N for each species is provided in Table 14.



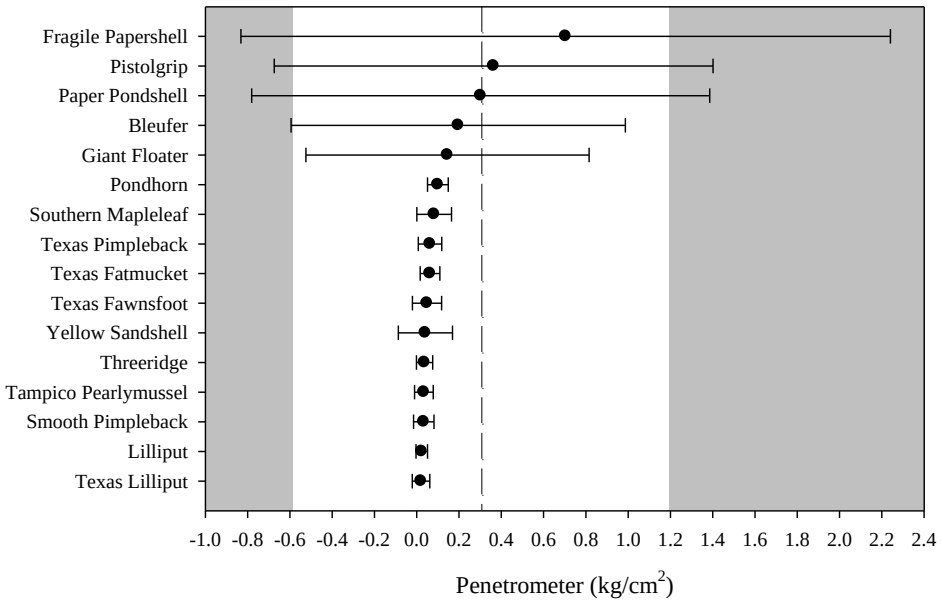
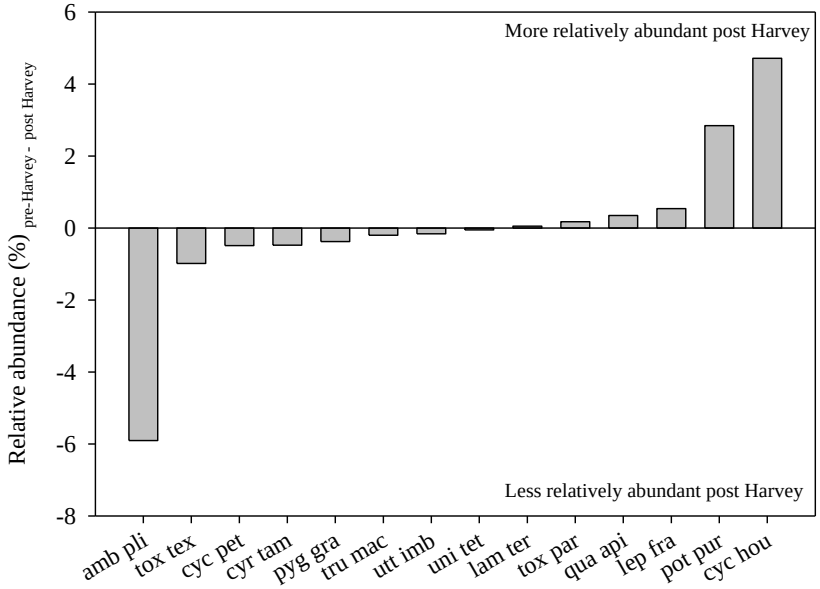


Figure 14. Differences in mussel community relative abundances before and after Hurricane Harvey and >150,000 cfs in the lower Colorado River (Georegion 5; top panel). Relative abundances of mussel species pre and post Harvey, ranked by magnitude of percent change (bottom table). Species names are abbreviated by the first three letters of their generic and specific epithets.



Species	Pre-Harvey	Post Harvey	Change in percent
amb pli	59	53	-5.91
tox tex	1.7	0.68	-0.99
cyc pet	1.4	0.91	-0.49
cyr tam	1.6	1.1	-0.48
pyg gra	0.38		-0.38
tru mac	0.43	0.23	-0.20
utt imb	0.16		-0.16
uni tet	0.05		-0.05
lam ter	17	17	0.05
tox par	0.05	0.23	0.17
qua api	0.11	0.45	0.35
lep fra	2.6	3.2	0.54
pot pur	0.11	2.9	2.84
cyc hou	16	21	4.71
N of mussels	1859	441	
N of habitats	179	66	

Part I & II References

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Task 2: Potential Factors Limiting Growth, Survival, and Reproduction of Freshwater Mussel Species of Interest

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Task 2. Objectives: The overall objective of this study was to conduct applied research experiments on up to three mussel species of interest to investigate impacts of the following stressors: temperature, hypoxia, suspended solids, salinity, and nitrogenous compounds. Specific objectives are listed under each sub-task.

Task 2.1 Sublethal effects of thermal and hypoxia stress

Task 2.1.A. Test microplate respirometry using early stage *Ligumia subrostrata*

2.1.A. Goal: The goal of this objective was to use a surrogate species to develop protocols for conducting microplate respirometry on early stage (glochidia and/or juveniles) mussels.

2.1.A. Methods: During the course of developing microrespirometry protocols, it became apparent that software needed to be updated in order to calibrate each individual microplate chamber rather than a general calibration for the microplate as a whole. Loligo Inc. made this software update available in December 2017. We are currently modifying our methodology to

incorporate the updated software and will provide a full description of updated methodology in the August 2018 addendum to this report.

2.1.A. Results: Despite the limitations of the original software, we were able to conduct initial trials and measure respiration rates of glochidia of *Ligumia subrostrata* (Fig. 1). Results were very promising and indicated that respiration patterns of glochidia subjected to hypoxia stress (declining dissolved oxygen) were similar to adults.

Task 2.1.B. Collection of focal species to be used in trials

Mussels for experiments were collected by BIO-WEST, Texas State University, and Auburn University personnel during surveys (see Table 1 for species list and collection information). Mussels were placed in coolers between moist cotton towels. Sufficient ice-packs were added above and below the toweling to try to maintain a shipping temperature intermediate between collection temperature in Texas and holding temperature (18^{o}C) at Auburn. All coolers were shipped overnight via FedEx. Upon arrival, mussels were tagged, measured (length), and placed in upwellers containing ~80 L of hard artificial freshwater (HAFW: 0.192 g $NaHCO_3$, 0.10 g $CaSO_4$*H20, 0.10 g $CaCl_2$, 0.06 g $MgSO_4$, and 0.008 g KCl per liter of reverse osmosis/deionized water; modified from Smith et al. 1997) at 18^{o}C. Biofilters in each upweller were allowed to establish for > 2 weeks prior to arrival of experimental mussels. Mussels in each upweller were fed 2 mL Shellfish Diet 1800 (Reed Mariculture Inc, Campbell, CA) in the morning and 1 mL in the afternoon on a daily basis. Water quality (ammonia, nitrites, nitrates) was measured 3 times/week using either Tetra 6-in-1 and Ammonia Aquarium Test Strips or API 5 in 1 and Ammonia Test Strips. Ammonia and nitrites remained at undetectable levels (< 0.5

mg/L) throughout the study. Nitrates were consistently detected and water changes were triggered when nitrate concentrations reached or exceeded 20-40 mg/L.

Newly arrived mussels were allowed to acclimate to HAFW and laboratory holding conditions for > 2 weeks. Following the lab acclimation, mussels were randomly assigned to one of six temperature treatments (13, 17, 23, 28, 32, and 36^{o}C). However, the lowest temperature was changed to 15°C after we found respiration rates at 13^{o}C were too low to reliably assess metabolic patterns. Mussels were acclimated in insulated upwellers (~70 L) equipped with chillers and/or heaters with temperature control (4 mussels/species/cooler, 2 coolers per temperature treatment). During acclimation, temperature was adjusted up or down at a rate of 1^{o}C/day until the target temperature was reached. Mussels were then acclimated to the temperature treatment for > 1 week. During the acclimation period, mussels were fed Shellfish Diet 1800 twice daily (2 mL morning, 1 mL afternoon per ~70 L upweller) and held at a 12h light: 12h dark cycle.

Task 2.1.C1. Thermal and hypoxia tolerance of adult mussels: Effects on metabolic patterns.

2.1.C1. Goals:

Following acclimation, we used closed respirometry to estimate resting metabolic rates (RMR) of freshwater mussels at different temperatures as dissolved oxygen (DO) decreased from near 100% saturation to anoxic conditions. RMR represents the oxygen demand of organisms while at rest and approximates the metabolic rate required for basic maintenance. We then used the relationship between RMR and DO to calculate a regulation index (RI) for each mussel. The regulation index provides an assessment of the ability of an organism to maintain a constant

respiration rate (i.e. meet its basic metabolic requirements) as oxygen declines. The closer the RI is to 1, the better an organism is able to continue to meet its energetic demands as DO declines (Fig. 2a,c). The closer the RI is to zero, the less an organism is able to meet its energetic demands as DO declines (Fig. 2b,c). We also used the relationship between RMR and DO to calculate critical dissolved oxygen levels (DO_{crit}). The DO_{crit} indicates the DO threshold below which an organism switches from aerobic to anaerobic respiration and thus is experiencing severe respiratory stress (Fig. 2a,b).

2.1.C1. Methods:

Respirometry experiments were conducted in 8-chamber fiber optic respirometry systems using AutoResp^{TM} 2.3.0 software (Loligo, Inc.). Chambers were made of acrylic and ranged in volume from ~200 – 700 mL. Each chamber was connected to two Eheim submersible 300 L/h pumps: one circulated fresh oxygenated water through the chamber during acclimation, and the other circulated water through the chamber during experiments. A fiber-optic sensor was inserted in the closed recirculation line of each chamber. Respirometry chambers and associated pumps and sensors were submerged in a ~300 L tub filled with HAFW. Temperature was controlled by means of a TECO 1/3 hp chiller/heater unit. Chambers, tubing, and gravel associated with the respirometry setup were chlorinated (5 ml bleach/gallon tap water) to reduce bacteria and then rinsed thoroughly before each trial.

We measured respiration rates of 8 randomly selected individuals per temperature for *Cyclonaias houstonensis* and *C. petrina* (4 individuals per species/run * 2 runs per temperature). Acclimated individuals were removed from temperature-controlled upwellers, scrubbed lightly with a brush to remove any algae, and weighed (gWW). Mussels were then set in PVC cups

filled with gravel within the respirometry tub, and held without food for ~24 hours to prevent feeding and digestion from affecting estimates of RMR.

Following the 24 hr starvation period, mussels were assigned to an appropriately sized respirometry chamber (chamber that accommodated a mussel's shell without touching sides or lid). A PVC cup (1.5" H) half full of pea gravel was placed in each chamber to provide substrate for the mussels to burrow into. Cups had 4-mm mesh screening on the bottom to allow for water recirculation and reduce the chance of 'dead zones'. Flush pumps associated with each chamber were turned on and mussels allowed to acclimate to respirometry chambers for 5 hrs – a period of time which equaled or exceeded the amount of time required for mussels to reach a stable RMR as determined by previous experiments (Haney and Stoeckel, unpublished data). During this time, DO levels were near 100% saturation levels. Respirometry rooms were held at a 12h light: 12h dark cycle.

Acclimation periods were always initiated in late afternoon/early evening, and respirometry initiated before midnight. Following acclimation, the flush pumps were turned off and closed pumps turned on – creating a closed system where the volume of water recirculating within each chamber and associated tubing was constant, and no new, oxygenated water entered the system. Pumps were controlled remotely using TeamViewer software to minimize any disturbance to the mussels within the respirometry rooms. Mussels were allowed to respire until dissolved oxygen levels fell below ~0.2 mgO_2/L in the chambers or until mussels exhibited valve closure (abrupt cessation of respiration). Valve closure rendered respiration data unusable for RI or DO_{crit} analyses. At the end of each experimental run, mussels were removed from their testing chambers and then returned to upwellers to recover. Duration of each run was temperature dependent. At the coldest temperature, it generally took > 8 hrs for DO to fall below 0.2 mgO_2/L

whereas at the warmest temperature, DO typically declined to < 0.2 mgO_2/L within 8 hours or less.

Additional respiration experiments were conducted for *Amblema plicata, Fusconaia mitchelli,* and *Lampsilis teres* at a single temperature of 21°C using the methodology described above. Results will be included in a later draft of this report as an addendum.

Correction for background (bacterial) oxygen demand

Chlorination, followed by rinsing, was used to reduce/eliminate bacteria in chambers and associated tubing prior to each respiration run. However, bacteria populations tend to grow quickly and may have accounted for a significant portion of chamber oxygen demand by the end of a given run (i.e. background respiration). To account for this, we measured background respiration rate of each chamber before and after each run, under normoxic conditions (DO ≥ 5 mgO_2/L), for ~1.5 hrs without mussels present. The mean background oxygen demand was then divided by the mean observed respiration rate under normoxic conditions with mussels present in order to determine the proportion of total chamber respiration that was due to background respiration. This proportion was referred to as the correction factor. We assumed that this proportion remained constant as dissolved oxygen declined below normoxia and corrected our respirometry data in each chamber by multiplying the observed respiration rate by 1 minus the correction factor.

Regulation Index and DO_{crit}

Regulation indexes (RI's) were calculated using the methodology of Mueller and

Seymour (2011). Corrected RMR ($mgO_2/g/hr$) values were plotted against DO (mgO_2/L) for each respirometry run. The upper limit of the DO range for which RI was calculated was held constant at 6 mg O_2/L to avoid bias in colder temperature runs where initial DO could be much higher than warmer runs (Mueller and Seymour 2011). Data were fitted with the curve (3-parameter exponential rise to maximum, 2-parameter hyperbola, or 2-segment piecewise regression) that showed the lowest Akaike information criterion adjusted for small sample size (AICc: SigmaPlot 13.0). We then used the Sigma Plot area under the curve (AUC) macro to calculate AUC for 1) the observed data, 2) a horizontal line that represented perfect regulation, and 3) a linear decrease that represented perfect conformation (see Fig. 2c). RI was calculated as (Observed AUC-Conformation AUC)/(Regulation AUC-Conformation AUC). The RI provided a quantitative measure of the degree to which mussels were able to regulate oxygen consumption as ambient DO declined from 6 to < 0.2 mg O_2/L. DO_{crit} was calculated as the dissolved oxygen concentration showing the greatest distance between the observed RMR and the perfect conformation line (Mueller and Seymour 2011).

2.1.C1: Results:

Resting metabolic rate increased linearly with increasing temperature for *Cyclonaias houstonensis* from the Colorado (RMR = 0.0007*temp – 0.0053; R^2 = 0.9744, P = 0.0002) and Navasota rivers (RMR = 0.0004*temp – 0.0046; R^2 = 0.9423, P = 0.0013). The regulation index did not show a significant linear relationship with temperature for *C. houstonensis* from the Colorado (R^2 = 0.6328, P = 0.0584) or Navasota (R^2 = 0.0164, P = 0.8088) rivers. Similarly, DO_{crit} did not show a significant linear relationship with temperature for *C. houstonensis* from the Colorado (R^2 = 0.1166, P = 0.5078) or Navasota (R^2 < 0.01, P = 0.9922) rivers (Fig. 3).

Cyclonaias petrina exhibited similar patterns. Resting metabolic rate increased linearly with increasing temperature for *C. petrina* from the Colorado (RMR = 0.0004*temp – 0.0038; R^2 = 0.9757, P = 0.0002) and Guadalupe (RMR = 0.0005*temp – 0.0070) rivers. The regulation index did not show a significant linear relationship with temperature for *C. petrina* from the Colorado (R^2 = 0.4592, P = 0.2088) or Guadalupe (R^2 = 0.4574, P = 0.2100) rivers. DO_{crit} did not show a significant linear relationship with temperature for *C. petrina* from the Colorado (R^2 = 0.6336, P = 0.1072) or Guadalupe (R^2 = 0.5517, P = 0.1504) rivers (Fig. 4).

There was no significant difference in mean RI (calculated across all temperatures) among species and locations (ANOVA, P = 0.079). There were significant differences in DO_{crit} among species and locations (ANOVA, P = 0.025) with *C. houstonensis* from the Navasota River exhibiting a significantly lower DO_{crit} than *C. houstonensis* from the Colorado River (Tukey's, P = 0.026). DO_{crit} of *C. petrina* did not differ between locations or from *C. houstonensis* collected from either location (Tukey's, P > 0.05) (Fig. 5).

The proportion of animals exhibiting at least one episode of valve closure, as evidenced by a sudden drop of RMR to 0, increased at high temperatures. *C. petrina* tended to show higher proportions of valve closure than did *C. houstonensis* (Fig. 6).

2.1.C1 Conclusions:

Results suggest that the main impact of increasing temperatures to a maximum of 36ºC is to increase metabolic demand for basic maintenance. Thus, mussels of both species require more food, and are likely to become more susceptible to food limitation, at warm temperatures. *C. houstonensis* from the Colorado River is likely the most sensitive to food limitation as its RMR increased at a faster rate with increasing temperature than did *C. houstonensis* from the Navasota

River or *C. petrina* from either location. This result emphasizes the importance of understanding mussel food resource availability, particularly during the warm summer months.

Valve closure results further support the potential for food limitation at high temperatures. Previous studies have shown bivalves exhibit valve closure in response to stressful temperatures (e.g. Anestis et al. 2007). In our study, all species/location combinations showed a trend of increasing episodes of valve closure as temperatures increased, with 40-87% of all mussels exhibiting at least one episode of closure when temperatures reached 36ºC. Because increased frequency of valve closure would reduce feeding and aerobic respiration activities while demand for energy is increasing, mussels would become increasingly susceptible to growth limitation as temperatures approach and exceed 36ºC. However, mussels appeared to exhibit tradeoffs to potentially offset this effect. *C. houstonensis* (Colorado River) exhibited the greatest increase in energy demand as temperatures increased, but kept valves open until temperatures reached 36ºC. *C. petrina* exhibited a greater frequency of closed valves than *C. houstonensis* as temperatures approached 36ºC, but exhibited a lower energy demand. Studies examining the effects of intermittent valve closure on mussel energy budgets at high temperatures would help determine which species is more strongly affected by food limitation at high temperatueres.

Surprisingly, there was little evidence that increasing temperatures up to 36ºC increased sensitivity to hypoxia for any species/location tested. Although some weak trends in RI and DO_{crit} with increasing temperature were apparent, none were significant. The ability of mussels to obtain oxygen from the water column (RI) remained fairly constant as temperatures increased, with a switch from aerobic to anaerobic respiration (DO_{crit}) only becoming apparent when DO levels fell below ~2.0 mgO_2/L. Short term tolerance of low DO, even at high temperatures, is

further supported by the lack of mortality when mussels remained in respirometry chambers at 36^{o}C at DO concentrations < 1 mgO_2/L for several hours prior to termination of trials.

2.1.C1. Ongoing work

Respirometry results for *Lampsilis bracteata* will be added to this report by the end of April 2018.

Task 2.1.C2. Thermal tolerance of adult mussels: Effects on respiratory enzymes.

2.1.C2. Objectives:

The electron transport system (ETS) assay measures the activity of enzymatic complexes I and III of the respiratory chain within the mitochondria. It provides excess substrate (NADH and NADPH) for the enzyme complexes to act upon and utilizes INT dye as the electron acceptor. Originally developed by Packard (1971) it has since been used as a proxy for in-situ respiration rates of marine and freshwater organisms (Owens and King 1975, Madon et al. 1998, Elderkin et al. 1998). It yields an estimate of the potential oxygen consumption rate of an organism if all enzymes function maximally by quantifying ETS activity in the presence of excess substrates (Fanslow et al. 2001). Recently, Simcic et al. (2014) showed that the relationship between ETS enzyme activity and temperature can be used to estimate optimal thermal temperatures for organisms at the cellular level. They also showed that ETS activity shows a high degree of correlation with scope for growth at the organismal level, with optimal temperatures for organism growth being a few degrees cooler than optimal temperature for ETS enzymes. We used the ETS assay to determine optimal enzymatic temperatures for acclimated

and non-acclimated mussels and to compare intra and interspecific variation in optimal temperatures among species and locations.

2.1.C2. Methods:

We used two different approaches to examine the relationships between ETS activity and temperature. In the first approach, mussels were acclimated for >1 week to each of nine experimental temperatures (see *Acclimated Approach* below). ETS activity at each temperature was measured as the mean of four acclimated mussels, and tissue sampling was non-lethal. This approach yielded a single, composite, thermal performance curve for a given species and was limited to a non-lethal temperature range because it requires acclimation of mussels to each temperature for > 1 week with minimal mortality. It also required a large number of mussels (e.g. ≥ 4 mussels/temperature x 9 temperatures = ≥ 36 mussels).

In the second approach (non-acclimated), mussels were acclimated to only a single temperature (21^{o}C), and tissue sampling was lethal. However, this approach required fewer mussels because the enzymes extracted from a single mussel were tested across all temperatures, yielding a separate thermal performance curve for each individual mussel. The temperature range tested could include and exceed the lethal range for mussels because only the extracted enzymes, not the mussels themselves, are exposed to each temperature. Methods for the two approaches are described in detail below.

Acclimated Mussels

Within 24 hours of respirometry measurements (see 2.1.C1), we randomly selected four mussels from each of the original six temperature treatments, gently pried their shells open, and

collected two, ~10 mg tissue plugs from the foot of each mussel using a nasal biopsy tool (Karl Storz nasal biopsy tool #453733) (Fritts et al. 2015). Tissue plugs were placed in cryovials and immediately frozen at -80^{o}C. An additional two mussels were randomly selected from each temperature, placed in a temperature-controlled upweller, and assigned to a new temperature of 20, 25, or 30^{o}C. Temperatures were raised or lowered at a rate of 1^{o}C/day until the target temperature was reached. Mussels were then acclimated for >1 week at the new target temperature and tissue plugs collected and stored in the same manner as described previously. Thus, we collected tissue plugs from four mussels/species acclimated for >1 week to each of 9 temperatures (15, 17, 20, 23, 25, 28, 30, 32, 36^{o}C). After tissue collection, all mussels were cooled back down to 18^{o}C at a rate of 1^{o}C/day and transferred back to the original upwellers to allow them to recover.

ETS activity of acclimated mussels was measured using standard methodologies adapted from Packard (1971) and Simcic et al. (2014). Frozen tissue plugs collected from a single mussel were weighed and placed in a 5 mL vial (note: vial could actually hold up to 7 mL) (self-standing sample tube, 5 mL, Globe Scientific via VWR, number 89497-730) filled to the 4 mL mark with 1.0 mm diameter glass beads (Biospec Products, Cat. No. 11079110) and containing 4 mL of homogenization buffer (0.1 M sodium phosphate buffer pH=8.4; 75 uM $MgSO_4$; 0.15% (w/v) polyvinyl pyrrolidone; 0.2% (v/v) Triton-X-100). Tissue was then homogenized with a BeadBeater (MiniBeadBeater-24; BioSpec Products, Inc., Bartlesville, OK) for 1 min and chilled for 1-2 min in a freezer. The beadbeating/chilling cycle was repeated for 3-4 cycles until tissue was thoroughly homogenized. The vial was then centrifuged for 4 min, at 10,000 rpm, at 0^{o}C in a refrigerated centrifuge (Allegra X-30R, Beckman Coulter, Brea, CA). Homogenate generated from a given mussel was placed in a flask, diluted to 2.5 mg tissue/mL using reagent grade DI

water (Ricca, cat# 9150-1), mixed with a stir bar, distributed amongst ~ 2 mL vials (Eppendorf$^{(R)}$ Safe-Lock microcentrifuge tubes (MCT), polypropylene) and frozen at -80^{o}C. Homogenate was stored for ≤ 6 weeks prior to measurement of ETS activity.

To measure ETS activity, two, replicate, 0.5 mL subsamples of thawed homogenate were each incubated in 1.5 mL substrate solution (0.1M sodium phosphate buffer pH = 8.4; 1.7mM NADH; 0.25 mM NADPH; 0.2% (v/v) Triton-X-100) with 0.5 mL INT solution (2.5mM 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride) for 30 minutes, in the dark, at the temperature to which the mussel had been acclimated. The reaction was then stopped by adding 0.5 mL of stopping solution (Formalin: H_3PO_4 = 1:1). A blank for the replicate samples was made by combining 1.5 mL substrate solution with 0.5 mL INT solution, and incubated and stopped along with the samples. Following addition of stopping solution, 0.5 mL of the corresponding homogenate was added to the blank. Absorbance (490 nm) of the replicate samples was measured with a spectrophotometer (Genesys 10S UV-VIS, ThermoScientific, Waltham, MA) and corrected for absorbance of the blank. ETS activity was calculated according to the following formula (Kenner and Ahmed, 1975):

$$\text{ETS activity } (\mu l\ O_2\ g^{-1}\ WW\ h^{-1}) = (ABS^{490nm} * V_h * V_r * 60)/(V_a * S * t * 1.42)$$

where ABS^{490nm} is the absorption of the sample corrected for blank; V_h is the volume of the homogenate (4 mL) prior to removal of subsamples; V_r is the volume of the reaction mixture (homogenate subsample + substrate solution + INT solution + stopping solution = 3mL); V_a is the volume of the homogenate subsample (0.5 mL); S is the mass of the tissue sample (g); t is the incubation time (min); 60 is a correction factor to convert the rate to hours, and 1.42 is the factor for conversion to volume O_2.

The mean ETS activity was then calculated for each acclimation temperature (~4 mussels per temperature), graphed against temperature, and fitted with a four-parameter Gaussian curve (SigmaPlot 13.0; Systat Software, Inc., San Jose, CA). Optimal temperature was defined as the temperature which exhibited the highest ETS activity. Optimal temperature range was defined as the temperature range within which ETS activity was within 10% of the maximum value.

Non-acclimated mussels

Following respiration measurements (see 2.1.C1), two mussels were randomly selected from each of six experimental temperatures, placed in temperature controlled upwellers, and brought to 21^{o}C at a rate of 1^{o}C / day. All mussels were held at 21^{o}C for at least 1 week. Following the > 1 week holding period, each mussel was sacrificed by severing the adductor mussels with a scalpel and opening the shell. Approximately 100 mg of foot tissue was immediately collected from each mussel using the nasal biopsy tool, and frozen at -80^{o}C. Tissue from each mussel was subsequently removed from the freezer and homogenized using the previously described beadbeater technique. Homogenate generated from a given mussel was combined in a flask, diluted to 2.5 mg tissue/mL using reagent grade DI water (Ricca, cat# 9150-1), mixed with a stir bar, distributed amongst ~ 1.8 mL vials and refrozen at -80^{o}C. ETS activity was subsequently measured following the same methodology as described above.

For each mussel, two replicate enzyme samples were incubated for 30 minutes at each temperature of interest, yielding a complete thermal performance curve (ETS activity vs temperature) for each individual. Initially, incubation temperatures were 12, 15, 17, 20, 23, 25, 28, 30, 32, and 36°C. Optimal temperatures for each individual was calculated using a four parameter Gaussian regression. Because optimal temperature data was not normally distributed,

we used a Kruskal-Wallis ANOVA on rank-transformed data to test for significant differences among species/location combinations (SigmaPlot 13.0, Systat Software, Inc., San Jose, CA, USA). Pairwise comparisons were conducted using Dunn's Method (SigmaPlot 13.0).

While analyzing samples for optimal temperature, we conducted some exploratory runs at temperatures >36ºC and found ETS activity did not decline symmetrically with increasing temperatures as would be described by a Gaussian curve. We hypothesized that the post-peak decline pattern might yield additional information regarding thermal stress. We therefore added temperatures of 39, 42, 45, 48, 51, 54, and 57ºC to the non-acclimated assay for remaining species and populations. We also added a cooler temperature (9ºC). We then used a 5-segment piecewise regression (SigmaPlot 13.0) to characterize the relationship between ETS activity and temperature during the decline following peak activity.

2.1.C2. Results

Acclimated Mussels

Optimal temperatures for ETS enzyme activity were higher for *C. petrina* from the Colorado (35.3ºC) and Guadalupe (34.6ºC) rivers than for *C. houstonensis* from the Colorado (31.6ºC) and Navasota (27.6ºC) rivers. Optimal range estimates predicted mussels would begin to experience enzymatic thermal stress at some point >36ºC for Colorado and Guadalupe River *C. petrina*, >35.8ºC for Colorado River *C. houstonensis*, and > 30.6ºC for Navasota River *C. houstonensis* (Fig. 7).

Because the acclimated approach generates only a single, composite, thermal performance curve for each species/location, we were not able to test for significant differences in optimal temperature between species or locations, nor were we able to assess variability in

optimal temperature among individuals within the same species/location group. To address these issues, we analyzed thermal performance curves for individual mussels using the non-acclimated approach.

Non-acclimated Mussels

ETS activity was strongly correlated with temperature for individual, non-acclimated mussels, with four parameter Gaussian regressions typically yielding an $R^2 > 0.90$ (see Fig. 8 for example; full set of graphs for other species/location combinations available upon request). Summary graphs (Fig. 9) show variation in curve height and optimal temperature (temperature at which curve peaks) within and among each species/location combination. Intraspecific variation in optimal temperature was highest for *C. petrina* from the Colorado River and lowest for *L. bracteata* (Llano River) and *C. houstonensis* (Navasota River) (Fig. 10). We expect curves for Llano Lake *Lampsilis bracteata* to be available prior to April 30, 2018.

There were significant differences in mean optimal temperature among the four candidate species tested (Kruskal-Wallis ANOVA: H=18.836, d.f.= 4, $P < 0.001$). Optimal temperature for *C. houstonensis* from the Colorado River was significantly higher than *C. houstonensis* (Navasota River), *C. petrina* (Guadalupe River), *L. bracteata* (Llano River), and *F. mitchelli* (Guadalupe River) (Dunn's Method, $P < 0.05$; Fig. 11). *Fusconaia mitchelli* (Guadalupe River) and *L. bracteata* had significantly lower optimal temperatures than *C. houstonensis* or *C. petrina* from the Colorado River (Dunn's Method, $P < 0.05$; Fig. 11). However, their optimal temperatures did not differ significantly from *C. houstonensis* from the Navaota River or C. *petrina* from the Guadalupe River (Dunn's Method, $P > 0.05$; Fig. 11).

There was evidence of differences in thermal optima between subpopulations of *C.*

houstonensis, but not between subpopulations of *C. petrina*. Optimal temperature of *C. houstonensis* from the Colorado River was significantly higher than that of *C. houstonensis* from the Navasota River (Dunn's Method, $P < 0.05$; Fig. 11), but optimal temperature of *C. petrina* from the Colorado River was not higher than that of *C. petrina* from the Guadalupe River (Dunn's Method, $P > 0.05$; Fig. 11).

There was no evidence that the four candidate species had lower thermal optima than two common species tested. *Amblema plicata* (Colorado River) had an intermediate optimal temperature, and *Lampsilis teres* (Colorado River) had a low optimal temperature relative to the four candidate species (Tables 3 and 4).

We measured activity of ETS enzymes from non-acclimated adult mussels at temperatures expected to exceed 24-hr lethal temperature (LT_{50}) thresholds for five species (Fig. 12). As temperatures increased above the thermal optimum, ETS activity did not decline in a linear fashion. Rather, each species exhibited a "shoulder" pattern where enzyme activity initially declined, then leveled off, then declined again. In a previous study, Marshall et al. (2011) showed that a bimodal pattern of snail respiration with increasing temperature was correlated with the onset of heat shock protein production and 24-hr LT_{50} thresholds. Because ETS activity represents the maximum potential respiration rate of an organism (Fanslow 2001), it is likely that similar endpoints are correlated with ETS activity patterns. We hypothesize that the point at which the decline in ETS activity begins to level off represents the activation of heat shock proteins – signaling the onset of major thermal stress and the transition from sublethal to lethal thermal stress. Preliminary comparison of independent LT_{05} (temperature at which 5% of animals die) data from the lab of Dr. Charles Randklev (Texas A&M University) is providing support for this hypothesis. We plan to continue this line of inquiry with Dr. Randklev in 2018.

The hypothesized breakpoint between sublethal and lethal effects was 38.9°C for *C. petrina* from the Guadalupe River, 37.1°C for *C. houstonensis* from the Navasota River, 29.4°C for *L. teres* from the Colorado River, 31.0°C for *F. mitchelli* from the Guadalupe River, and 36.0°C for *A. plicata* from the Colorado River (Fig. 12).

2.1.C2. Conclusions:

Mussels acclimated to warm temperatures generally exhibited higher optimal temperatures than non-acclimated mussels (Table 4). However, optimal temperatures of non-acclimated mussels still fell within the optimal range of acclimated mussels – usually near the lower end of the range (Fig. 13, Table 4). The primary effect of acclimation appeared to be an increase in the upper portion of the optimal range rather than shifting the entire range. This suggests that in natural populations, a given mussel species will enter its optimal thermal range at approximately the same temperature threshold regardless of previous thermal history. However, mussels subjected to rapid, flashy increases in temperature will leave their thermal optima and start to experience thermal stress at lower temperatures than mussels subjected to gradual, stable increases in temperature. Intraspecific variation in non-acclimated optimal temperature (Fig. 10) was highest for *C. petrina* from the Colorado River, suggesting they may have a greater capacity to adapt to future fluctuations in temperature than species such as *L. bracteata* (Llano River) and *C. houstonensis* (Navasota River), which exhibited relatively low variation in optimal temperatures.

A summary of the estimated optimal and stressful temperatures for ETS enzymes of acclimated and non-acclimated mussels can be found in Table 4. While the ETS assay estimates optimal temperatures at the enzymatic level, corresponding estimates of optimal temperature

measured at the more complex organismal level (e.g. optimal scope for growth) may be cooler by 2-3^{o} C (Simcic et al. 2014). Therefore, temperature ranges that are optimal or stressful to the organism as a whole are likely to be a few degrees lower than temperatures optimal or stressful to the organism's ETS enzymes.

Task 2.1.D. Effect of temperature on respiration of glochidia and juveniles.

Preliminary respirometry runs on *Ligumia subrostrata* glochidia have been conducted (see 2.1.A). Respirometry data from target species will be added as an addendum to this report by Aug. 31 2018 if glochidia and/or juveniles are available before that deadline.

Task 2.3 Effect of turbidity/suspended solids on valve closure of adult mussels

2.3 Objectives:

Mussels must keep their shells open to obtain food and oxygen from the surrounding waters. However, they often respond to stressors by closing their shells. Electromagnetic sensors attached to each valve can be used to monitor gaping and closing behavior and set off alarms when behavior indicates the presence of stressful toxicants in the water (Manley and Davenport 1979, Kramer et al. 1989, Gnyubkin 2009). Systems such as the MosselMonitor (www.mosselmonitor.nl; Kramer et al. 1989) and the Dreissena Monitor (Envicontrol Köln Germany; Borcherding, 1994) have been used to monitor stressors in fresh and saltwater environments in Europe. In this study, we used a MosselMonitor to determine whether mussels fully or partially close their valves in response to high turbidity/suspended solids – indicating negative impacts on feeding and respiration.

2.3. Methods:

Sediments

Sediments were obtained from a drained, 0.1 ha, earthen pond at the South Auburn Fisheries Research Station of Auburn University, Alabama. The top two inches of sediment were collected, mixed in a 5 gallon bucket, distributed into baking pans, and dried for 24 hrs at 105^{o}C. Dried sediment was passed through a #60 (250 µm) Fisher Scientific Company sieve and stored in an air tight 5-gallon bucket. This process was repeated until we had obtained enough sieved sediments to complete all trials. Stored sediment was mixed thoroughly via rolling and shaking in a closed container to ensure even distribution of particles immediately prior to each use. Soil analysis (T. Knappenberger, Auburn University) showed the sediments were composed of 53.5% sand (63 – 2000 µm), 46.6% silt (2.0-63 µm) and 0.0% clay (< 2.0 µm).

Experimental animals

Following respirometry experiments (see previous section), mussels were allowed to recover for > 4 weeks at 18^{o}C. Water temperature was then raised by 1^{o}C/day to the experimental temperature of 28^{o}C. Mussels were acclimated to this temperature for ≥ 1 week prior to initiation of experiments. During this time mussels were fed Shellfish Diet 1800 twice daily (2 mL morning, 1 mL afternoon per ~70 L upweller) and held at a 12h light: 12h dark cycle.

Experimental protocol

To monitor valve movements, mussels were held in individual, mesh bottom, plastic baskets (8 X 5 X 4 cm) screwed to the walls of a MosselMonitor (Flow through version,

AquaDect B. V.Brouwershaven, Netherlands). MosselMonitor settings, data downloads, and data display were controlled via PresentIT^{TM} 3.0 software on a connected desktop computer. The MosselMonitor was filled with HAFW and held at 28^{o}C. One valve of each mussel was glued (Unifast Trad Methylmethacrylate two part Resin GC America Inc. Alsip IL) to the plastic basket wall parallel to the side of the MosselMonitor. This ensured the mussel remained at a fixed distance from an electromagnetic sensor in the MosselMonitor wall. A second sensor was glued directly to the other valve of the mussel. Aquarium pea gravel was then added to the basket until half of the mussel was embedded in the substrate. Throughout the subsequent experiment, mussels were fed Shellfish Diet 1800 at the same rate as during the acclimation period. The Light:Dark cycle was held constant at 12:12.

Mussels were acclimated to the system for three days, during which time the MosselMonitor monitored distance between sensors and assessed the baseline maximum and minimum valve opening exhibited by each mussel. During the subsequent portions of the experiment, distance between valves (sensors) was reported as percent gape, based on the baseline maximum and minimum distances calculated during the acclimation period. Percent gape was calculated and recorded every 10 seconds for the remainder of the experiment. The first 24 hours following acclimation (Day 4) served as a control period, during which time food, but no sediment, was added to the MosselMonitor. The experimental period began on day 5. Sediment was added to a belt feeder located above a cone tank that was connected to the MosselMonitor. The belt feeder dropped sediment into the cone tank at a constant rate over a period of 10 hours. Sediment was suspended in the cone tank via a submersible Resun King-2 pump (1000 L/hr) and multiple air stones. A second submersible Resun King-2 pump transferred water and suspended sediments to the MosselMonitor at a constant rate of 360 L/hr.

Flexible tubing returned water at the same rate to the cone tank via ambient head pressure. A LaMotte 2020we turbidimeter was used to measure turbidity in replicate 10 mL samples collected immediately after mussel attachment (0 h), at the start of the control period (72 h), every hour for the first 12 hours of the experimental period (hrs 96-108), and at the end of the experimental period (hr 120). Supplemental samples were periodically collected to quantify TSS concentrations. A ~250 mL sample was siphoned from the center of the MosselMonitor. The siphon tube was attached to the MusselMonitor prior to the experiment so that subsequent samples could be collected without disturbing mussels. Sample water was filtered through a pre-ashed (1 hr at 550^{o}C) 1.2 µm Whatman 47 mm glass microfiber filter to collect sediments. Filters were then dried overnight at 105^{o}C and weighed. Filter weight was subtracted from total weight and then divided by sample volume to calculate TSS in mg dry weight/liter.

This protocol resulted in an initial ~6-hour ramping period (9:00 – 15:00 hrs) on Day 5 during which suspended solid concentration in the MosselMonitor increased with time as the rate at which sediment was added to the system exceeded the rate at which sediment settled within the system. Equilibrium between sediment addition and settlement was reached within 5-6 hrs, after which time total suspended solids (TSS) concentrations remained relatively stable for the remaining four hours (15:00 – 19:00 hrs) of the run (Fig. 14 bottom panels). Mussels were exposed to one of two treatments, with two runs per treatment. Different mussels were used for each run. Within each run, sufficient sediment was added to the belt feeder to allow turbidity to ramp up to the target level. During our first three runs, we set a high turbidity target of ~25 NTU (~70 mg TSS/L), intending to reduce turbidity in subsequent runs. However, due to the lack of obvious effects on valve closure even under high turbidity conditions (see results), we changed

our subsequent runs to target excessive turbidity levels of ~70 NTU (~ 250 mg TSS/L) for the two final runs (Fig. 14).

2.3. Results

Individuals of both species exhibited highly variable relationships between percent gape and turbidity during the ramping periods when turbidity increased from 0 to 25 or 65-75 NTU's over a 6-hr period (Figs. 15-18). During the excessive turbidity ramping period, mean percent gape of all *C. petrina* combined exhibited a negative linear relationship with turbidity (Figure 19 top panel: $R^2 = 0.53$, $P < 0.0001$, mean percent gape = 59.3214 – 0.1478*NTU). Mean percent gape during the high turbidity ramping period showed a similar negative relationship with turbidity, albeit with a steeper slope (Fig 19 top panel: $R^2 = 0.83$, $P < 0.0001$, mean percent gape = 62.7746 – 0.4654*NTU).

During the excessive turbidity ramping period, mean percent gape of all *C. houstonensis* combined exhibited a significant positive linear relationship with turbidity, but turbidity explained very little of the variation in valve gape (Figure 19 bottom panel: $R^2 = 0.064$, $P < 0.0001$, mean percent gape = 45.8459 + 0.0451*NTU). Mean percent gape during the high turbidity ramping period showed a significant, negative linear relationship with turbidity, but, again, turbidity explained very little of the variation in gape (Fig. 19 bottom panel: $R^2 = 0.040$, $P < 0.0001$, mean percent gape = 63.9433 - 0.1675*NTU).

During the constant turbidity period of the high treatment (~25 NTU), there was little to no evidence that turbidity resulted in decreased gape for either species. Exposed *C. petrina* exhibited less frequent gape values exceeding 65% than control mussels but percent gape peaked

at 65% in both groups. Gape of exposed *C. houstonensis* peaked at a higher value (75%) than control *C. houstonensis* (55%) (Fig. 20).

During the constant turbidity period of the excessive treatment (60-75 NTU), there was evidence that gape decreased slightly for both species. Gape peaked at 65% in exposed *C. petrina* compared to 75% for control mussels. Gape peaked at 65% in exposed and control *C. houstonensis*, but very few exposed mussels gaped more than 65% compared to control mussels (Fig. 21).

2.3. Conclusions:

Because mussels obtain food and oxygen from surrounding water, there was concern that high concentrations of suspended solids may trigger valve closure by mussels, with subsequent, negative effects on feeding and respiration. However, we found little to no evidence that exposure to suspended solids, even at high (turbidity ~25 NTU; TSS ~70 mg/L) or excessive (turbidity ~75 NTU, TSS ~250 mg/L) concentrations resulted in valve closure. As turbidity increased, *C. petrina* exhibited only a small reduction in mean percent gape. Increasing turbidity explained very little of the variation in percent gape for *C. houstonensis*. Under conditions of excessive turbidity, mussels appeared to close valves slightly, but the peak in percent gape remained high at 65% as compared to a peak of 65-75% during the preceding control period. If high suspended solids have a negative effect on mussel feeding rates or ability to obtain oxygen, the mechanism behind negative effects is not likely to be valve closure.

2.2 Sublethal effects of nitrogenous compounds and salinity on adult mussels

2.2A. Effect of Ammonia on Respiration.

2.2A. Objectives:

In 2013, the U.S.EPA updated its Aquatic Life Ambient Water Quality Criteria for Ammonia – Freshwater in order to take into account data for highly sensitive unionid mussel and non-pulmonate snail species that had not previously been tested (USEPA 2013). Because ammonia toxicity issues are fairly complex, a brief explanation is provided here.

Ammonia in surface waters is typically reported as total ammonia nitrogen (TAN). This refers to the combined concentration of nitrogen (mg/L) occurring in two co-existing forms of ammonia – ionized (NH_4^+) and un-ionized (NH_3). Un-ionized ammonia is the most toxic form. The proportion of un-ionized to unionized (NH_3: NH_4^+) ammonia increases with increasing pH and temperature. Thus ammonia becomes more toxic with increases in temperature and/or pH even if the concentration of ammonia, measured as TAN, remains the same. The U.S.EPA 2013 ammonia benchmark is 17 mg TAN/L for acute (1 hour average) exposure and 1.9 mg TAN/L for chronic (30-d rolling average) exposure. These benchmarks are referred to as "criterion maximum concentrations" (CMC) and represent a concentration that is expected to be lethal to <50% of individuals in sensitive species. They specifically apply to a pH of 7 and a temperature of 20^{o}C. In many Texas rivers, pH is typically ≥8 and temperatures rise well above 20^{o}C during the summer months. The toxicity of 17 (acute) and 1.9 (chronic) mg TAN/L benchmark concentrations would therefore increase and may no longer be sufficiently protective of unionid mussels. The USEPA is cognizant of this issue and provides tables to adjust benchmark concentrations for specific temperature and pH values (see tables 5b, 6 in USEPA 2013).

Unionized ammonia can affect organisms such as mussels via multiple mechanisms that include increased ventilation rates (volume of water passing through gills per unit time), gill damage, and a reduction in the ability of blood (hemolymph) to carry oxygen. Thus it is

reasonable to expect that metabolic and respiration patterns would be sensitive to ammonia. The objectives of this study were to determine whether ammonia affected metabolic patterns of mussels by 1) reducing their ability to regulate oxygen consumption, 2) increasing their DO_{crit}, and 3) altering their resting metabolic rate. Note that this task was completed last due to the high probability of significant sublethal and lethal effects on experimental mussels when exposed to ammonia.

2.2A. Methods:

Following turbidity assays described in previous sections, *C. petrina* and *C. houstonensis* from the Colorado River were allowed to recover for ≥ 2 weeks at 28°C prior to initiation of ammonia experiments. During this time, they were fed Shellfish Diet 1800 according to the standard feeding regime (2 mL morning, 1 mL afternoon, per 70 L upweller). Due to a limited number of mussels remaining from the original collections, we were only able to test 5-6 individuals of *C. petrina*, and 6 individuals of *C. houstonensis* per ammonia treatment, prior to preparation of this report. Each mussel was exposed to only a single ammonia treatment. Thus we tested a total of 16 *C. petrina* and 18 *C. houstonensis*.

Resting metabolic rates (RMR) were measured at 28°C using the same respirometry system described in section B of this chapter. Mussels were starved for 24 hrs prior to experiments to prevent feeding and digesting from affecting metabolism. Following the starvation period, sufficient ammonia from a stock solution (Hach ammonia standard, 1,000 mg TAN/L) was added to the respirometry trough to bring it to the target concentration of 0.5 or 2.0 mg TAN/L. No ammonia was added to the trough for the control runs. Ammonia concentrations in the respirometry trough were measured using a YSI 9300 Photometer (YSI Inc. 2017) at the

beginning and end of each experiment to ensure the target concentration had been reached and remained stable throughout the duration of each trial. The moderate concentration, (0.5 mg TAN/L) was selected to represent the average concentration that we observed in our respirometry chambers following a standard, closed respirometry experiment (section B). The higher concentration represents the 2013 USEPA CCC chronic criteria of 1.9 mg TAN / L at pH 7, 20^{o}C. Note that in our experiments temperature was 28^{o}C, and pH was ~8.5. Under these conditions, the recommended acute and chronic benchmarks (CMC; Tables 5b and 6 in USEPA 2013) are adjusted downward to 0.77 (acute) and 0.21 (chronic) mg TAN/L. Thus the highest TAN concentration in our study exceeded both the acute and chronic benchmarks adjusted for pH and temperature.

Mussels were acclimated to respiration chambers for ≥ 5 hrs. During this time both the flush and closed pumps were turned on to ensure oxygenated water containing the appropriate ammonia concentration circulated through chambers and tubing. Following acclimation, flush pumps were turned off, and only the closed pumps remained on - creating a closed system where the volume of water recirculating within each chamber and associated tubing was constant, and no new, oxygenated, water entered the system.

Because mussels excrete ammonia as a waste product, and this ammonia can build up in closed respirometry chambers over time, we periodically flushed chambers during a respirometry run to ensure that ammonia levels remained fairly constant during each trial. The modified respirometry protocol was as follows: After allowing closed respirometry to run for 50 minutes, nitrogen gas was bubbled into the trough water (external to the submerged respirometry chambers) using DO-SET software (Loligo Inc.), until DO had fallen from 7 to 5 mg O_2/L. Previous closed respirometry trials at 28^{o}C (section 2.1.C1) using the same two mussel species

yielded a decrease of approximately 2 mg O_2 per hour within the respiration chambers. At 60 minutes, the flush pumps were turned on and chambers flushed for 5 minutes with ~5 mg O_2/L water from the trough in order to flush out any ammonia excreted by the mussels that would otherwise increase ammonia levels above the targeted trial concentration. Flush pumps were then turned off to once more create a closed system at the target ammonia concentration. After 50 minutes DO in the trough was reduced from 5 to 3 mg O_2/L, and ten minutes later chambers were flushed again for 5 minutes. Flush pumps were then turned off and mussels allowed to draw DO down from 3 to < 0.2 mg O_2/L at which time the trial was terminated. This technique yielded a relationship between resting metabolic rate (RMR) and dissolved oxygen (e.g. Fig. 22) that could be analyzed for regulation index and DO_{crit} using the same methodology as described in task 2.1.C1, while at the same time avoiding problems associated with accumulating ammonia in closed respiration chambers.

2.2A Results:

TAN of the respirometry water matched the nominal treatment TAN concentrations fairly well and remained stable throughout the experiment. Background levels within the control treatment ranged from 0.04 to 0.1 mg TAN / L. Respirometry water pH ranged from 8.4 to 8.6 (Table 5).

The proportion of individuals exhibiting valve closure (cessation of respiration) increased with increasing TAN for both species, and was more frequent in *C. petrina* than *C. houstonensis* (Fig. 23). Because accurate RMR, RI, and DO_{crit} values could not be calculated for individuals that closed during respirometry, we did not have enough usable respiration curves to test for effects of TAN on these endpoints for *C. petrina*. For *C. houstonensis*, valve closure eliminated

only four individuals from the experiment, yielding 4-5 replicate estimates of these endpoints for each TAN treatment. There was no significant effect of TAN on RMR (ANOVA: $F = 2.528$, df = 2, 11; $P = 0.125$), RI (ANOVA: $F = 1.988$; df = 2, 11; $P = 0.183$), or DO_{crit} (ANOVA: $F = 1.178$, df = 2, 11; $P = 0.344$) (Fig. 24).

2.2A Conclusions:

At a pH of 8.5 and temperature of 18 C, the USEPA ammonia benchmarks are revised downward from 17 to 0.77 mg TAN/L for acute (1 hour average) exposure and from 1.9 to 0.21 mg TAN/L for chronic (30 day rolling average) exposure (see Tables 5b and 6 in USEPA 2013). In our study, mussels were exposed to treatment TAN concentrations exceeding both of these benchmarks for ~10 hours. Results suggest that the revised benchmarks are sufficient to protect *C. houstonensis* from short term effects of ammonia on metabolic rate (RMR) and ability to extract oxygen even under low oxygen conditions (RI and DO_{crit}). However it remains to be tested whether chronic (30 day) exposure would affect metabolism. Also, the revised benchmarks may not be sufficient to protect mussels from increased frequency of valve closure (see Fig. 23) which could affect respiration, filtration, and fertilization efficiency during long term exposure. Future studies examining effects of chronic exposure to TAN concentrations matching and exceeding the revised chronic benchmarks on metabolism and valve closure are warranted.

A major challenge of working with rare species is having a sufficient sample size to be able to detect significant differences between treatments. In the case of the ammonia studies, the sample sizes were low, increasing the chances of us not finding an effect of ammonia when one existed. We performed a power analysis (G*Power 3.1.9.2; Faul et al. 2007) to determine 1) the

difference between treatments that we had a ≥ 80% chance of detecting with the current sample size, and 2) the minimum number of samples (mussels) required to have a ≥ 80% chance of detecting a specific difference between treatments. Given our sample size of 5 individuals/treatment and the observed variance among individuals, we had a ≥ 80% chance of detecting a change of 0.004 mgO_2/gWW/hr in RMR, a change of 0.15 in RI, and a change of 1.0 mg O_2/L in DO_{crit} among treatments. In future studies, if we want to double the sensitivity of our assays (i.e. reduce the detectable difference by half) and thus have a ≥ 80% chance of detecting a change of 0.002 mgO_2/gWW/hr in RMR, 0.075 in RI, and 0.5 mg O2/L in DO_{crit}, we would need sample sizes of at least 17, 10, and 18 mussels/treatment respectively. We are currently evaluating our remaining mussel stocks and may be able to conduct additional ammonia trials with *C. petrina* and *houstonensis*. If so, data will be included in a future addendum, no later than August 2018.

A legitimate concern regarding closed respirometry techniques, such as those employed in task 2.1.C1 (thermal tolerance), is that a buildup of metabolic wastes might affect respiration rates and patterns measured in the chambers. However, these potential effects are not well understood and have not been previously tested for freshwater mussels. In our closed respirometry experiments, accumulation of metabolic-waste ammonia in closed chambers rarely exceeded 0.5 mg TAN/L and never reached 2mg TAN/L. The lack of a significant effect of 0.5-2 mg TAN/L on respiration rates, RI, or DO_{crit} in of *C. houstonensis* in the current experiment (Task 2.2A) suggests that accumulation of metabolic wastes did not affect our estimates of these parameters in the thermal tolerance experiments (Task 2.2.C1).

2.2B. Effect of salinity on adult mussel valve closure

Mussels must keep their shells open to obtain food and oxygen from the surrounding waters. However, they often respond to stressors by closing their shells. Electromagnetic sensors attached to each valve can be used to monitor gaping and closing behavior, setting off alarms when behavior indicates the presence of stressful toxicants in the water (Manley and Davenport 1979, Kramer et al. 1989, Gnyubkin 2009). Systems such as the MosselMonitor (www.mosselmonitor.nl; Kramer et al. 1989) and the Dreissena Monitor (Envicontrol Köln Germany; Borcherding, 1994) have been used to monitor stressors in fresh and saltwater environments in Europe. Valve movements have been recommended as a sublethal, behavioral endpoint for stressors such as chloride (Hartmann et al. 2016). In this experiment, we use a MosselMonitor to determine whether mussels fully or partially close their valves in response to increasing salinity – indicating negative impacts on feeding and respiration.

Experimental animals

Following respirometry experiments (see section 2.1.C1), *C. petrina* (Guadalupe River) and *C. houstonensis* (Navasota River) were allowed to recover for > 4 weeks at 18^{o}C. Water temperature was then raised by 1^{o}C/day to the experimental temperature of 28^{o}C. Mussels were acclimated to this temperature for ≥ 1 week prior to initiation of experiments. During this time mussels were fed Shellfish Diet 1800 twice daily (2 mL morning, 1 mL afternoon per ~70 L upweller) and held at a 12h light: 12h dark cycle.

Experimental protocol

To monitor valve movements, four mussels of each species (eight mussels total) were placed in the MosselMonitor and sensors attached to their valves following the same methodology as described for the suspended solids experiment (section 2.3). Mussels were acclimated to the system for three days, during which time the MosselMonitor assessed distance between sensors and calculated the baseline maximum and minimum valve opening exhibited by each mussel. During the subsequent portions of the experiment, distance between valves (sensors) was reported as percent gape, based on the baseline maximum and minimum distances calculated during the acclimation period. Percent gape was calculated and recorded every 10 seconds for the remainder of the experiment.

The first 24 hours following acclimation (Day 4) served as a control period, during which time food, but no salt, was added to the MosselMonitor. The experimental period began on Day 5. A belt feeder dropped salt (Diamond Crystal pool salt; Cargill Inc., Minneapolis, MN) into the cone tank at a constant rate of 27.3 g/hr over a period of 11 hours. Salt was mixed in the cone tank via a submersible Resun King-2 pump (1000 L/hr) and multiple air stones. A second submersible Resun King-2 pump transferred saltwater to the MosselMonitor at a constant rate of 360 L/hr. Flexible tubing returned water at the same rate to the cone tank via ambient head pressure. A PinPoint Salinity Monitor (American Marine Inc., Ridgefield, CT) was used to measure salinity at the time of attachment, start of the control period, and every hour during the beginning of the experimental period. The salinity meter's probe was held in the cone tank to make sure the mussels were not disturbed. Previous trials determined that the salinity in the cone tank and the MosselMonitor were equivalent due to constant flow between the two units. This protocol resulted in an 11-hour ramping period (9:00 – 20:00) during which salinity rose linearly with time from a low of <1 ppt to a high of ~4ppt at a rate of ~0.3ppt/h (Fig. 25).

2.2B Results

Six of the eight *C. petrina* (Guadalupe River) were >50% open at the beginning of the experimental period. Each exhibited a period of steady decline in percent gape with increasing salinity until valves were completely closed or nearly so. One individual subsequently re-opened to 50%, but quickly closed again (Fig. 26). Similarly, six of the eight *C. houstonensis* (Guadalupe River) were >50% open at the beginning of the experimental period. Each exhibited a subsequent period of steady decline in percent gape with increasing salinity, but degree of closure was more variable than for *C. petrina*, with more individuals exhibiting >10% gape at high salinities (Fig. 27). On average, *C. petrina* exhibited a steady decline in percent gape as salinity levels increased beyond 2.0 ppt, with a final mean gape of < 5% whereas *C. houstonensis* exhibited a steady decline in percent gape as salinity increased beyond 2.5 ppt and mean gape subsequently leveling out at ~35% as salinity increased from 3.0 to 4.0 ppt (Fig. 28).

2.2B Conclusions:

Previous studies have shown that adult mussels (*Elliptio complanata*) exposed to 6 and 4 ppt exhibited 50% mortality by day 3 and 4 respectively, while mussels exposed to 2 ppt exhibited reduced metabolic rates but no mortality after 28 days (Blakeslee et al. 2013). Our results suggest that a reduction in gape and/or complete closure, and resultant reduction and/or cessation of water flowing past the gills is a likely mechanism driving reduced metabolic rates (i.e. respiration) as salinity increases. Valve closure would also be expected to interfere with feeding and fertilization success. These sublethal impacts of salinity >2 ppt are likely greater for *C. petrina* than *C. houstonensis* as they exhibited an earlier and steeper decline in gape.

In our study, mussels were exposed to salinities ≥2 ppt for less than 6 hours and exhibited zero mortality during the experiment or within 7 days of being transferred back to freshwater. However, if high salinity conditions were sustained over a long period of time, lethal effects of high salinity might occur more quickly for *C. houstonensis* due to increased exposure. They did not close valves as tightly and appeared to reopen to a greater degree than *C. petrina* as salinity approached LC_{50} (4ppt, 7d) concentrations reported by Blakeslee et al (2013).

We are currently analyzing results of a similar salinity experiment run on *L. bracteata* (Llano Lake). Results will be included in an addendum to this report prior to August 2018.

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Table 1. Species, collection sites, and shipping information for mussels used in thermal and hypoxia tolerance experiments at Auburn University.

Species	Drainage	Site	Collection Date	# shipped	Collection Temp. (°C)	Receiving Temp. (°C)
Cyclonaias petrina	Colorado River	Altair	4/28/2017	6		*NT*
			5/17/2017	33		16.2
		Lometa	6/1/2017	11	26.6	22.0
	Gauadalupe River	Gonzales	11/01/2017	14	18	16.9
		Gonzales	8/17/2017	32	30.9	23.5
Cyclonaias houstonensis	Colorado River	Altair	4/27/2017	6		*NT*
			5/17/2017	50		16.4
	Navasota River	Easterly	7/17/2017	50		22.6
Lampsilis bracteata	Llano River	Mason	5/31/2017	20	23	15.3
		Llano Park Lake	11/01/2017	50	Stranded, 13.7	15.1, 14.4
Fusconaia mitchelli	Guadalupe River	Gonzales	8/17/2017	6	30.9	23.5
			11/01/2017	4	18	16.9
Truncilla macrodon	Brazos River	Highbank	12/4/2017	7	19	*NT*
Amblema plicata	Colorado River	Altair	4/27/2017	12		*NT*
			8/4/2017	20	31	21.7
Lampsilis teres	Colorado River	Altair	11/02/2017	12	18	*NT*

Table 2. Optimum temperature and optimum range for acclimated ETS activity. Prior to ETS measurement, individuals were acclimated for >1 week to each of 10 temperatures from 15 – 36ºC.

Species	**Drainage**	**Optimum**	**Range**
Cyclonaias petrina	Colorado River	35.3	28.2 - >36
	Gauadalupe River	34.6	26.5 - >36
Cyclonaias houstonensis	Colorado River	31.6	27.5 – 35.8
	Navasota River	27.6	24.8 – 30.6

Table 3. Optimal temperature data for non-acclimated mussels. Individuals were all acclimated for >1 week to 21ºC. Enzymes were then extracted from foot tissue of each individual and incubated at each of 9 temperatures ranging from 12 – 36ºC, generating a separate thermal performance curve for each individual (see Fig. 9). Min and max refer to the minimum and maximum optimal temperatures among all individuals within each species X drainage combination.

Species	**Drainage**	**Mean**	**Min**	**Max**	**Stdev**	**CV**	**n**
Cyclonaias petrina	Colorado River	30.2	28.6	34.8	1.7	5.7	12
	Gauadalupe River	28.5	27.6	29.5	0.7	2.6	6
Cyclonaias houstonensis	Colorado River	30.5	28.3	32.1	1.1	3.7	11
	Navasota River	28.8	27.7	29.8	0.6	2.0	12
Lampsilis bracteata	Llano River	28.4	27.6	29.1	0.6	2.1	11
	Llano Lake	*April 2018*					
Fusconaia mitchelli	Guadalupe River	27.8	26.5	28.8	0.9	3.3	6
Amblema plicata	Colorado River	28.4	27.5	29.0	0.5	1.6	10
Lampsilis teres	Colorado River	27.1	27.9	26.2	0.6	2.1	12

Table 4. Summary of ETS enzyme thermal tolerance endpoints from acclimated and non-acclimated experiments. Optimal Range for acclimated mussels represents the temperature range where ETS activity was within 10% of the observed maximum rate, whereas for non-acclimated mussels it represents the range in optimal temperatures estimated for individual mussels. The upper end of the acclimated range indicates the temperature threshold above which we predict the initiation of thermal stress at the enzymatic level for at least some individuals in the population. The upper end of the non-acclimated range represents the maximum optimal temperature observed for enzymes subjected to a sudden increase in temperature. Onset of lethal effects indicates the temperature threshold beyond which we predict the onset of mortality due to thermal stress. Months indicate when we expect additional information will be available. Blank cells indicate no results due to a lack of samples and/or animals. All temperatures are ^{o}C.

Species	**Drainage**	**Optimal Temp.**	**Optimal Range**	**Estimated onset of lethal effects**	**Assay Type**
Cyclonaias petrina	Colorado River	35.3	28.2 - >36		Acclimated
		30.2	28.6-34.8		Non-acclimated
	Gauadalupe River	34.6	26.5 - >36		Acclimated mussels
		28.5	27.6-29.5	38.9	Non-acclimated
Cyclonaias houstonensis	Colorado River	31.6	27.5 – 35.8		Acclimated mussels
		30.5	28.3-32.1		Non-acclimated
	Navasota River	27.6	24.8 – 30.6		Acclimated mussels
		28.8	27.7-29.8	37.1	Non-acclimated
Lampsilis bracteata	Llano River	28.4	27.6-29.1		Non-acclimated
	Llano Lake				Acclimated mussels
		May	***May***	***May***	Non-acclimated
Fusconaia mitchelli	Guadalupe River	27.8	26.5-28.8	31.0	Non-acclimated
Amblema plicata	Colorado River	28.4	27.5-29.0	36.0	Non-acclimated
Lampsilis teres	Colorado River	27.1	26.2-27.9	29.4	Non-acclimated

Table 5. Total ammonia nitrogen (TAN) and pH measurements in respirometry water for individual runs within ammonia treatments.

		TAN (mg/L)		**pH**	
Run	*Treatment*	*Initial*	*Final*	*Initial*	*Final*
1	0	0.05	0.4	8.6	8.6
2	0	0.1	0.1	8.6	8.6
1	0.5	0.60	0.43		8.4
2	0.5	0.66	0.30	8.6	8.5
1	2.0	2.40	2.08	8.6	8.4
2	2.0	2.16	1.76	8.5	8.4

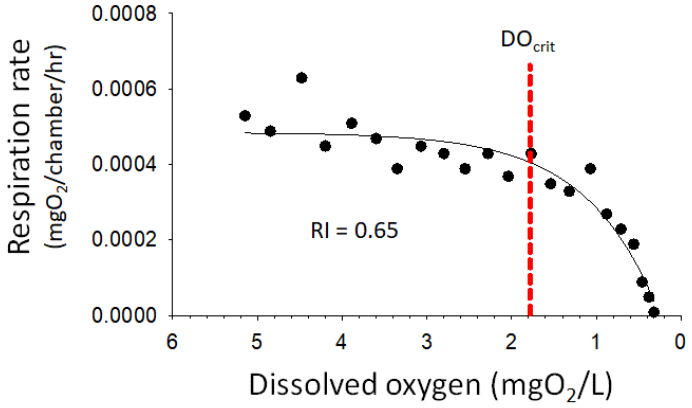

Figure 1. Example of preliminary data showing respiration pattern of *L. subrostrata* glochidia subjected to declining dissolved oxygen. Regulation index (RI) and DO_{crit} values could be calculated for glochidia and were similar to that observed for adult mussels.

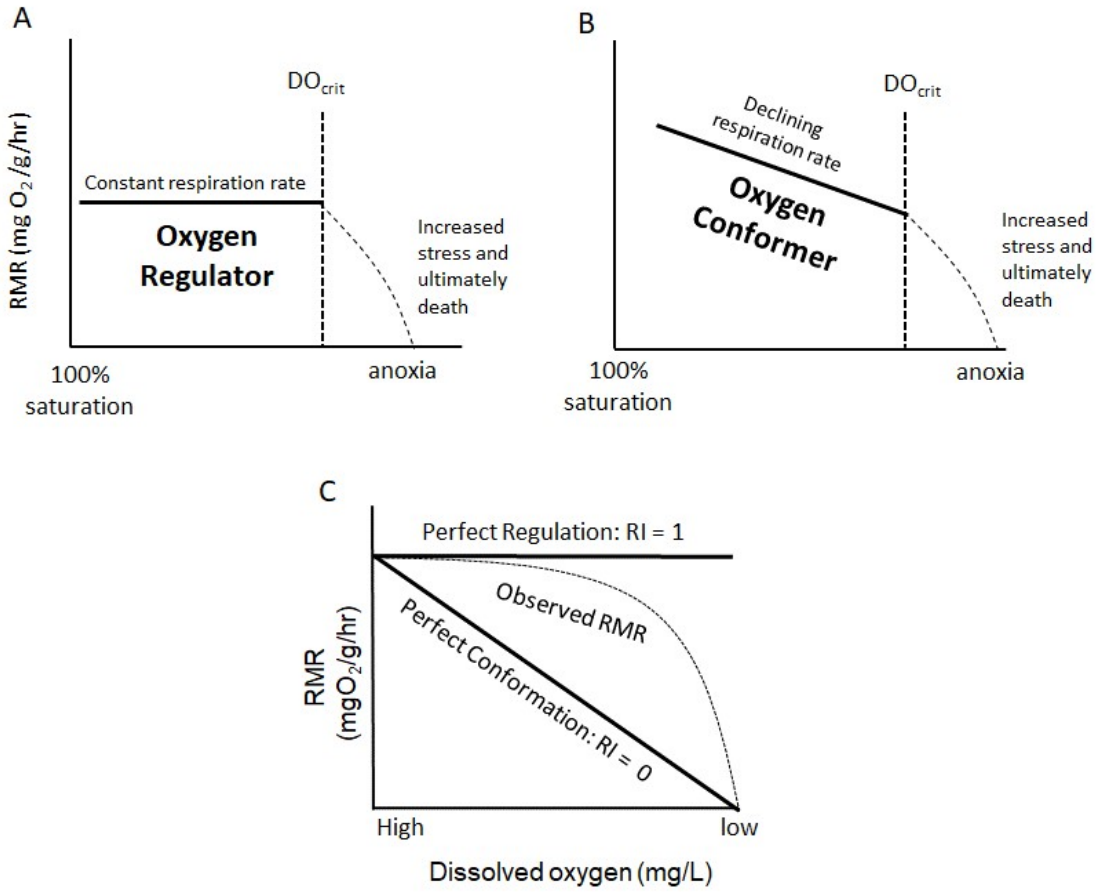

Figure 2. Resting metabolic rates (RMR) graphed as a function of declining dissolved oxygen showing A) an oxyregulator and B) an oxyconformer. DO_{crit} is the DO threshold below which respiration rates show a marked decrease or increase, indicating the switch from aerobic to anaerobic respiration. C) RMR graphed as a function of DO indicating the range of values of the Regulation Index (RI; Mueller and Seymour 2011). Solid lines indicating either perfect regulation (RI = 1) or perfect conformation (RI = 0) and dashed line indicates an intermediate, typically observed pattern that falls between perfect regulation and perfect conformation.

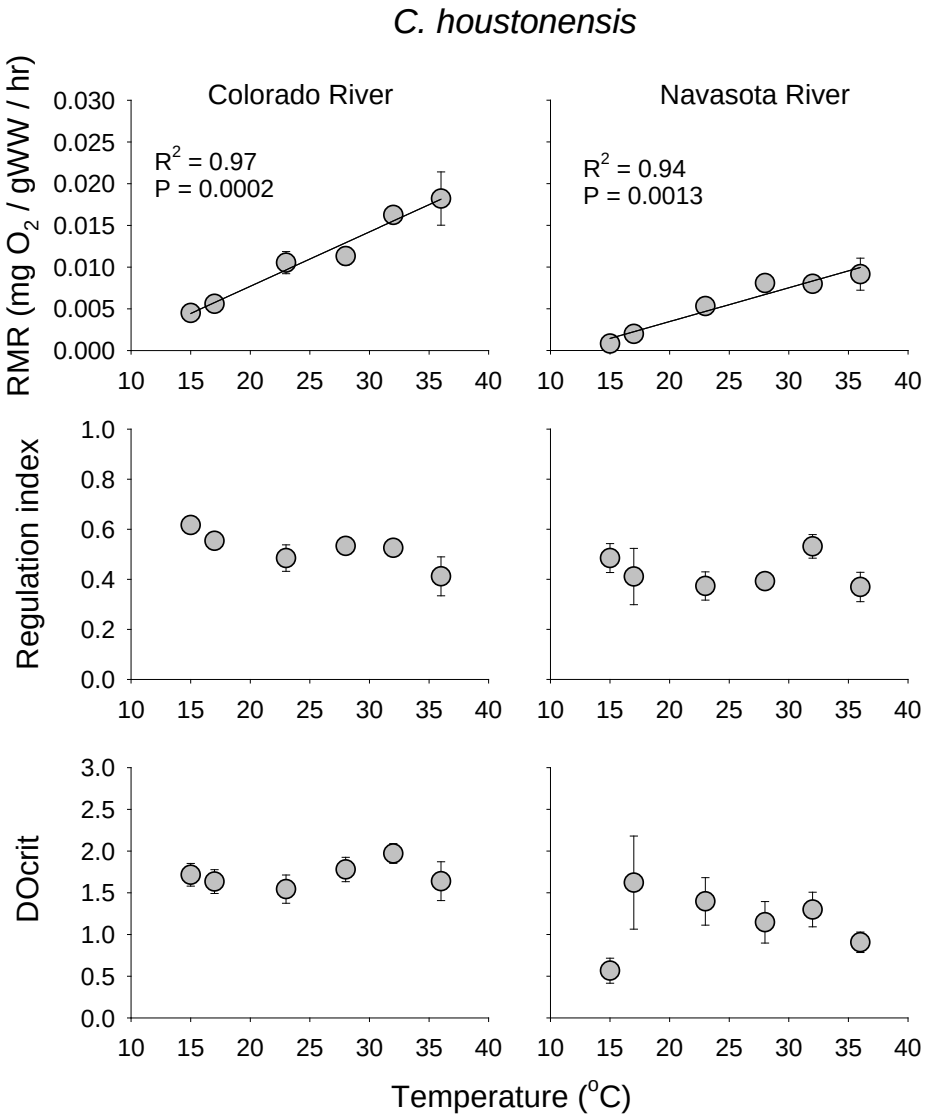

Figure 3. Relationships between resting metabolic rate (RMR), regulation index, DO_{crit} and temperature for *C. houstonensis* collected from two drainage basins. Neither regulation index nor DO_{crit} yielded a significant linear relationship with temperature.

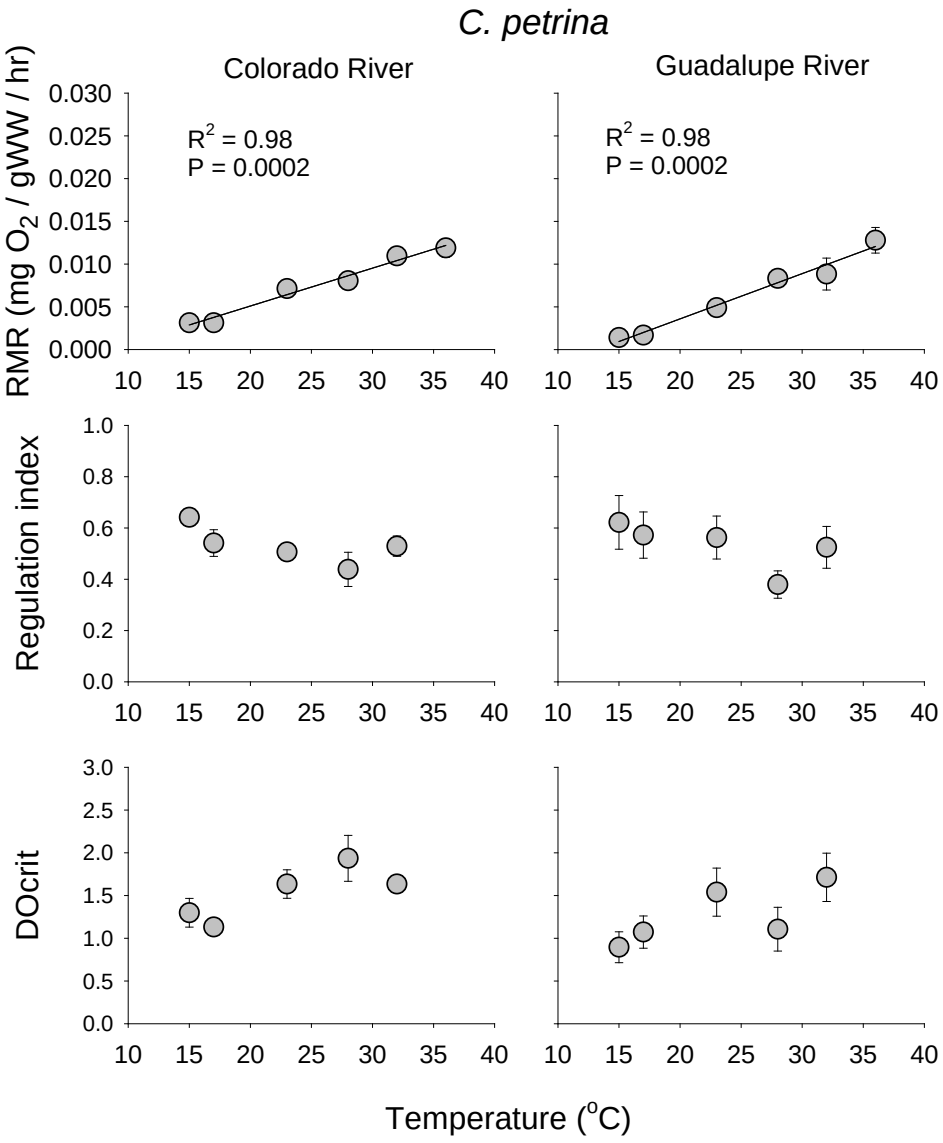

Figure 4. Relationships between resting metabolic rate (RMR), regulation index, DO_{crit} and temperature for *C. petrina* collected from two drainage basins. Neither regulation index nor DO_{crit} yielded a significant linear relationship with temperature.

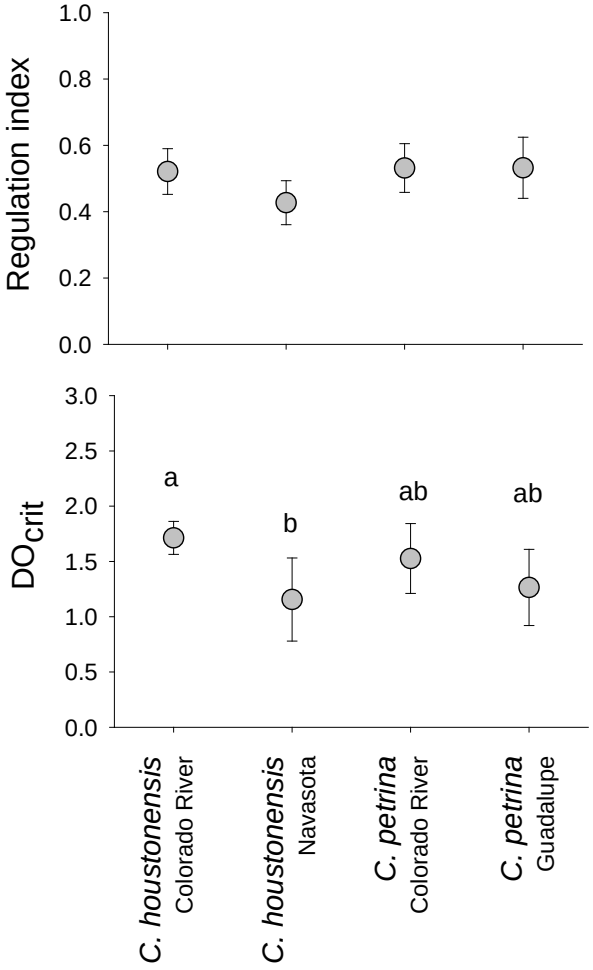

Figure 5. Mean regulation index and DO_{crit} across all temperatures for *C. houstonensis* and *C. petrina* collected from different drainage basins. No significant differences among mussel groups were found for mean regulation index. Letters indicate significant differences in DO_{crit} among mussel groups. Error bars represent ± 1 standard deviation.

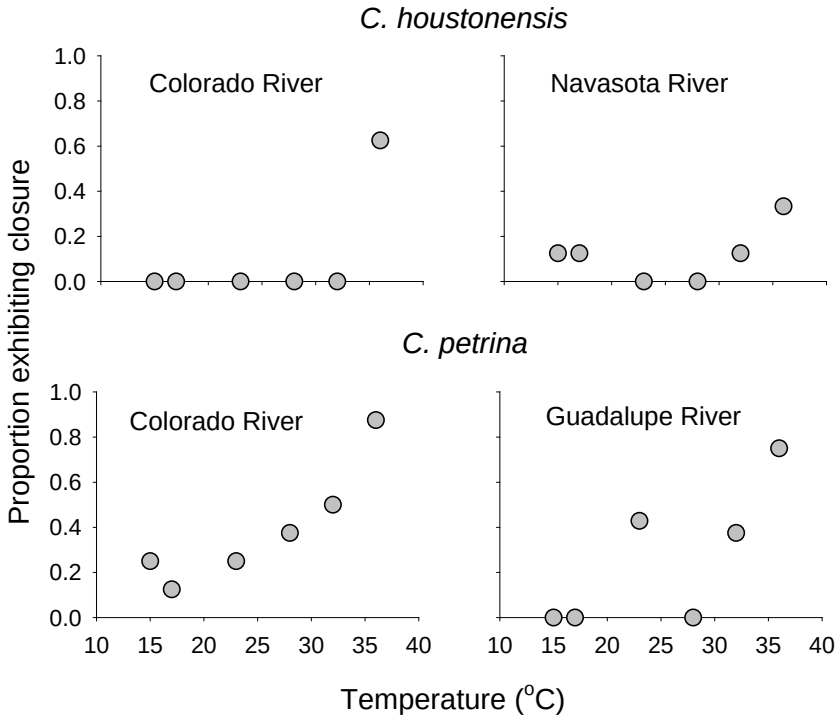

Figure 6. Proportion of individuals exhibiting at least one closure event during respirometry runs at six temperatures.

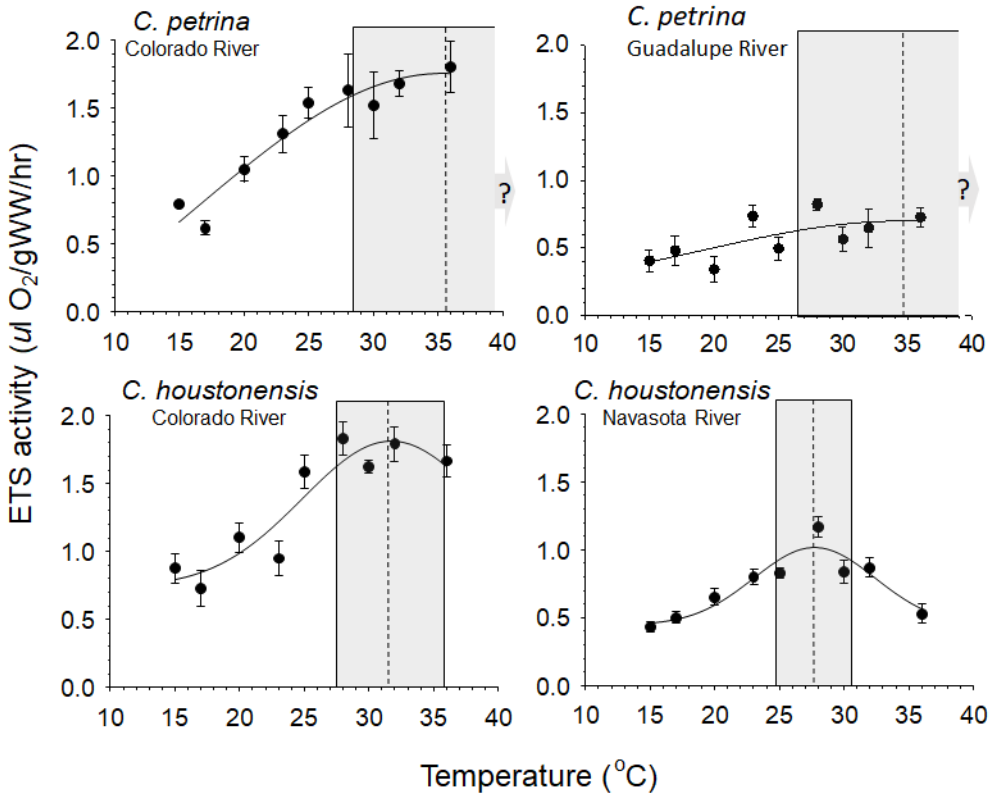

Figure 7. Relationship between ETS activity and temperature for acclimated mussels. Each data point represents the mean ETS activity of all mussels acclimated to that particular temperature. Mussel groups at each temperature were unique. ETS activity of a given mussel was measured only for the temperature to which it had been acclimated. Dashed lines indicate the optimal temperature for enzymatic activity. Grey rectangles represent the optimal temperature range within which ETS activity is within 10% of the maximum.

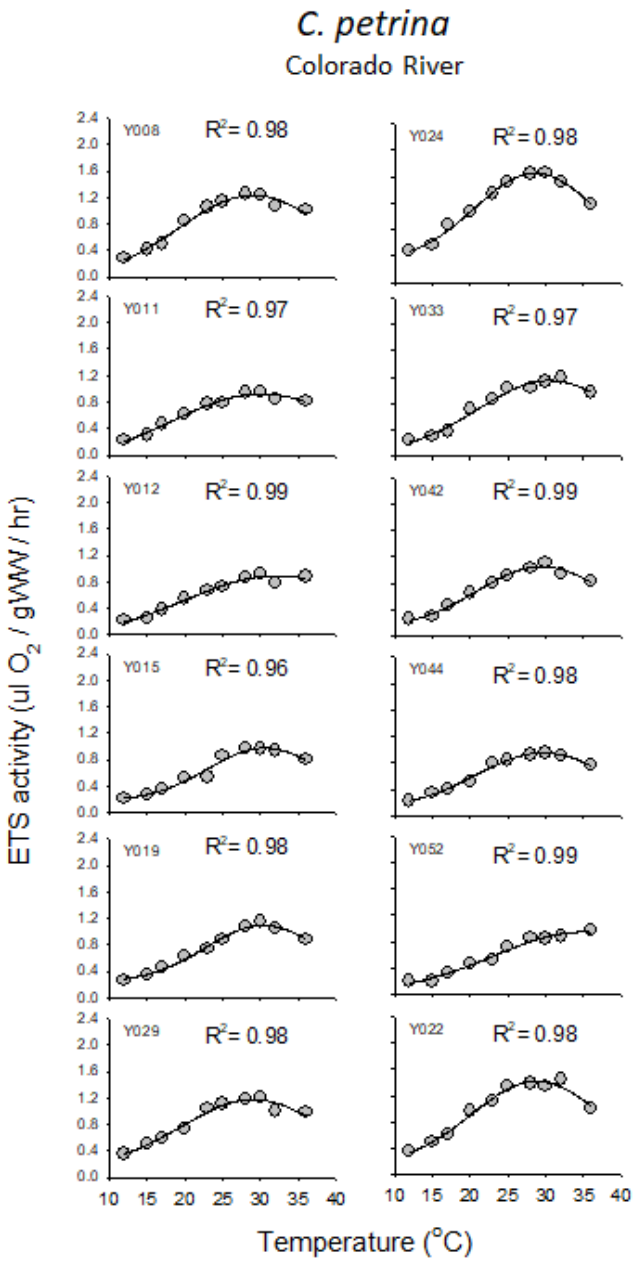

Figure 8. Relationship between ETS activity and temperature for non-acclimated *C. petrina* from the Colorado River. The tag number identifying each mussel is given in the upper left corner of each graph. Each data point represents ETS activity of enzymes collected from the same individual and incubated for 30 minutes at each temperature. Solid lines represent a four parameter Gaussian curve fitted through the data points. Optimal temperature for each individual was calculated as the temperature at the peak of each Gaussian curve.

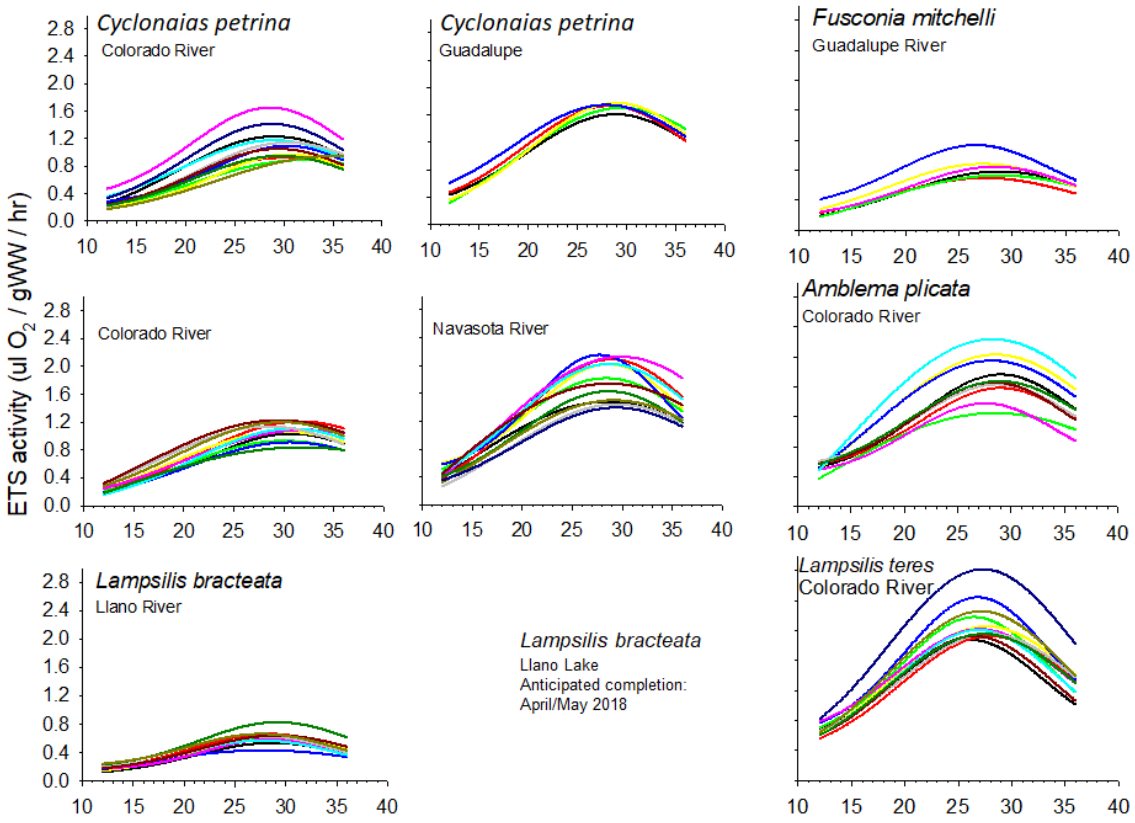

Figure 9. Summary graphs of relationship between ETS enzyme activity and temperature for non-acclimated mussels. Each curve represents a single mussel. ETS enzymes were extracted from each mussel and incubated at nine temperatures to which the mussel had not been acclimated. Colored lines represent a four parameter Gaussian curve fitted through the data for each individual mussel.

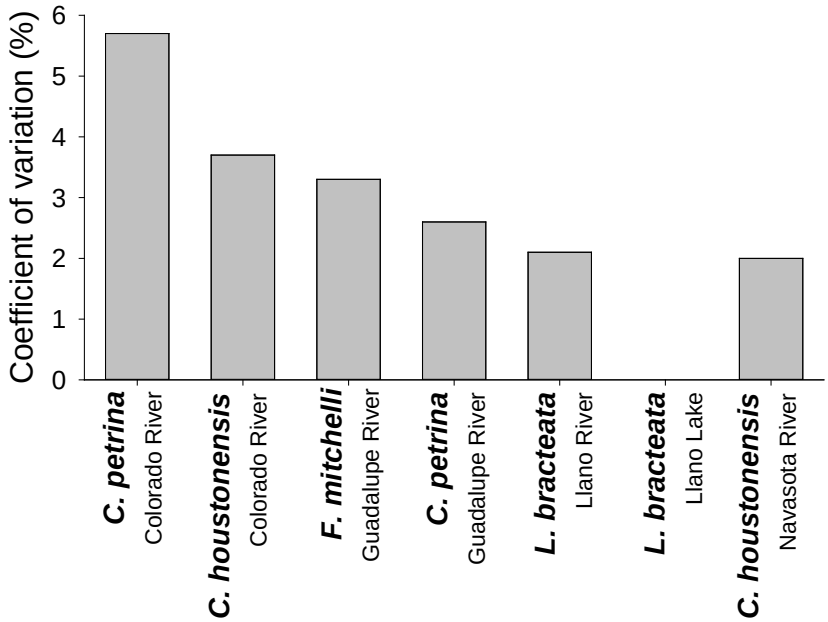

Figure 10. Coefficient of variation for optimal ETS temperatures of non-acclimated mussels from a given species and drainage, arranged in order from highest to lowest. Data for Llano Lake *L. bracteata* are expected to be available by May 2018.

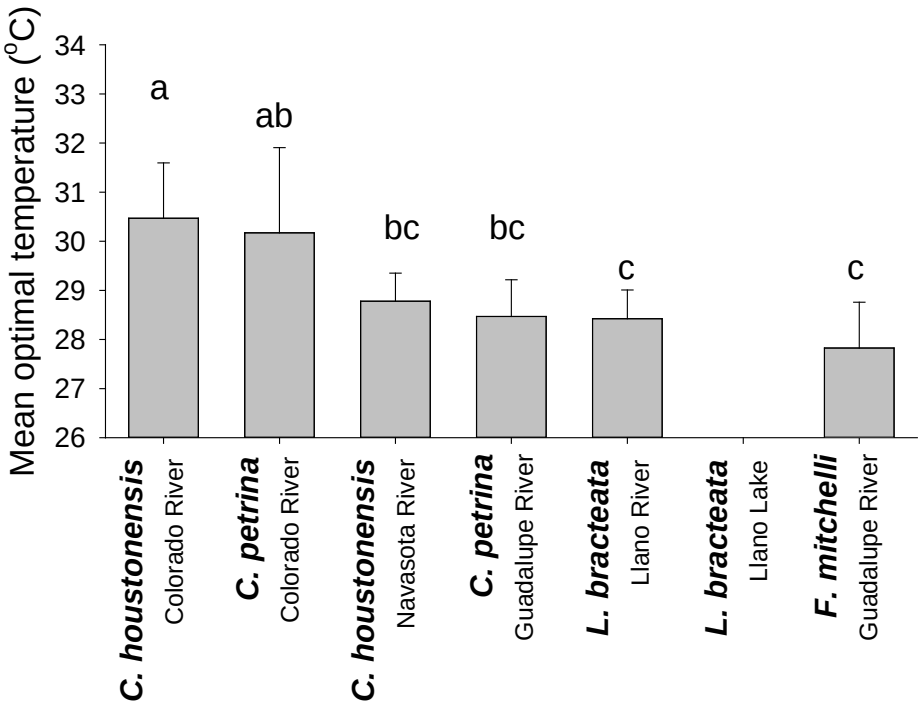

Figure 11. Mean optimal ETS temperatures of non-acclimated mussels determined from individual curves shown in Figure 2, arranged from highest to lowest. Error bars represent ± 1 SD. Letters indicate significant differences. Data for Llano Lake *L. bracteata* is expected to be available by May 2018

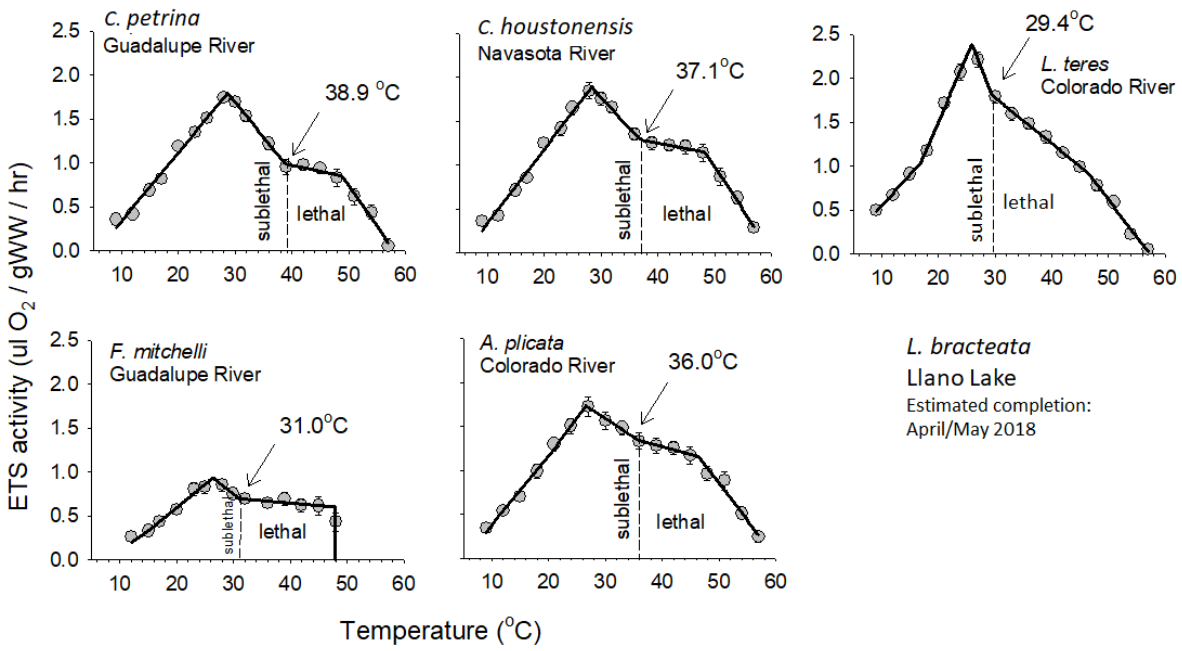

Figure 12. Relationship between ETS activity and temperature for non-acclimated mussels across the full range of experimental temperatures. Each data point within a panel represents the mean activity of enzymes extracted from the same group of 6-12 mussels. Error bars represent ± 1 standard error. Solid lines represent 5-segment piecewise regressions. Dotted lines indicate temperature at which we hypothesize sublethal effects transition to lethal effects.

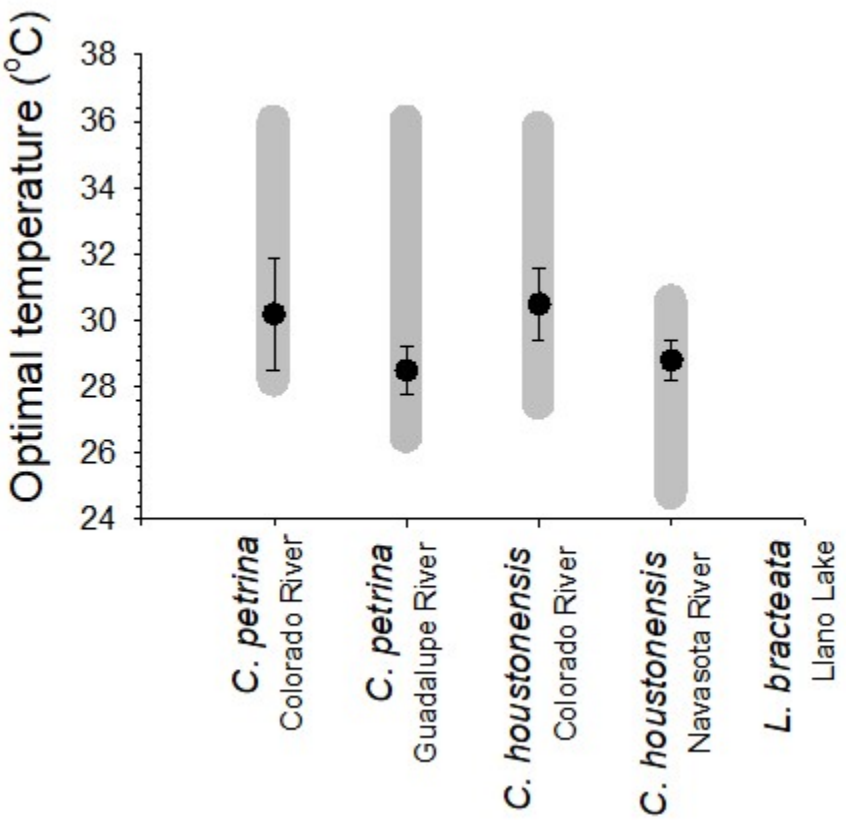

Figure 13. Comparison of optimal temperature range (grey bars) obtained from acclimated mussels and mean optimal temperature (black circles) obtained from non-acclimated mussels. Error bars represent ± 1 SD. Data for *L. bracteata* is expected to be available by May 2018.

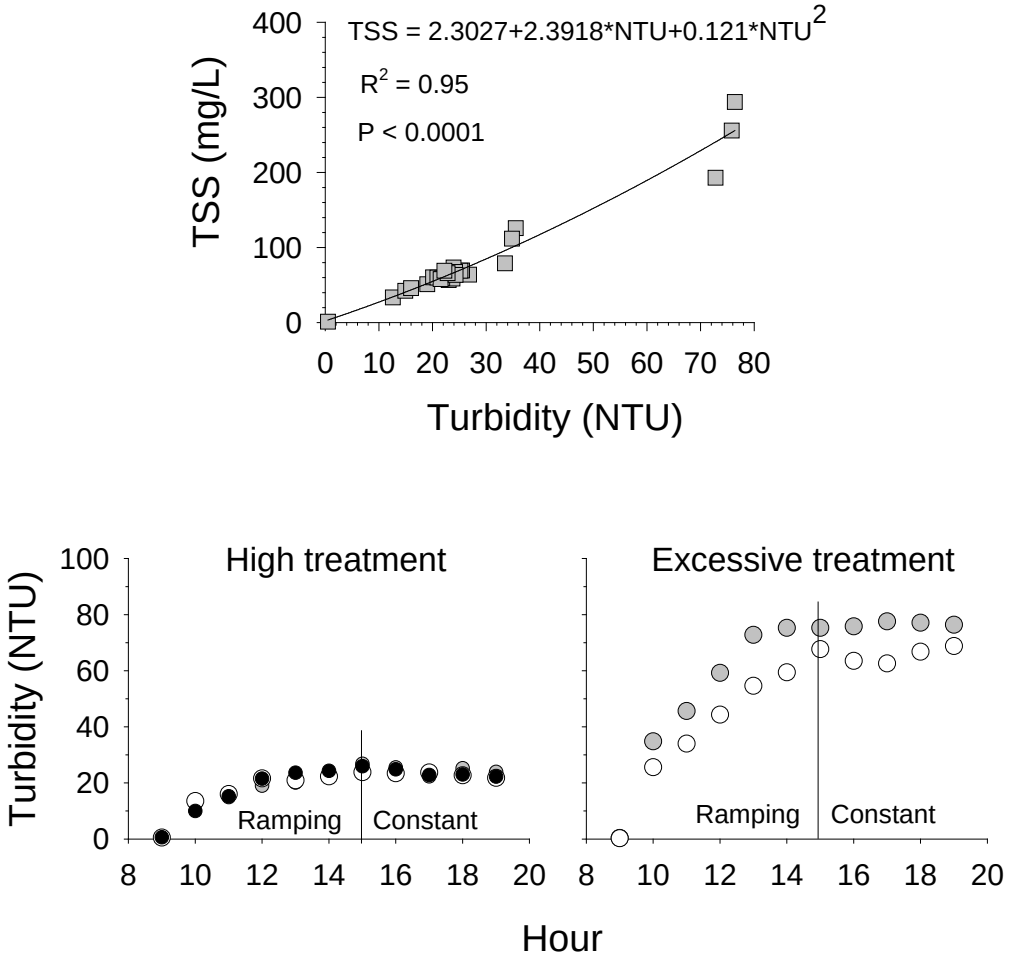

Figure 14. Top panel: Predictive relationship between turbidity (NTU) and total suspended solids (TSS mg/L). Bottom panel: Changes in turbidity through time in the High and Excessive turbidity treatments. Solid vertical line indicates the break between the ramping turbidity and the constant turbidity periods.

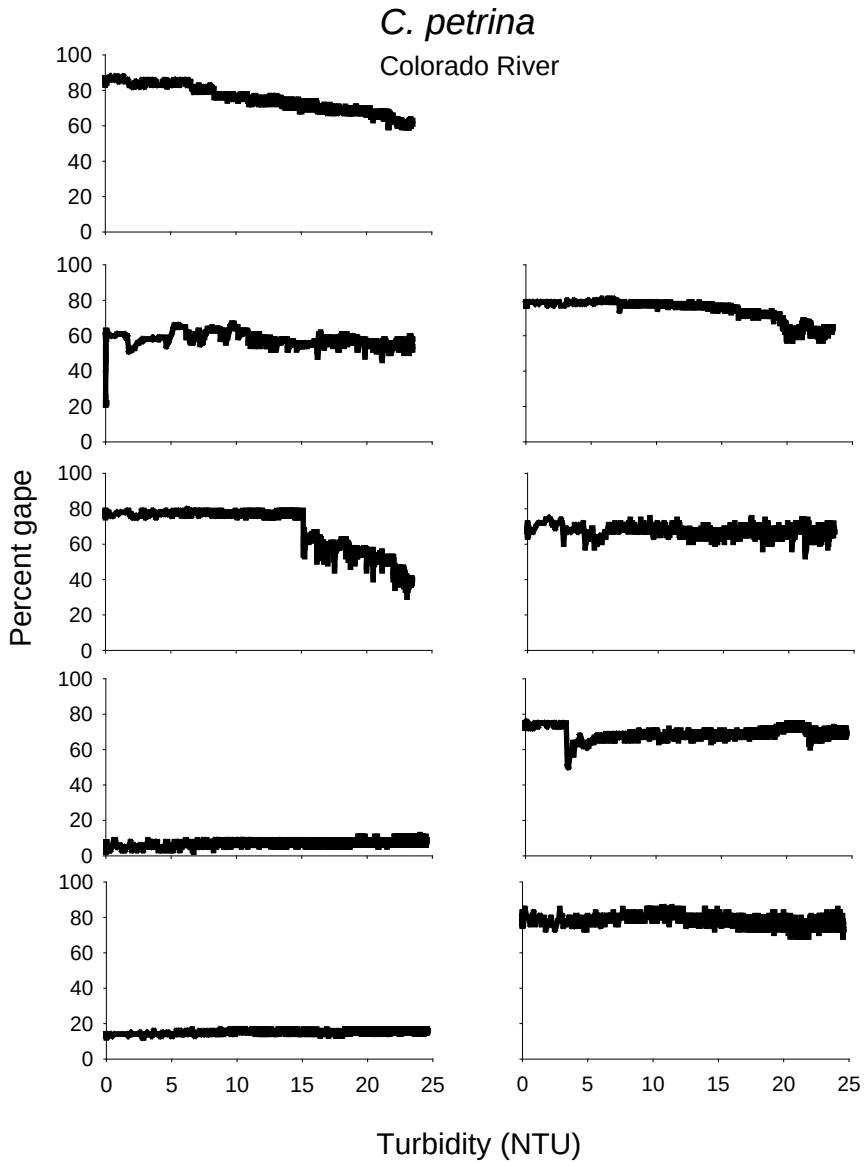

Figure 15. Percent gape for *C. petrina* from the Colorado River as turbidity increased over a ~6-hr period to a maximum of ~25 NTU. Each graph represents a unique individual.

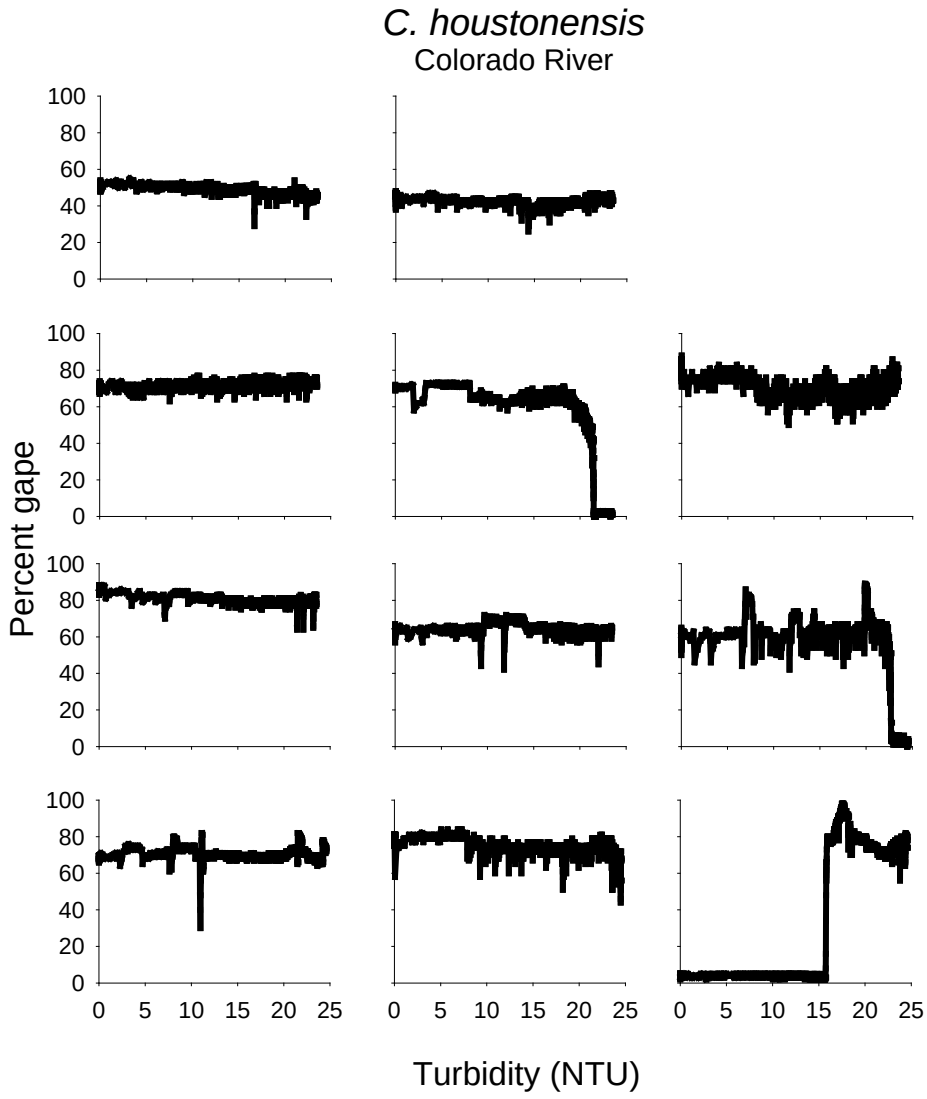

Figure 16. Percent gape for *C. houstonensis* from the Colorado River as turbidity increased over a ~6-hr period to a maximum of ~25 NTU. Each graph represents a unique individual.

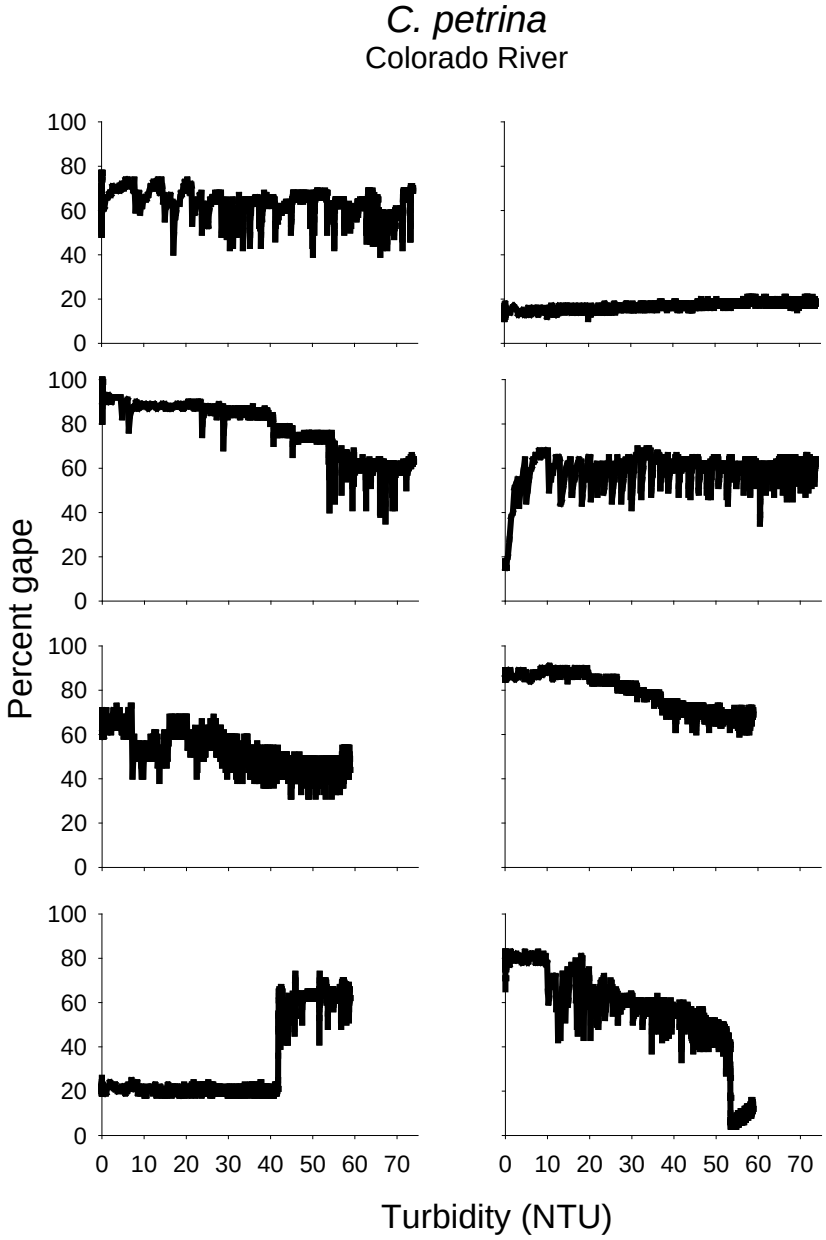

Figure 17. Percent gape for *C. petrina* from the Colorado River as turbidity increased over a ~6-hr period to a maximum of 60-75 NTU. Each graph represents a unique individual.

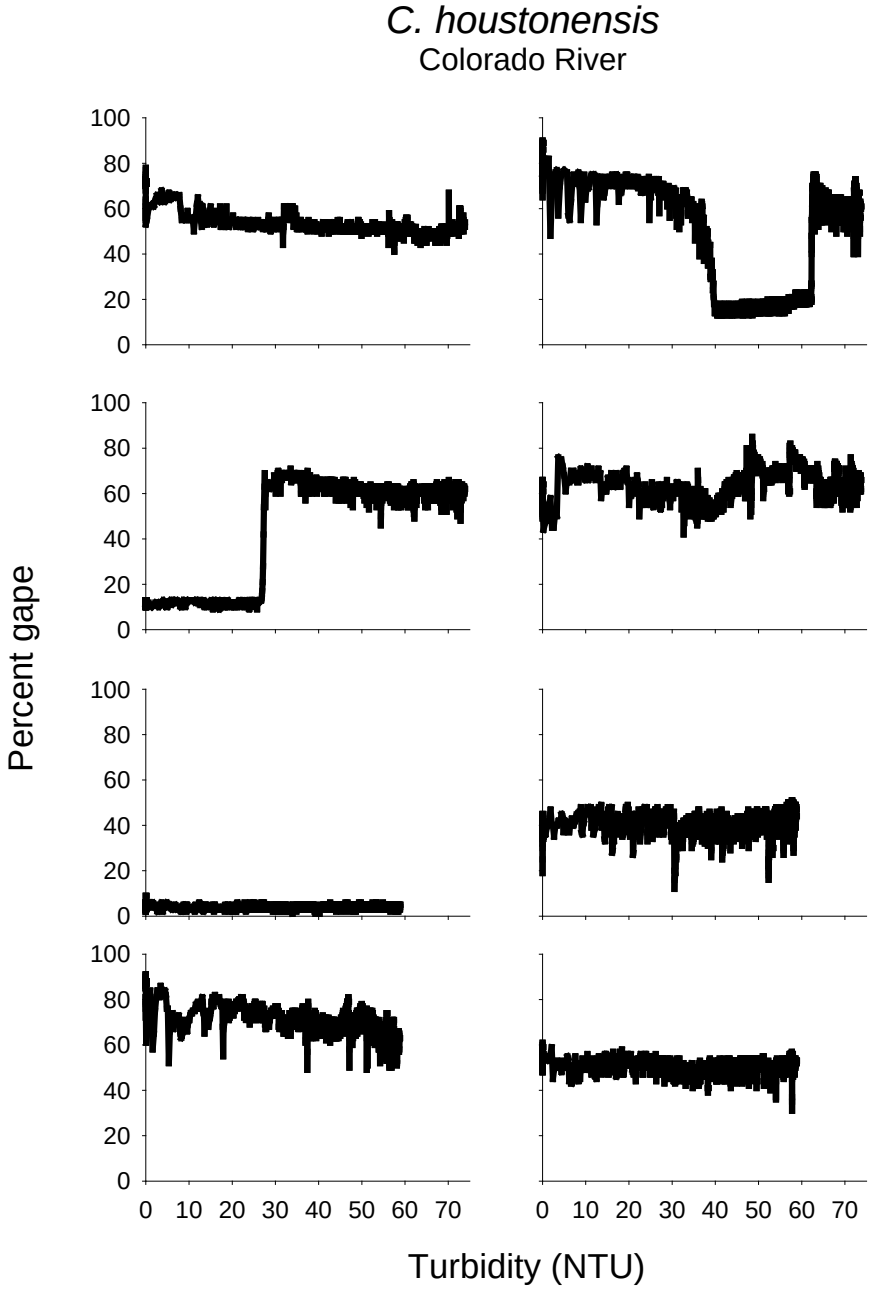

Figure 18. Mean percent gape for *C. houstonensis* from the Colorado River as turbidity increased over a ~6-hr period to a maximum of 60-75 NTU. Each graph represents a unique individual.

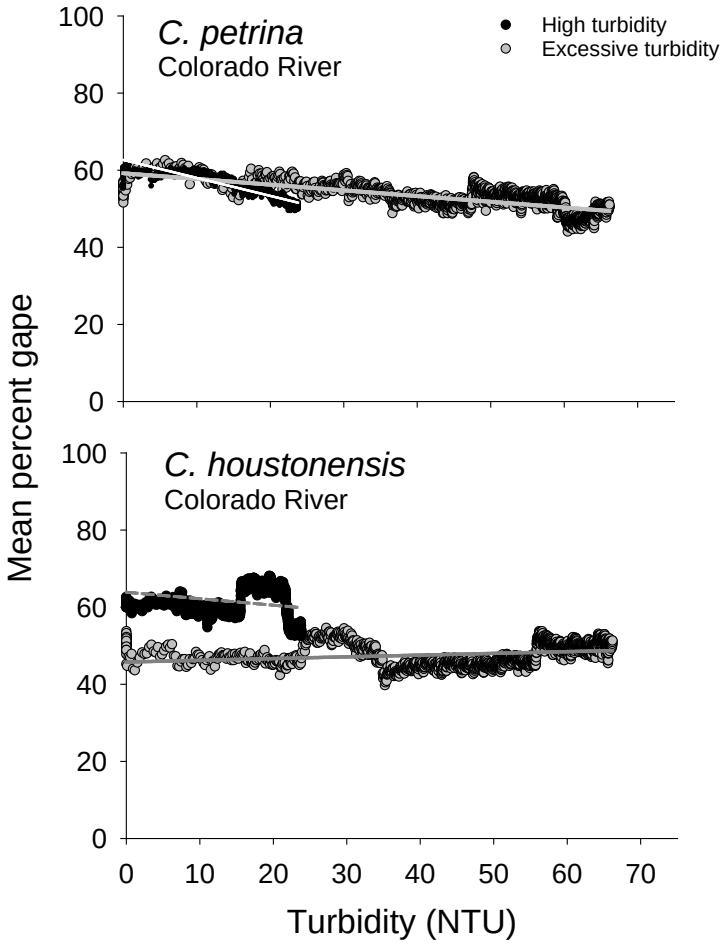

Figure 19. Mean percent gape for all individuals within each species as turbidity increased over a ~6-hr period to a high (25 NTU) or excessive (60-75 NTU) turbidity level. Solid grey lines represent linear regressions through the excessive turbidity dataset. Solid white line and dashed grey line represent linear regressions through the high turbidity datasets for top and bottom panels respectively.

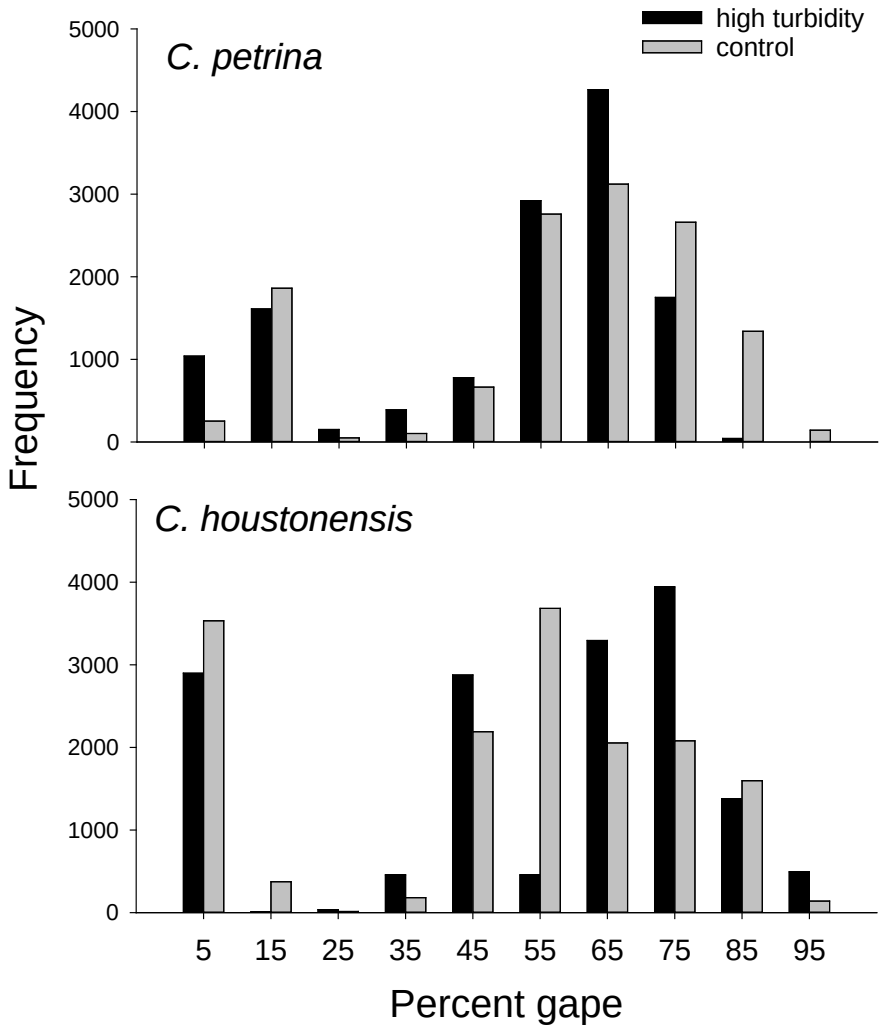

Figure 20. Frequency (# of occurrences) of a range of percent gape measurements for mussels during an initial, pre-exposure period (control: < 1 NTU) and subsequent constant turbidity period (treatment: High ~25 NTU). Only data from the same time frame (15:00 – 19:00 h) were compared between control and constant periods.

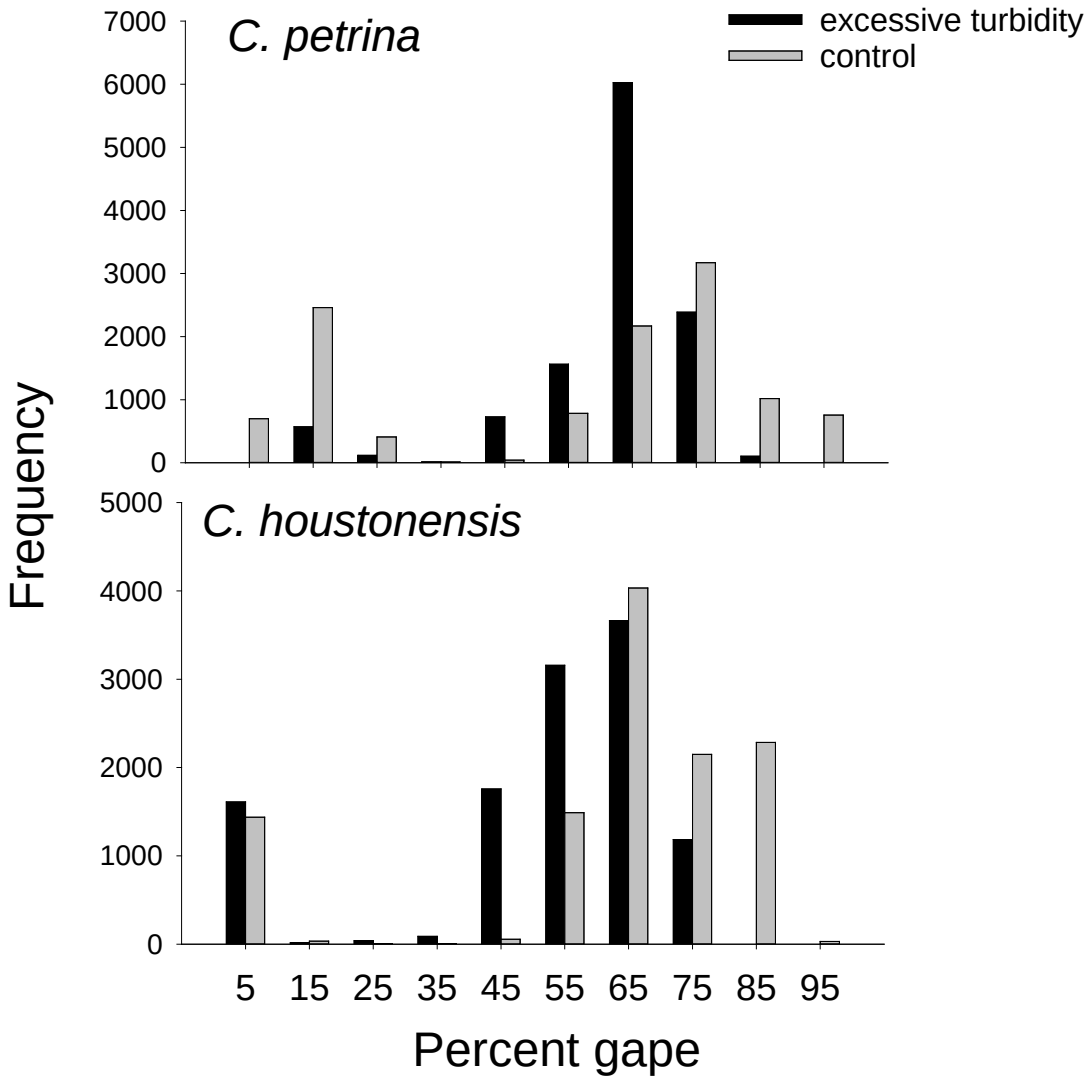

Figure 21. Frequency (# of occurrences) of a range of percent gape measurements for mussels during an initial, pre-exposure, control period (< 1 NTU) and subsequent constant turbidity period (Treatment: excessive 60-75 NTU). Only data from the same time frame (15:00 – 19:00 hrs) were compared between control and constant periods.

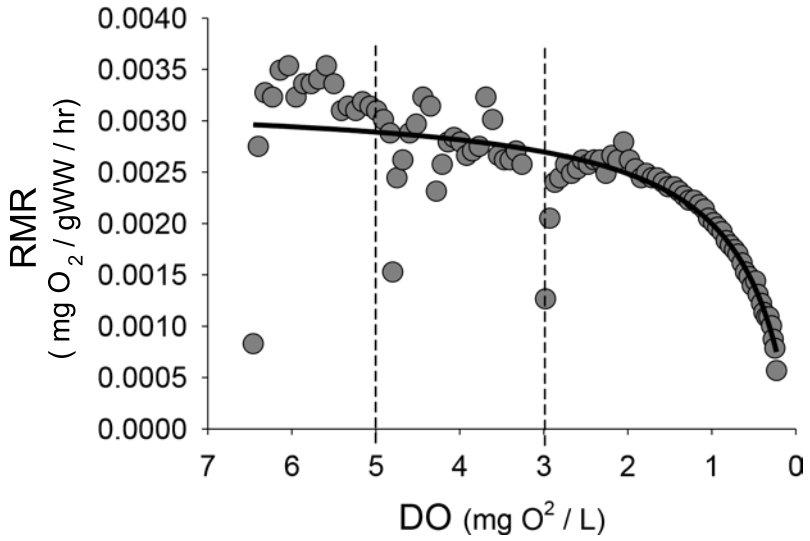

Figure 22. Typical pattern of RMR changing with declining DO when using periodic flushes to prevent excreted ammonia from accumulating in chambers. Dotted lines show when flushing occurred.

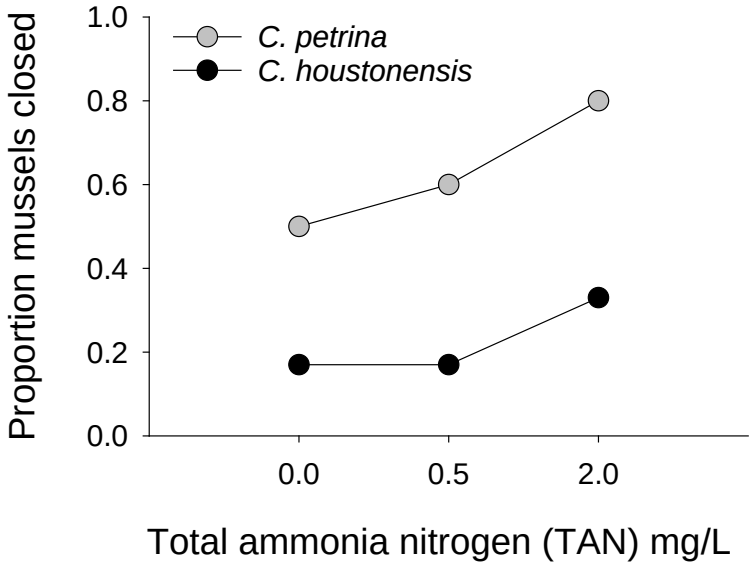

Figure 23. Proportion of mussels that exhibited at least one valve closure during respirometry at each of three TAN concentrations.

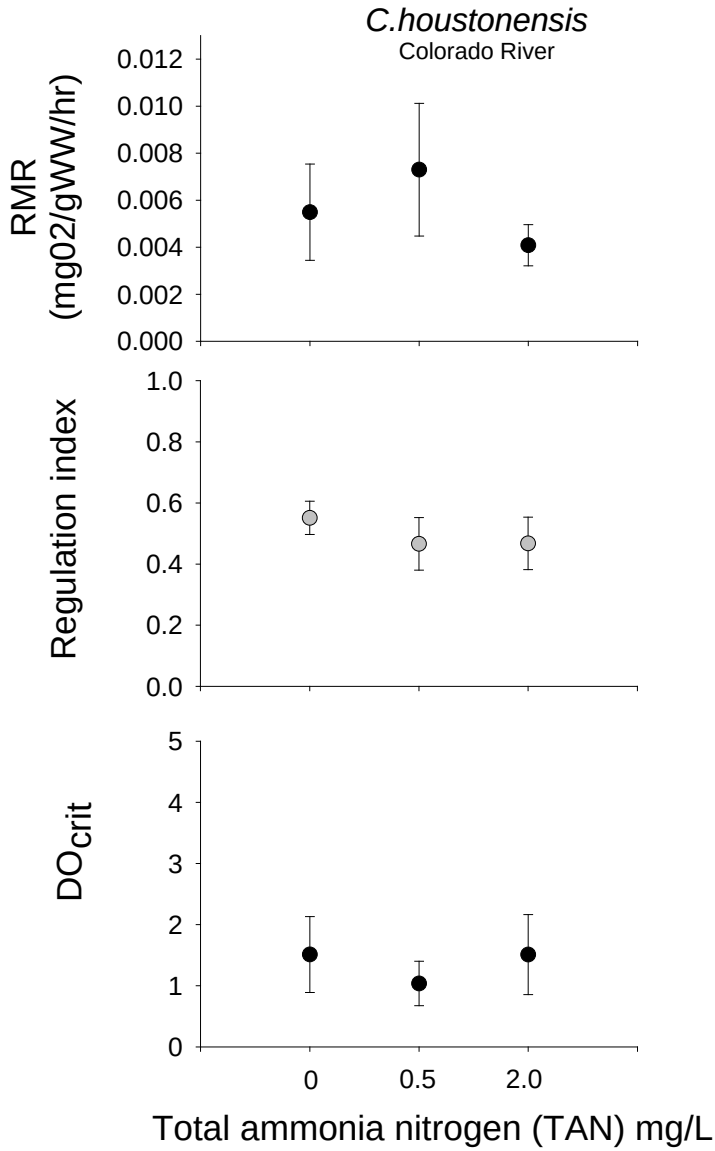

Figure 24. Mean resting metabolic rate (RMR), regulation index, and critical oxygen concentration (DO_{crit}) at three total ammonia nitrogen (TAN) concentrations. No significant differences in any response variable was found among different TAN concentrations.

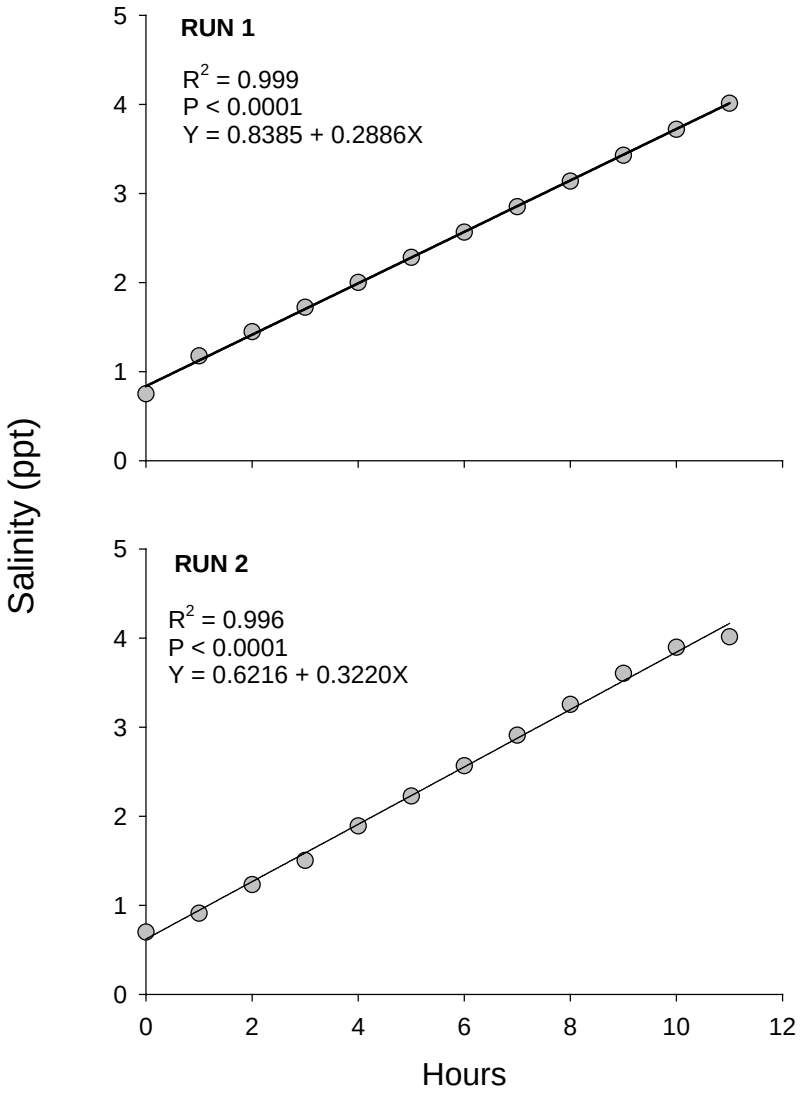

Figure 25. Increase in salinity over time for runs 1,2 of salinity experiments for *C. petrina* and *C. houstonensis*

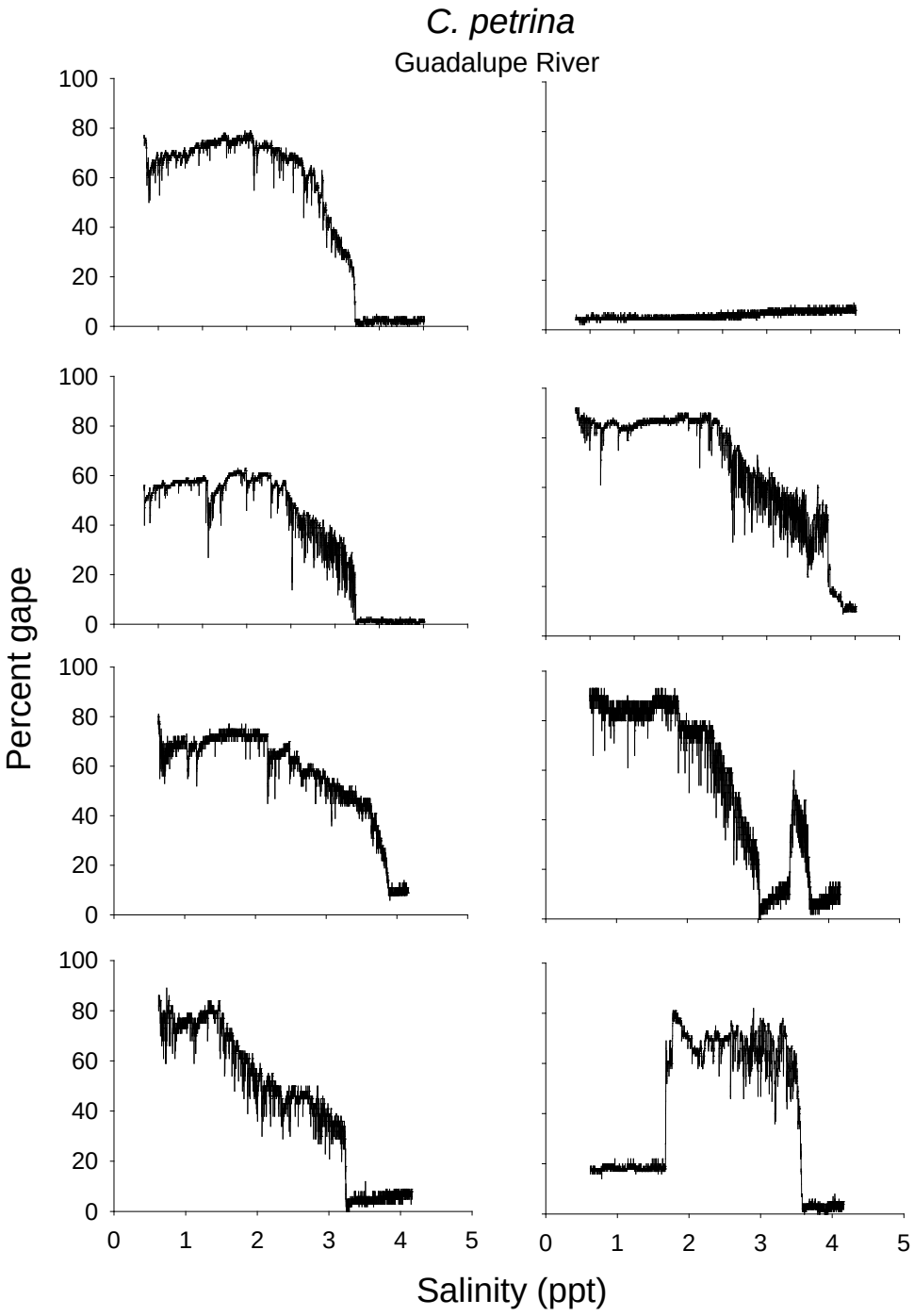

Figure 26. Change in percent gape with increasing salinity for C. petrina collected from the Guadalupe River. Each graph represents an individual mussel.

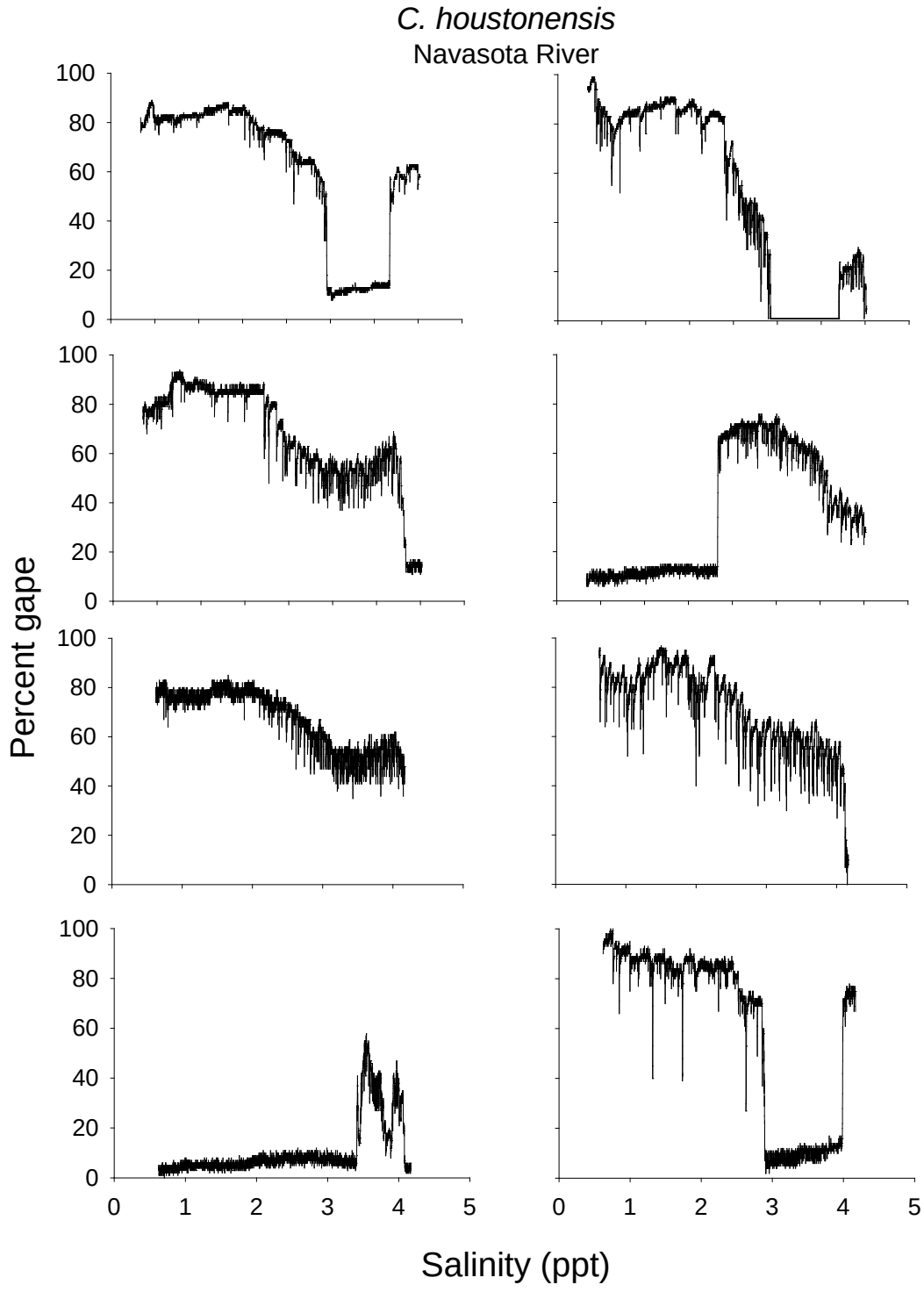

Figure 27. Change in percent gape with increasing salinity for C. houstonensis collected from the Colorado River. Each graph represents an individual mussel.

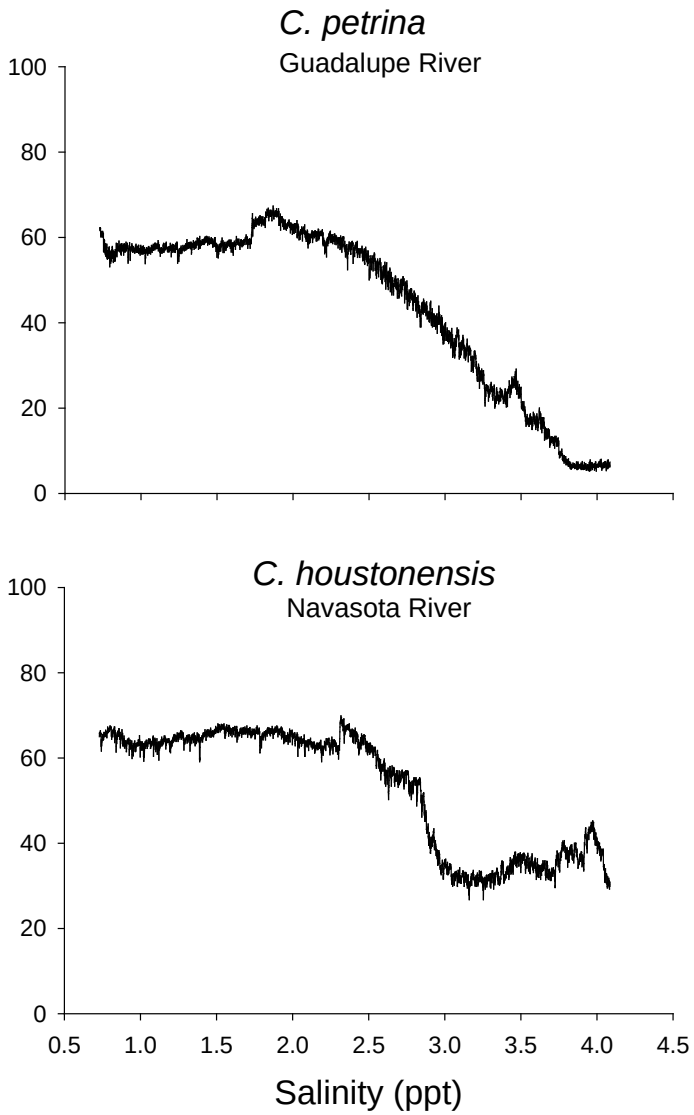

Figure 28. Change in mean percent gape (averaged across all mussels) with increasing salinity for *C. petrina* and *C. houstonensis*.

Task 2.4: Desiccation Tolerances and Behavioral Responses to Dewatering of Central Texas Endemic Mussels

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Freshwater mussels are a highly imperiled fauna that provide important ecosystem services (Vaughn and Hakenkamp 2001). Most species of mussel are largely sessile, and have limited ability to escape threats. Some species will exhibit varying degrees of vertical and horizontal movement in response to environmental and reproductive cues (Amyot and Downing 1997, Watters et al. 2001, Perles et al. 2003, Schwalb and Pusch 2007). Drought conditions can have severe negative consequences for mussels (Galbraith et al. 2010); extreme low flow events expose mussels to high temperatures, low dissolved oxygen, and often high ammonia levels, which can result in sublethal affects such as reduction in growth and cessation of reproduction, and in severe cases, high mussel mortality (Gagnon et al. 2004, Golladay et al. 2004, Haag and Warren 2008). In worst-case scenarios, extreme low flows can lead to stream dewatering, which results in mussel emersion, and, if prolonged, can lead to mortality. Therefore, the objectives of this study are to determine the desiccation tolerances of three mussel species endemic to Central Texas, and to study the behavioral responses to dewatering.

Methods

<u>Laboratory desiccation trail</u>

Species Collection and Holding

Three endemic Central Texas mussel species were examined for desiccation tolerances: *Lampsilis bracteata, Cyclonaias houstonensis,* and *Cyclonaias petrina.* Both *Cyclonaias* species were collected from the Colorado River near Altair, Texas, and the *L. bracteata* were collected from the Llano River near Mason, Texas. At the time of collection, all mussels were wrapped in towels saturated with source water within an insulated cooler and transported to San Marcos, Texas. Upon arrival to the San Marcos Aquatic Resources Center (SMARC), all mussels were placed in a coarse sand mixture within recirculating indoor raceways. Mussels were acclimated to 25 °C slowly, over a four week period, and held at this temperature for at least two weeks prior to testing. During this acclimation period, all mussels were fed daily a 2:1 mixture of Shellfish Diet 1800 and Nannochloropsis 3600 (Reed Mariculture, Campbell, California) targeting a final concentration of ca. 300,000 cells/mL.

Laboratory Desiccation

Laboratory desiccation trials were conducted at the SMARC during July through September 2017. Fifteen adult mussels of each species were placed in an environmental chamber (Powers Scientific; Pipersville, PA) maintained at 25 °C on a 16:8 hour light-dark cycle. Prior to entering the environmental chamber, all mussels were blotted dry to remove excess moisture and placed in separate 14 x 14 cm plastic weigh dishes. We assessed mussel condition daily by manually stimulating abductor mussel retraction via probing. Once mortality occurred, we measured shell length to the nearest 0.01 mm and extracted gonadal fluid to determine gender. Gender was determined by the presence of sperm or eggs (Galbraith and Vaughn 2009). Gills were also visually inspected to determine gravidity.

Statistical Analysis

Lethal time to desiccation (LT_{50}) was calculated (in days) for each species using probit analysis. A one-way analysis of variance (ANOVA) was used to examine differences in survival time between species. All statistical analysis was performed in R (version 3.4.1; R Project for Statistical Computing, Vienna, Austria) and Excel (Microsoft Corporation, Redmond, Washington) software.

Simulated field dewatering trial

Study Species

Twenty-three *L. bracteata* were collected from the San Saba River (n=11) and the Llano River (n=12) in September 2017. Twenty-four *Q. petrina* were collected from the Llano River in September 2017. All mussels were transferred to the SMARC wrapped in source-water saturated towels inside coolers. All mussels were then held in quarantine systems and acclimated to the hatchery water temperature of 19 °C not exceeding more than 1 °C temperature change per day. After a two week quarantine and acclimation period, all mussels were held under hatchery conditions for 6 weeks. All mussels were fed daily a 2:1 mixture of Shellfish Diet 1800 and Nannochloropsis 3600 (Reed Mariculture Inc. Campbell, CA) with a target dilution of 300,000 cells/mL. Prior to beginning experiment, all mussels were weighed, measured and tagged with Passive Integrative Transponders (PIT) (Biomark®, Boise, ID). PIT tags were affixed with dental cement to the left valve of each mussel, directly posterior to the medial axis. A 15 cm length of nylon fishing line was also affixed to the posterior tip of left valve of each mussel. A duct tape flag was attached to the free end of fishing line with the last 3 digits of the corresponding mussels PIT tag identification number.

Dewatering Methodology

To examine the differences in behavior between *L. bracteata* and *Q. petrina*, a simulated field-dewatering trial was conducted in November and December 2017 following the modified methods of Galbraith et al. (2015). The trials were conducted in two outdoor concrete raceways (5 m x 2 m x1 m) filled with coarse sand to simulate a small stream (Figure 2). Each raceway was filled with water to a depth of 1 m. Four gallons per minute of fresh well water were continuously added to each raceway. Twenty animals of each species were assigned without bias to one treatment and one control raceway. All mussels were evenly spaced at the top of each simulated bank. Mussel position was marked for each individual using surveying flags labeled with the last three digits of the corresponding mussel's PIT tag identification number. Water was removed from the treatment raceway via submersible pump at a rate of 0.044 m per day. Water was not removed from the control raceway. Horizontal movement was assessed twice weekly and totaled for each individual upon reaching experimental endpoint. Vertical movement was also recorded by measuring the length of fishing line above the sediment surface.

Statistical Analysis

To determine the effects of dewatering on mussel behavior, t-tests were used to test for differences in horizontal and vertical movements compared between the control and treatment groups of each species. A one-way Analysis of Variance (ANOVA) was used to test differences in lengths and weights between treatment groups. All statistical analyses were conducted using Excel (Microsoft, Redmond, WA) software.

Results

Lethal times to desiccation (LT_{50}) were 2.86 days, 18.39 days, and 32.04 days for *Lampsilis bracteata, Cyclonaias houstonensis,* and *Cyclonaias petrina* respectively. Survival times differed significantly between species (F=98.1, p<0.001) (Figure 1). Of the other factors measured which could affect survival times, shell length (Table 1) was the only one which could be recorded among species and did not have a significant interaction with survival times. Gender determination was not possible with the *Cyclonaias* species because little to no fluid was extracted from the desiccated mussels. No gravid females were found in the *L. bracteata* test organisms.

Vertical movement did not significantly differ between control and treatment groups of either species. Horizontal movement did not differ between control and treatment groups of *L. bracteata,* but did differ significantly between control and treatment groups of *Q. petrina* (t(11)=2.90, p =.007) (Figure 3). There were no significant differences in lengths and weights of mussels between treatment groups (Table 2).

Discussion

Based on the results of the laboratory desiccation trials, *L. bracteata* was predicted to display more horizontal movement than *Cyclonaias petrina.* With a greater than ten-fold time to mortality, when removed from water, it was hypothesized that *Q. petrina* would be more likely to remain stationary when exposed to dewatering. Despite these predictions, the opposite was observed. *Q. petrina,* within the dewatered group, displayed an almost four times greater average horizontal movement (2,237 mm) than that of their control counterparts (572 mm). At the

experimental endpoint, no *Q. petrina* were considered stranded, defined as an individual mussel remaining stationary out of water for greater than 48 hours. In contrast, horizontal movement of *L. bracteata* was not significantly different between treatment groups, and 30% of mussels in the dewatered group where considered stranded. These data help further elucidate the habitat niches of these two species.

Lampsilis bracteata have been found in the upper reaches of the Colorado and Guadalupe River basins and their tributaries (Morton et al. 2016, Howells 2010). Recent surveys have shown that *L. bracteata* is most commonly found in bedrock substrates with high water permanency (Burlakova and Karatayev 2010; Figure 4). Occupying habitat with low water capacity coupled with a low tolerance to desiccation make dewatering possibly a deleterious event for *L. bracteata* when compared to *Q. petrina.* This point was illustrated in a 2011 survey when a recently dewatered stretch of the San Saba River was found to contain 65 recently dead individuals of *L. bracteata* (Burlakova and Karatayev 2010). Unlike *L. bracteata*, *Q. petrina's* LT_{50} of 32 days and clear movement response make it likely more tolerant of drought induced dewatering events. *Cyclonaias petrina's* avoidance of stranding, when exposed to dewatering at the rate of 0.044 m per day, illustrates its ability to better likely respond to the conditions found at lower reaches of these systems.

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Table 1: Average lengths of mussels used in laboratory desiccation trial.

Species	**Average (mm)**	**Standard Deviation**
Q. petrina	69.86	4.73
Q. houstonensis	52.51	4.91
L. bracteata	49.05	6.49

Table 2: Average lengths and weights of mussels used in field dewatering trial.

	Lengths (mm)	Weights (g)
L. bracteata		
Treatment	61.64 (± 4.03)	37.19 (± 6.85)
Control	58.68 (± 3.67)	31.71 (± 5.97)
Q. petrina		
Treatment	45.75 (± 1.65)	20.69 (± 2.07)
Control	44.33 (± 2.23)	20.11 (± 2.85)

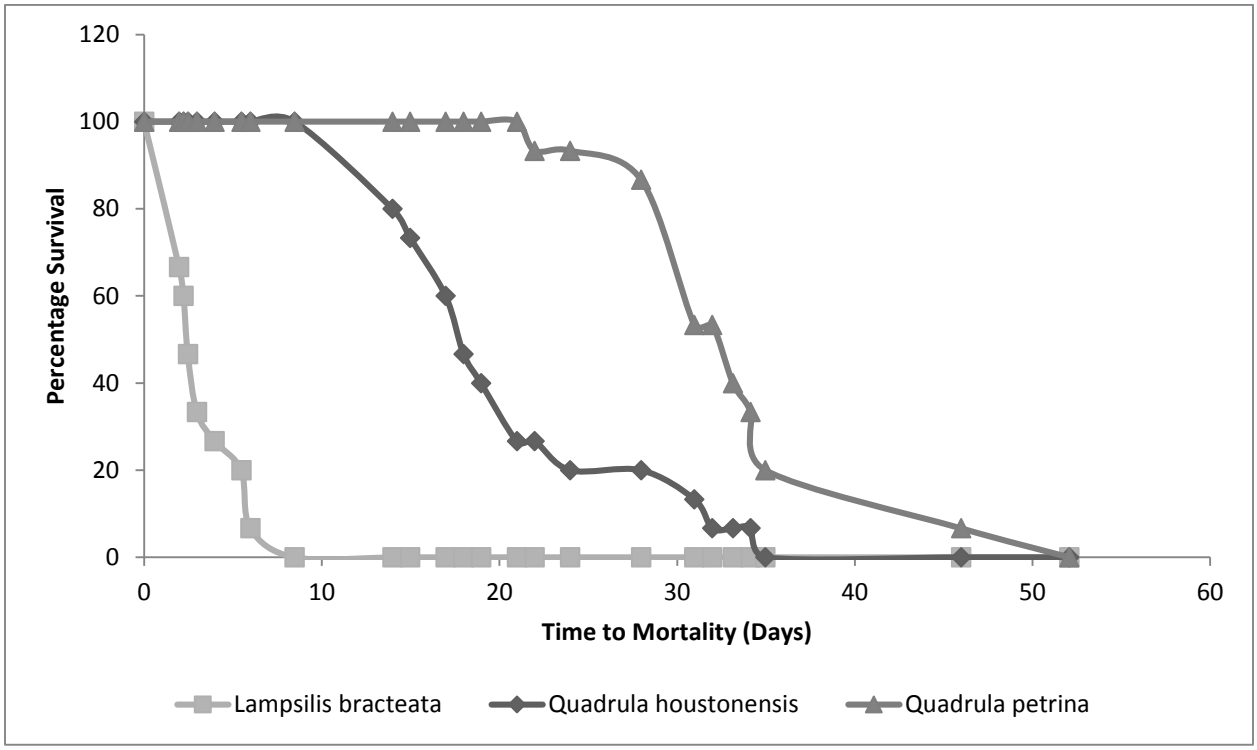

Figure 1: Percentage survival over time in laboratory desiccation trial.

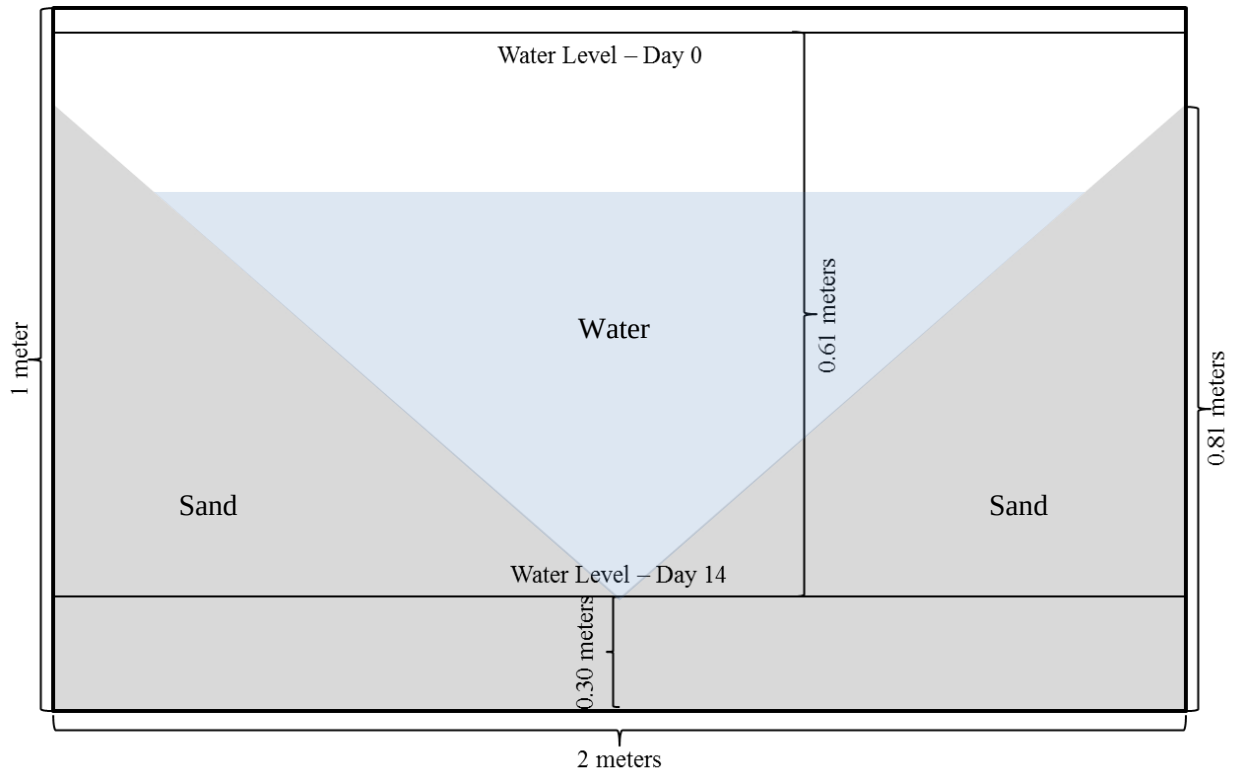

Figure 2: Cross-section view of raceway design used in simulated field dewatering trials.

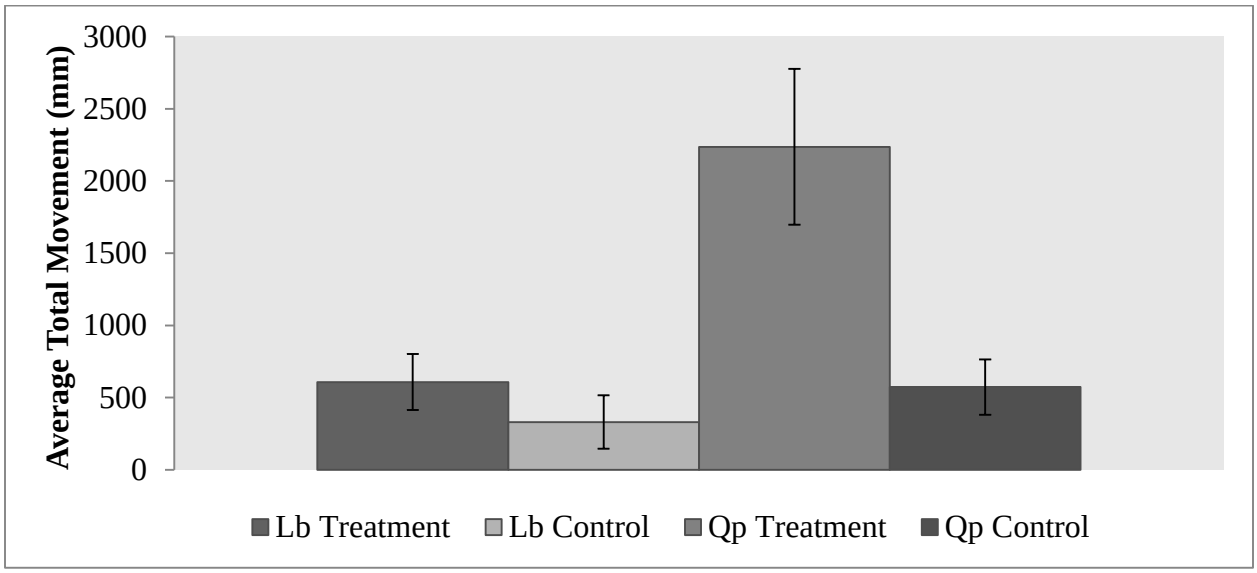

Figure 3: Average total horizontal movement of all test groups in simulated field dewatering trials. Lb = *Lampsilis bracteata*, Qp = *Cyclonaias petrina*.

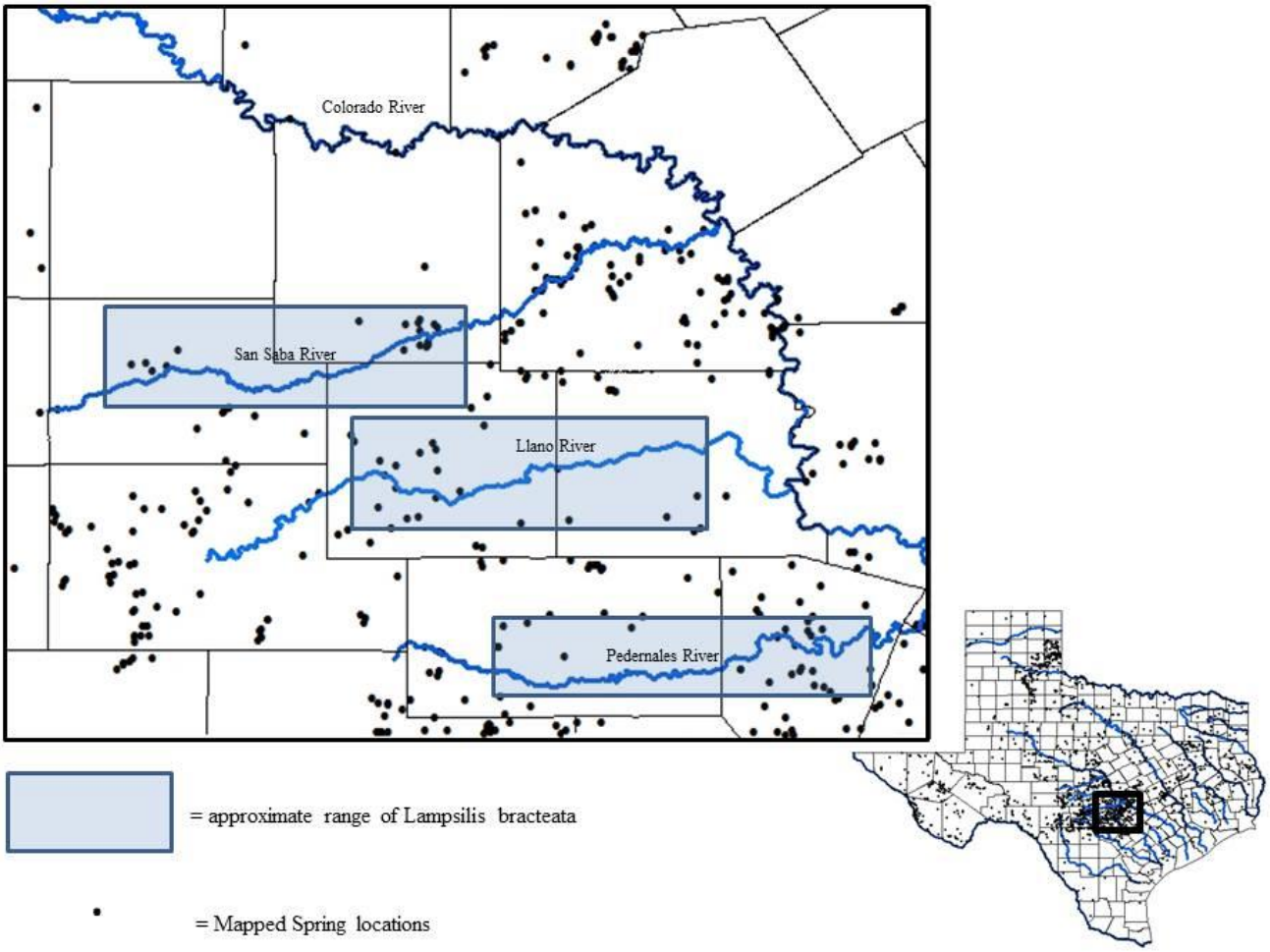

Figure 4: Map shows approximate *Lampsilis bracteata* range found in surveys by Morton et al. (2016), in relation to mapped spring locations in survey area.

Task 2.5. Stable isotope analysis to determine relative mussel food sources

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Mussels have long been considered to be suspension feeders (i.e., feeding on material suspended in the water column), with a diet comprised of phytoplankton, protozoans, detritus, bacteria, and dissolved organic carbon (Strayer et al. 2008). Previous studies using stable isotope analysis, fatty acid analysis and biochemical markers have elucidated the more prevalent components of suspended particulate organic matter (SPOM) that mussels use for food. Bacteria (Nichols & Garling 2000; Christian & Smith 2004), algae and phytoplankton (Weber et al. 2017; Raikow and Hamilton 2001) have been identified as important contributors to the freshwater mussel diet. While individual components of the mussel diet have become clearer, what they are actually assimilating from their food resources is poorly understood. Although mussels are largely considered suspension feeders, burrowing habits and associated pedal feeding (sweeping their ciliated foot through sediments) allow access to benthic food sources such as sediment-based organisms and detritus (Yeager et al. 1994; Nichols et al. 2005; Raikow and Hamilton 2001). While evidence shows that juveniles can consume benthic organic matter (Yeager et al. 1994; Haag 2012), the extent to which adult mussels utilize non-suspended food sources through pedal feeding is not fully understood.

A highly efficient method of determining mussel feeding ecology is stable isotope analysis. This approach is effective in inferring both ultimate energy sources and trophic position in organisms and can help elucidate the relative assimilation of suspended or benthic food sources (Cabana and Rasmussen 1996; Raikow and Hamilton 2001; Post 2002; Christian et al. 2004; Vuorio et al. 2007; Vaughn et al. 2008; Weber et al. 2017). Ultimate energy source is determined through carbon stable isotope ratios ($^{13}C/^{12}C$, or $\delta^{13}C$) and trophic position is determined through nitrogen stable isotope signatures ($^{15}N/^{14}N$, or $\delta^{15}N$) (Cabana & Rasmussen 1996; Vuorio et al. 2008; Newton et al. 2013). Although ^{13}C of many primary producers vary, the stable C isotope ratios of consumers are similar to that of their food reflecting the ultimate carbon source (DeNiro and Epstein 1978). However, the N pools of animals are enriched with ^{15}N relative to their food and this enrichment is on average +3.4 $^{O}/_{OO}$, i.e. 3.4 $^{O}/_{OO}$ difference in trophic levels (Deniro and Epstein 1981, Minagawa and Wada 1984). Sulfur ($^{34}S/^{32}S$) stable isotope ratios have also been useful in marine bivalve food web studies, and can potentially separate producers when stable C and N cannot (Connolly et al. 2004). Analysis of the stable isotope signatures of potential food resources for freshwater mussels in an aquatic system, including suspended organic matter, benthic sediment containing detritus, algae, bacteria and fungi mixed with sand, detritus (decaying organic leaf material), and primary producing plants, can ultimately identify the major contributors to the mussel diet in a specific system or season.

Several drivers for population decline of endemic mussels in Texas include habitat loss and destruction through impoundments, sedimentation, dewatering, and pollution (Howells et al. 1996; Randklev et al. 2013a; Randklev et al. 2013b). The status of three Texas endemics, *Cyclonaias petrina*, *Cyclonaias houstonensis* and *Lampsilis bracteata* is currently being assessed to identify conservation needs and inform management efforts. Incomplete knowledge of mussel

food resources can inhibit successful conservation and a better understanding of mussel feeding ecology can help elucidate causes of their imperilment and inform management efforts such as propagation and relocation. Thus the objectives of this study are to 1) determine the potential food resources of focal taxa through stable isotope analysis and, 2) assess spatial and temporal variations in feeding.

Methodology

Study Sites

Study sites were chosen from previously identified sites containing beds of target mussel species (*C. houstonensis*, *C. petrina* and *L bracteata*). Sites in the Colorado River watershed were located on the lower Colorado (LC; 29.556197N, -96.402160W) near Altair, Texas and on the Llano River, a tributary of the middle Colorado River (MC; 30.39267N, -99.19214W), located near Mason, Texas. The Guadalupe River drainage was sampled on the upper Guadalupe (UG; 29.93953N, -98.94846W) in Comfort, Texas and the Brazos River was sampled on the Navasota River, a tributary of the Lower Brazos (LB, 31.15155N, -96.19501W), near Easterly, Texas.

Mussel Sampling

Each mussel species (and its respective potential food resources) was sampled from two different basins each. *C. petrina* was sampled in the lower Colorado and upper Guadalupe Rivers, *C. houstenensis* was sampled in the lower Colorado and Navasota Rivers and *L. bracteata* was sampled in the upper Guadalupe and Llano Rivers. Ten individuals were collected per species at the site by snorkeling and searching benthic sediments by hand. Individual mussels were collected and measured for length, width, height and weight. Mussels were opened using

reverse action pliers and two sublethal tissue samples were taken from the foot using a 1.5 x 4.5 mm biopsy punch (Karl Storz 453733) (Fritts et al. 2015). Mussels were subsequently returned to the streambed where they were collected and tissue samples were transported on ice from the field and subsequently frozen and transported to Auburn University. Tissue samples were dried at 80°C to a constant mass, ground using a mortar and pestle, weighed (nearest 10^{-5} g) and placed in 4 x 6 mm tin capsules and sent to Washington State University (WSU) Stable Isotope Core Laboratory for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ stable isotope analysis (see below).

Potential Food Source Sampling

Hypothesized food sources were suspended particulate organic matter (SPOM), fine particulate organic matter associated with benthic sediments (FPOM), and coarse particulate organic matter (CPOM). Five 1-2 L water column samples were collected in spring (April 2017), summer (July 2017) and fall (October 2017) at all four sites to sample for SPOM. To isolate the particulate organic matter (primarily phytoplankton and detritus) in the water column that mussels could utilize as a food source, sample water was passed through 55 µm mesh to remove larger particles, as mussels utilize items <55 µm for food (Newton et al. 2013). The filtered water was then filtered again through a precombusted 47 cm Whatman GF/F filter (nominal pore size = 0.7 µm) using vacuum filtration. This procedure isolates suspended solids between 55 and 0.7 µm, which reflects the size fraction of identified potential food sources for mussels (Christian et al. 2004; Post 2002; Newton et al. 2013). Filters were then frozen for transportation back to Auburn University where they were dried at 80°C to a constant mass. The filters were fumigated in 3N H_3PO_4 for 8 hours to remove carbonates (Harris et al. 2000). Whole filters were sent to WSU Stable Isotope Core for analysis of stable C, N and S (see below).

Ten mid-channel benthic sediment samples for FPOM (which includes detritus, algae, bacteria and fungi mixed with sand) were collected in spring and summer in the same habitats where mussels were collected to reflect the available food resources mussels could access through pedal feeding. Five mid-channel, five bank and five bedrock surface sediment samples were taken from each site during fall sampling to further differentiate between benthic food sources being utilized. Sediment FPOM samples were prefiltered through an 80 and 55 □m sieve to remove gravel and debris larger than the previously established size fraction for potential mussel food resources. Samples were filtered through Whatman GF/F filters using vacuum filtration, frozen and transported to Auburn University as above with the SPOM filters. Samples were dried to a constant mass at 80°C and the sediment layer was then removed from the filter. Sediment was fumigated in 3N H_3PO_4 for 8 hours to remove carbonates and was then ground to a fine powder using a mortar and pestle, weighed (nearest 10^{-5} g) and encapsulated in 4 x 6 mm tin capsules and sent to WSU Stable Isotope Core for analysis of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ (see below).

Coarse particulate organic matter (CPOM, a mixture of decaying terrestrial leaf litter and organic aquatic material, i.e. "detritus") was collected from each site seasonally. Previous studies confirm that mussels produce the necessary enzymes to digest detrital food resources (Christian et al. 2004; Newton et al. 2013), and CPOM may be an important carbon source for bacteria production (Besemer et al. 2009). Additionally, filamentous algae and representative emergent (*Justicia*), submerged (*Myriophyllum*), and riparian ("grass") vascular plants were collected when present as further potential basal food resources and to provide primary producer context. Vascular algae and vascular plant samples were identified and separated in the field and all samples were frozen for transportation to Auburn University. Samples were dried at 80°C to a constant dry mass, ground to a fine powder using a mortar and pestle, weighed (nearest 10^{-5} g)

and placed in 4 x 6 mm tin capsules and sent for analysis of stable $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$. Water temperature, conductivity and pH were measured and discharge from the nearest USGS gauge was recorded at the time of sample collection for each site to inform any potential patterns identified in feeding ecology.

Stable Isotope Analysis

Stable carbon, nitrogen and sulfur isotopic analyses were conducted at Washington State University Stable Isotope Core Laboratory. Samples for carbon and nitrogen isotopic analysis are converted to N_2 and CO_2 with an elemental analyzer (ECS 4010, Costech Analytical) and the two gases are separated with a 3m GC column and analyzed with a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen). Isotopic reference materials are interspersed with samples for calibration. Isotope ratios are reported in parts per thousand (‰) relative to standards (Vienna Peedee belemnite (VPDB) for carbon, atmospheric N for nitrogen and Vienna-Canyon Diablo Troilite (VCDT) for sulfur), defined in delta notation as:

$$\delta_{13}C \text{ or } \delta_{15}N \text{ or } \delta_{34}S = (R_{sample} / R_{standard} - 1) \times 10^3$$

where $R = {}^{13}C / {}^{12}C$ or ${}^{15}N / {}^{14}N$ or ${}^{34}S/{}^{32}S$ (Craig 1957, Jepsen and Winemiller 2002).

Statistical Analysis

Size data for species collected were averaged across all three seasons and compared across basins (Tables 1-3). Mean ($\bar{X}$) and standard deviation (σ) were calculated for each species across all seasons. Two-tailed *t*-tests were performed to compare length, width, height and length of species across basins. $\delta^{13}C$ and $\delta^{15}N$ data was averaged for each species for spring and summer seasons across basins and a two-tailed *t*-test was performed to determine within species

variation between seasons. We compared mussel length and stable isotope ratios with Pearson Product-Moment correlations.

To estimate feeding patterns, data initially were visually inspected using biplots. We then applied linear mixing models following the IsoSource procedure (Phillips and Greg 2003) to model the contribution of potential source materials to mussels. Since isotopic ratios of baseline resources are often different across systems, we analyzed each basin independently. We only used potential sources that were represented across all basins in IsoSource models for comparative purposes. These sources were SPOM, CPOM, and FPOM. Due to difficulties in the laboratory analysis of $\delta^{34}S$ and as a conservative measure until all seasonal isotope data are received from the analytical lab (see below), we constrained modeling to $\delta^{13}C$ for this report. Thus a 3 source single-isotope model was run for each mussel species for each site with a source increment set at 1% and tolerance initially set at 0.1‰ and increased incrementally (up to 2‰) until a solution was obtained. This model effectively assesses the relative contribution of CPOM, SPOM and FPOM to the basal carbon source(s) of mussel diet (i.e., this model may or may not reflect *direct* feeding). Reflecting this approach, and as a conservative measure, we did not correct consumer $\delta^{13}C$ in these models due to lack of information on the true trophic position of these species.

Results

Mussel Size

C. petrina was significantly larger in length, width, height and weight in the lower Colorado River compared to the upper Guadalupe River with mean length difference of 17.78 mm, width difference of 19.03 mm, height difference of 14.63 mm and weight difference of

70.31 g (two-tailed *t*-test; p = 1.23E-08 for length, p = 1.74E-12 for width, p = 1.69E-14 for height, p = 1.66E-07 for weight) (Table 1).

C. houstonensis was significantly longer and wider in the lower Colorado River compared to the lower Brazos River basin. Mean length difference was 6.12 mm and mean width difference was 5.88 mm (two-tailed t-test; p = 0.00094 for length; p = 0.00034 for width). There was no difference found in height and weight across basins (two-tailed *t*-test; p = 0.79 for height, p = 0.16 for weight) (Table 2).

L. bracteata was significantly larger in length, width, height and weight in the upper Guadalupe compared to the middle Colorado River basin with mean length difference of 5.11 mm, width difference of 2.46 mm, height difference of 1.46 mm and weight difference of 2.77 g (two-tailed *t*-test; p = 0.0047 for length, p = 0.019 for width, p = 0.018 for height, p = 0.022 for weight) (Table 3).

Environmental Sampling

The upper Guadalupe consistently had lower water temperatures due to being spring-fed. pH had low variability across seasons and sites. Conductivity and discharge were greatest in the lower Colorado River and lowest in the lower Brazos River basin during all seasons sampled (Table 4).

Stable Isotope Analysis

Stable isotope $\delta^{13}C$ and $\delta^{15}N$ analysis was completed for mussel foot tissue, SPOM, FPOM, CPOM and primary producing plants for samples collected in April 2017. Stable isotope $\delta^{13}C$ and $\delta^{15}N$ analysis also was completed for mussel foot tissue, SPOM, FPOM, CPOM, and primary producing plants for samples collected in July 2017. Laboratory analysis of $\delta^{34}S$ from

April samples was unsuccessful due to low sample volume causing sulfur detachment. This was corrected in sampling protocol for future seasons, yet summer and fall data have not been returned from the WSU Stable Isotope Core Laboratory and thus are not available for statistical analysis at this time. For samples collected in October 2017, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotope data has not been returned from WSU at this date. Presumably due to the impacts of Hurricane Harvey on the lower Colorado River, only three *C. petrina* and four *C. houstonensis* were located for fall sampling, compared to the standard sampling protocol for this study of 10 individuals per species per site.

Average isotopic signatures (± SD) and C:N ratios for all sampled sources and potential resources are presented (Table 5). In general, mussels exhibited $\delta^{15}N$ enrichment and $\delta^{13}C$ depletion relative to their respective environments with minimal intraspecific variation in both $\delta^{13}C$ and $\delta^{15}N$ (Table 6, Figures 1-4). Despite this, mussel size and $\delta^{13}C$ were positively correlated for mussels in the Upper Guadalupe and Lower Colorado and $\delta^{15}N$ and size was positively correlated for *C. houstonensis* in the Upper Guadalupe (Figure 5-7). An enrichment of $\delta^{13}C$ as mussels increase in size may reflect ontogenetic feeding shifts. For the spring, average $\delta^{13}C$ signatures for mussels ranged from -29.91‰ – -26.63‰ and average $\delta^{15}N$ ranged from 7.38‰ – 13.41‰ (Table 6). For the summer, average $\delta^{13}C$ signatures for mussels ranged from -26.45‰ – -28.83‰ and average $\delta^{15}N$ ranged from 7.28‰ – 13.38‰ (Table 6). Across seasons, mussels were more $\delta^{13}C$ enriched in the Middle Colorado basin and more $\delta^{15}N$ enriched in the Lower Colorado (Table 6). Also, where multiple species were sampled at a given site (Upper Guadalupe and Lower Colorado), all mussels had nearly identical isotopic signatures for both $\delta^{13}C$ and $\delta^{15}N$ (Table 6, Figure 1 and 2).

We observed seasonal changes in mussel isotopic signature, indicative of potential shifts in feeding and/or assimilation. There were significant changes in $\delta^{13}C$ signatures of *C. petrina*

between spring and summer with a mean enrichment of 0.90 ‰ from spring to summer in the upper Guadalupe (two-tailed *t*-test; $p = 0.0015$) and a mean enrichment of 1.18 ‰ in the lower Colorado (two-tailed *t*-test; $p = 3.43E\text{-}06$). *C. houstonensis* also had significant changes of $\delta^{13}C$ between spring and summer with a mean enrichment of 1.54 ‰ in the lower Colorado (twotailed *t*-test; $p = 0.0021$) and a mean enrichment of 0.84 ‰ in the lower Brazos basin (two-tailed *t*-test; $p = 0.0021$). There were no seasonal differences in $\delta^{13}C$ of *L. bracteata* in the Upper Guadalupe (two-tailed *t*-test; $p = 0.58$) or in the middle Colorado basin (two-tailed *t*-test; $p = 0.35$). There were no seasonal differences in the $\delta^{15}N$ signatures of any species in any basin (two-tailed *t*-test; $p > 0.05$).

Linear Mixing Models

Linear mixing models revealed that food resources are comprised primarily of materials with a carbon base of CPOM (i.e., benthic detritus) and to a lesser extent SPOM (i.e., seston) and FPOM (i.e., sediment deposits). All mussels and all seasons had high proportions of CPOM contributions, with the exception of *L. bracteata* in the Middle Colorado basin during spring. In general, there was little seasonal variation in dietary contributions of basal C resources, with the exception of *C. houstonensis* in the Lower Brazos basin, which showed a decreased reliance on CPOM in the summer, and *L. bracteata* in the Middle Colorado, which showed a shift from SPOM to CPOM contributions (Table 8, Figure 8). It should be noted that mussel stable isotope ratios remained largely consistent throughout both seasons within a given system, but basal resource signatures were often variable between seasons. Thus linear mixing models likely reflect less of a feeding shift in mussels and more of a changing food base offering insight on the timing of assimilation. Also, several models were only attainable after increasing tolerance

above reliable levels (up to 2‰). These included *C. petrina* in the Upper Guadalupe during the summer and *L. bracteata* in the Upper Guadalupe in the spring and summer. These models should be interpreted with extreme caution. Other models had tolerance levels set at 0.1‰.

For *C. houstonensis*, the mean dietary contribution of CPOM ranged from a low of 62.5% in the Lower Brazos basin during the summer to 96%, also in the Lower Brazos basin during the summer (Table 8). FPOM-based food sources represented a slightly higher proportion of total during the spring in the Lower Brazos basin whereas SPOM had a higher representation in Summer. In the Lower Colorado, *C. houstonensis* had somewhat higher contributions of SPOM than FPOM in the spring and summer, but this was still <5% on average of total dietary contribution (Table 8, Figure 8).

For *C. petrina*, the mean dietary contribution of CPOM ranged from a low of 93.9% during the spring in the Lower Colorado to 99% in the Upper Guadalupe, also in the spring (Table 8). FPOM-based food sources contributed near 1% in the Lower Colorado in both seasons and less than 1% in the Upper Guadalupe in both seasons. In the Lower Colorado, SPOM contributed on average 3 - 4.9% to total *C. petrina* diet but only 1 - 1.7% to total diet in the Upper Guadalupe (Table 8, Figure 8).

The contribution of CPOM based carbon for *L. bracteata* was more variable than for the other mussel species, with a high of 100% in the Upper Guadalupe in the summer to 2% in the Middle Colorado basin during the spring. The contribution of FPOM was similarly low for *L. bracteata* in both seasons and both systems, with averages ranging from 0 in the Upper Guadalupe to 5.4% in the Middle Colorado basin during the summer. The dietary contribution of SPOM based sources ranged from 0 in the Upper Guadalupe in the summer to 97.8% in the Middle Colorado basin during the spring (Table 8, Figure 8).

Brief Interpretation

These data reflect samples from spring 2017 ($\delta^{13}C$ and $\delta^{15}N$, no sulfur) and summer 2017 (carbon and nitrogen, no sulfur). Sulfur results from summer and the entire fall field season have not been returned from WSU analytical lab at the time of writing. Further, based on preliminary analysis, additional food sources were captured/isolated in fall (e.g., periphyton on hard surfaces, additional primary producers, and additional size fractions in FPOM). Any interpretation based on these data presented should be considered *preliminary* and undoubtedly more insight will be available once all data are received and full analyses can be complete. Several preliminary conclusions however seem to be arising. First, all three species appear to feed similarly. This is evidenced by the consistent stable C and N signatures across systems, and particularly the nearly identical signatures of *L. bracteata* and *C. petrina* in the Upper Guadalupe, and *C. petrina* and *C. houstonensis* in the Lower Colorado. The only deviation from this similarity is *L. bracteata* in the Middle Colorado, which displayed a relatively less-enriched $\delta^{15}N$ pattern. This characterization was from the Llano River, and whether this reflects differential feeding or simply is a system artifact is unclear at this point. Also, all three species showed a general trend to become carbon-enriched with increasing body size. This could reflect the inherent shift in diet from the parasitic glochidial stage thru adulthood. However, these trends were found in mussels in the Upper Guadalupe and Lower Colorado, but not the Middle Colorado basin and Lower Brazos basin, so that, again, a system artifact cannot be ruled out at this time.

From the data presented, with the exception of *L. bracteata* in the Middle Colorado basin during the summer, a major interpretation is that a majority of the carbon assimilated by all species is derived from CPOM. Whether this is direct feeding on CPOM particles or associated bacteria and fungi is not clear. Further, the role of SPOM (i.e, suspended producers and other

associated organics) and FPOM (benthic diatoms, algae, and organic matter associated with sediment) seem to play a much more minor role in contribution to dietary C as compared to CPOM. This could be interpreted as a reduced dietary reliance on filter-feeding phytoplankton and pedal feeding in sediments. However, it should be noted that mussels in general showed minimal (albeit statistically significant) seasonal variation, while food sources often showed considerable variation. This could indicate mussels assimilate food resources that are produced seasonally, or that we have yet to capture the C sources for their food. Full analysis of the complete dataset ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$), including updated mixing models with additional food sources and size fractions, should elucidate these trends.

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Table 1. Size data for *C. petrina* sampled in the upper Guadalupe River and the lower Colorado River. Note significant p-values in bold (two-tailed t-test with unequal variance).

	Upper Guadalupe		**Lower Colorado**		
C. petrina	$\bar{X}$	σ	$\bar{X}$	σ	*p*
Length (mm)	48.12	5.19	65.90	10.12	**1.23E-08**
Width (mm)	33.46	4.55	52.49	7.41	**1.74E-12**
Height (mm)	18.83	2.75	33.46	4.84	**1.69E-14**
Weight (g)	18.40	7.02	88.71	41.61	**1.66E-07**

Table 2. Size data for *C. houstonensis* sampled in the lower Colorado River and the lower Brazos River basin. Note significant p-values in bold (two-tailed t-test with unequal variance).

	Lower Colorado		**Lower Brazos**		
C. houstonensis	$\bar{x}$	σ	$\bar{x}$	σ	*p*
Length (mm)	50.34	7.07	44.22	4.97	**0.00094**
Width (mm)	44.46	5.88	38.58	5.14	**0.00034**
Height (mm)	28.10	3.54	27.83	3.86	0.79
Weight (g)	44.61	18.9	37.52	14.16	0.16

Table 3. Size data for *L. bracteata* sampled in the upper Guadalupe River and the middle Colorado River basin. Note significant p-values in bold (two-tailed t-test with unequal variance).

	Upper Guadalupe		**Middle Colorado**		
L. bracteata	$\bar{X}$	σ	$\bar{X}$	σ	*p*
Length (mm)	50.69	4.53	45.58	8.39	**0.0047**
Width (mm)	32.13	3.21	29.67	4.50	**0.019**
Height (mm)	19.26	1.83	17.80	2.70	**0.018**
Weight (g)	15.12	4.40	12.33	4.56	**0.022**

Table 4. Seasonal environmental data collected at sites in spring, summer and fall of 2017 during mussel and food source sampling.

Season	Site	Temp (ºC)	pH	Conductivity (µS/cm)	Discharge (cfs)
Spring	UG	22.6	8.3	471	125
	LC	24.5	8.5	500	1310
	MC	24.1	7.9	329	138
	LB	21.7	7.5	340	38
Summer	UG	26.5	8.4	497	55
	LC	32.7	9.2	584	1225
	MC	31.5	8.7	354	88.7
	LB	27.7	7.9	283	23
Fall	UG	16	8.7	510	56
	LC	20.6	8.4	765	1230
	MC	19.7	8.5	388	60
	LB	12.7	8.3	248	11

Table 5. Stable isotope values for all consumers and potential food source samples.

		Spring							Summer				
	Source	**$\delta^{13}C$**	**σ**	**$\delta^{15}N$**	**σ**	**C:N**	**$\delta^{34}\Sigma$**	**σ**	**$\delta^{13}C$**	**σ**	**$\delta^{15}N$**	**σ**	**C:N**
Upper Guadalupe	*C. petrina*	-29.21	0.48	11.15	0.45	3.76	1.03	0.2	-28.3	0.36	10.96	0.16	3.66
	L. bracteata	-28.95	0.55	11.06	0.3	3.89	0.85	0.46	-28.78	0.67	10.75	0.68	7.81
	Seston	-24.84	0.41	2.75	0	8.94			-17.3	0.3	6.34	2.84	14.71
	CPOM	-29.26	0.54	1.85	1.46	42.99			-28.14	0	3	0	15.33
	Sediment	-9.53	0.56	4.86	0.28	38.01			-10.86	0.14	8.48	0.25	33.17
	Grass								-30.36	0	5.47	0	25.48
	Justicia	-25.79	0.46	8.41	0.55	11.05			-26.26	0	8.32	0	14.4
	Myriophyllum	-29.16	0.27	10.29	0.82	16.47							
Lower Colorado	*C. petrina*	-29.86	0.37	13.41	0.46	3.91	-0.13	0.2	-28.68	0.43	13.38	0.92	9.77
	C. houstonensis	-29.91	0.8	13.1	0.87	3.97	-0.14	0.2	-28.83	0.39	12.71	0.4	8.61
	Seston	-24.49	2.21	2.35	0.52	9.56			-22.3	0.29	8.47	0.49	8.15
	CPOM	-29.58	0.78	4.7	1.54	29.57			-29.1	0	10.97	0	16.16
	Sediment	-12.63	0.75	3.86	0.56	21.51			-13.95	0.25	7.79	0.37	29.51
	Grass	-30.54	0.06	10.92	0.27	20.16			-13.78	0	8.68	0	9.82
	Justicia												
	Myriophyllum								-27.97	2.88	10.25	4.44	12.16
	Algae								-19.7	0	2.17	0	21.75

Table 5 (continued).

		Spring							Summer				
	Source	**$\delta^{13}C$**	**σ**	**$\delta^{15}N$**	**σ**	**C:N**	**$\delta^{34}\Sigma$**	**σ**	**$\delta^{13}C$**	**σ**	**$\delta^{15}N$**	**σ**	**C:N**
Middle Colorado	*L. bracteata*	-26.63	0.55	7.38	0.55	3.75	4.5	0.19	-26.45	0.33	7.28	0.26	9.47
	Seston	-22.92	0.77	0.24	0.92	10.26			-19.02	0.7	2.88	1.49	15.26
	CPOM	-20.85	2.99	5.67	2.09	23.61			-28.26	0	0.48	0	16.16
	Sediment	-11.11	0.16	3.34	0.36	36.43			-11.01	0.19	5.3	0.22	38.75
	Grass	-28.81	0.58	4.97	0.31	22.1			-26.5	0	1.17	0	9.77
	Justicia	-15.74	0.59	6.06	0.28	22.13			-22.97	0	5.57	0	8.71
	Myriophyllum	-25	0.65	3.4	0.46	16.1			-18.89	6.5	2.68	4.12	11.19
	Algae								-17.68	0	10.1	0	9.09
Lower Brazos	*C. houstonensis*	-28.37	0.18	11.81	0.45	3.76	-0.93	0.4	-27.99	0.2	12.22	0.52	10.63
	Seston	-22.3	2.02	2.76	0.22	9.09			-25.89	0.23	4.06	1.31	9.59
	CPOM	-29.16	1.49	5.36	1	33.7			-29.97	0	3.98	0	30.46
	Sediment	-23.08	0.63	3.02	0.48	9.14			-22.85	0.62	-0.76	2.65	10.37
	Grass	-30.38	0.07	9.56	0.22	14.42							
	Justicia								-31.75	0	9.98	0	14.42
	Myriophyllum								-32.37	0	7.35	0	17.67

Table 6. Mean $\delta^{13}C$ and $\delta^{15}N$ signatures of tissue from focal taxa in spring and summer 2017.

Source	Season	Site	n	$\delta^{13}C$	$\delta^{15}N$
C. petrina	Spring	UG	7	-29.21	11.15
		LC	10	-29.86	13.41
	Summer	UG	11	-28.31	10.40
		LC	10	-28.68	13.38
C. houstonensis	Spring	LC	10	-29.91	13.10
		LB	12	-28.37	11.81
	Summer	LC	10	-28.83	12.71
		LB	10	-27.99	12.21
L. bracteata	Spring	UG	6	-28.95	11.06
		MC	12	-26.38	7.38
	Summer	UG	13	-28.78	10.75
		MC	10	-26.45	7.23

Table 7. Pearson product-moment coefficients and associated p-values for correlations between mussel size and $\delta^{13}C$ and mussel size and $\delta^{15}N$. Data were pooled across seasons for each species and each site. Bold values are statistically significant.

		$\delta_{13}C$		**$\delta^{15}N$**	
Site	*Species*	*r*	*p*	*r*	*p*
Upper Guadalupe	*C. petrina*	**0.47**	**0.05**	0.01	0.98
	L. bracteata	**0.46**	**0.05**	0.45	0.06
Lower Colorado	*C. houstonensis*	**0.58**	**0.01**	**0.48**	**0.03**
	C. petrina	**0.57**	**0.01**	0.22	0.36
Middle Colorado basin	*L. bracteata*	0.36	0.10	0.34	0.12
Lower Brazos basin	*C. houstonensis*	0.26	0.24	0.39	0.08

Table 8. IsoSource model estimates for the 3 mussel species for Spring and Summer.'C: N' is average carbon:nitrogen ratio, CPOM is coarse particulate organic matter, FPOM is fine particulate organic matter, SPOM is suspended particulate organic matter, and values are mean, minimum, maximum, and standard deviation estimated proportional composition of each C source.

Species	Site	C:N		Spring 2017				Summer 2017			
			C source	Mean	Min	Max	SD	Mean	Min	Max	SD
Cyclonais houstensis	LB	3.62	CPOM	0.876	0.87	0.88	0.005	0.625	0.53	0.72	0.062
			FPOM	0.074	0.03	0.13	0.047	0.145	0.02	0.27	0.083
			SPOM	0.05	0	0.09	0.042	0.23	0.01	0.45	0.146
	LC	3.89	CPOM	0.943	0.87	1	0.033	0.96	0.94	0.98	0.012
			FPOM	0.01	0	0.03	0.01	0.01	0	0.02	0.009
			SPOM	0.047	0	0.13	0.036	0.03	0	0.06	0.019
Cyclonais petrina	LC	3.86	CPOM	0.939	0.86	1	0.035	0.96	0.94	0.98	0.012
			FPOM	0.012	0	0.04	0.011	0.01	0	0.02	0.009
			SPOM	0.049	0	0.14	0.039	0.03	0	0.06	0.019
	UG	3.79	CPOM	0.99	0.99	0.99	0	0.974	0.95	1	0.014
			FPOM	0	0	0	0	0.009	0	0.03	0.01
			SPOM	0.01	0.01	0.01	0	0.017	0	0.05	0.016
Lampsilis bracteata	MC	3.82	CPOM	0.02	0	0.05	0.019	0.85	0.8	0.9	0.03
			FPOM	0.003	0	0.01	0.005	0.054	0	0.11	0.034
			SPOM	0.978	0.95	1	0.017	0.096	0	0.2	0.063
	UG	3.83	CPOM	0.93	0.93	0.93	0	1	1	1	0
			FPOM	0	0	0	0	0	0	0	0
			SPOM	0.07	0.07	0.07	0	0	0	0	0

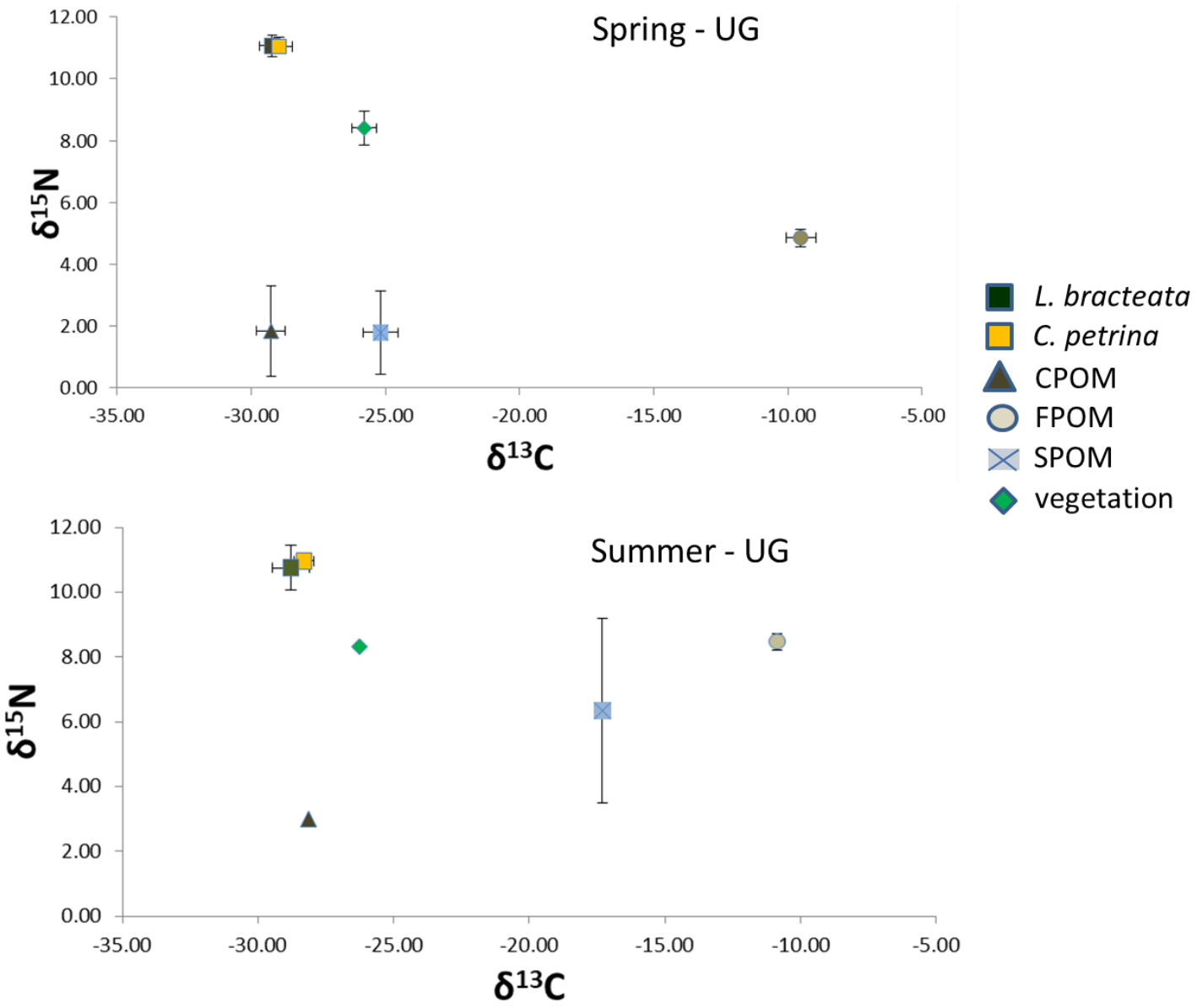

Figure 1. Stable CN isotope biplots for the Upper Guadalupe River site in April (Spring) and July (Summer) 2017. CPOM is coarse particulate organic matter (benthic detritus), FPOM is fine particulate organic matter from benthic sediments, SPOM is suspended particulate organic matter (seston), and 'vegetation' is *Justicia*.

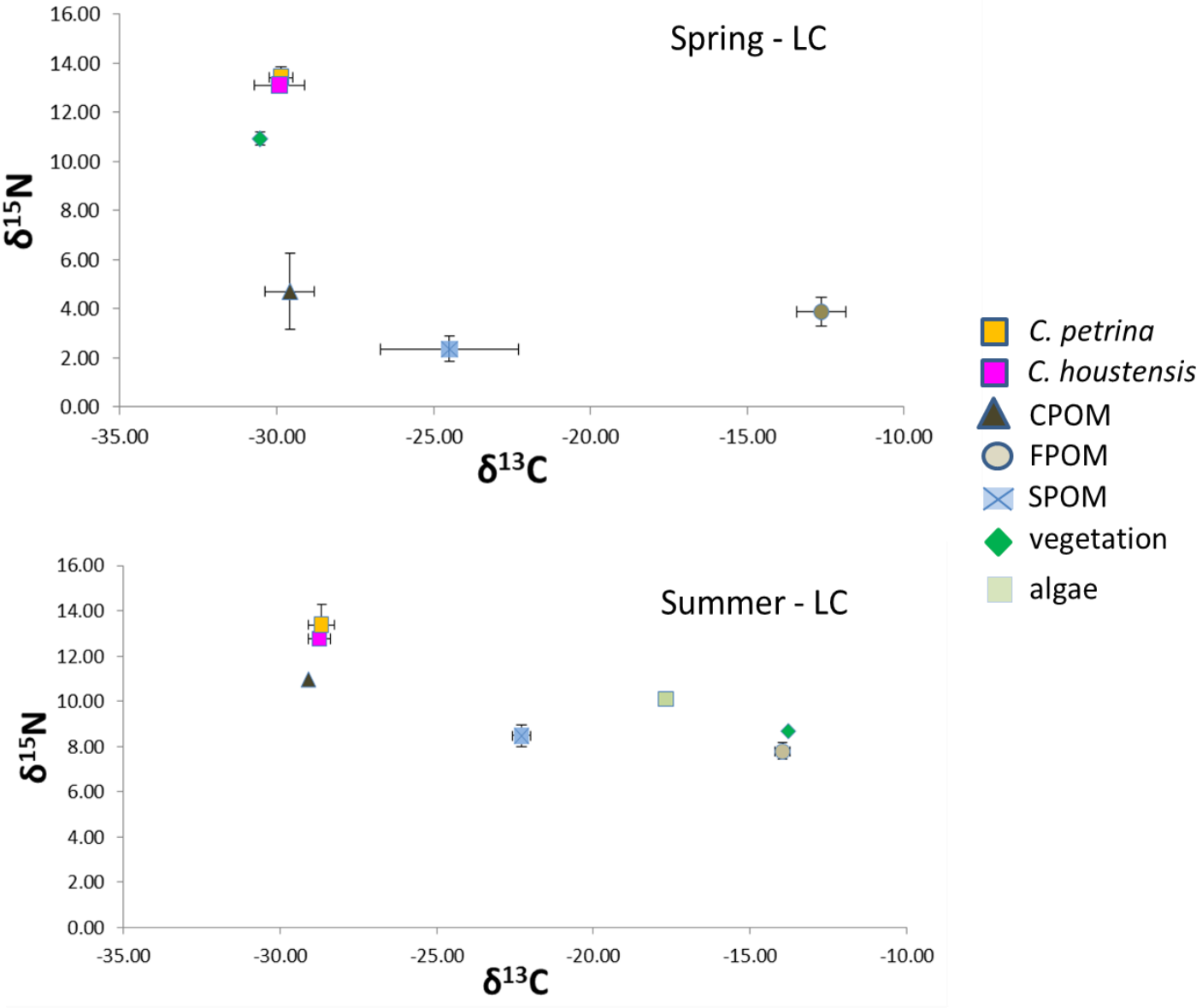

Figure 2. Stable CN isotope biplots for the Lower Colorado River site in April (Spring) and July (Summer) 2017. CPOM is coarse particulate organic matter (benthic detritus), FPOM is fine particulate organic matter from benthic sediments, SPOM is suspended particulate organic matter (seston), 'vegetation' is *Justicia* in the spring, riparian grass in the summer, and 'algae' is benthic filamentous algae

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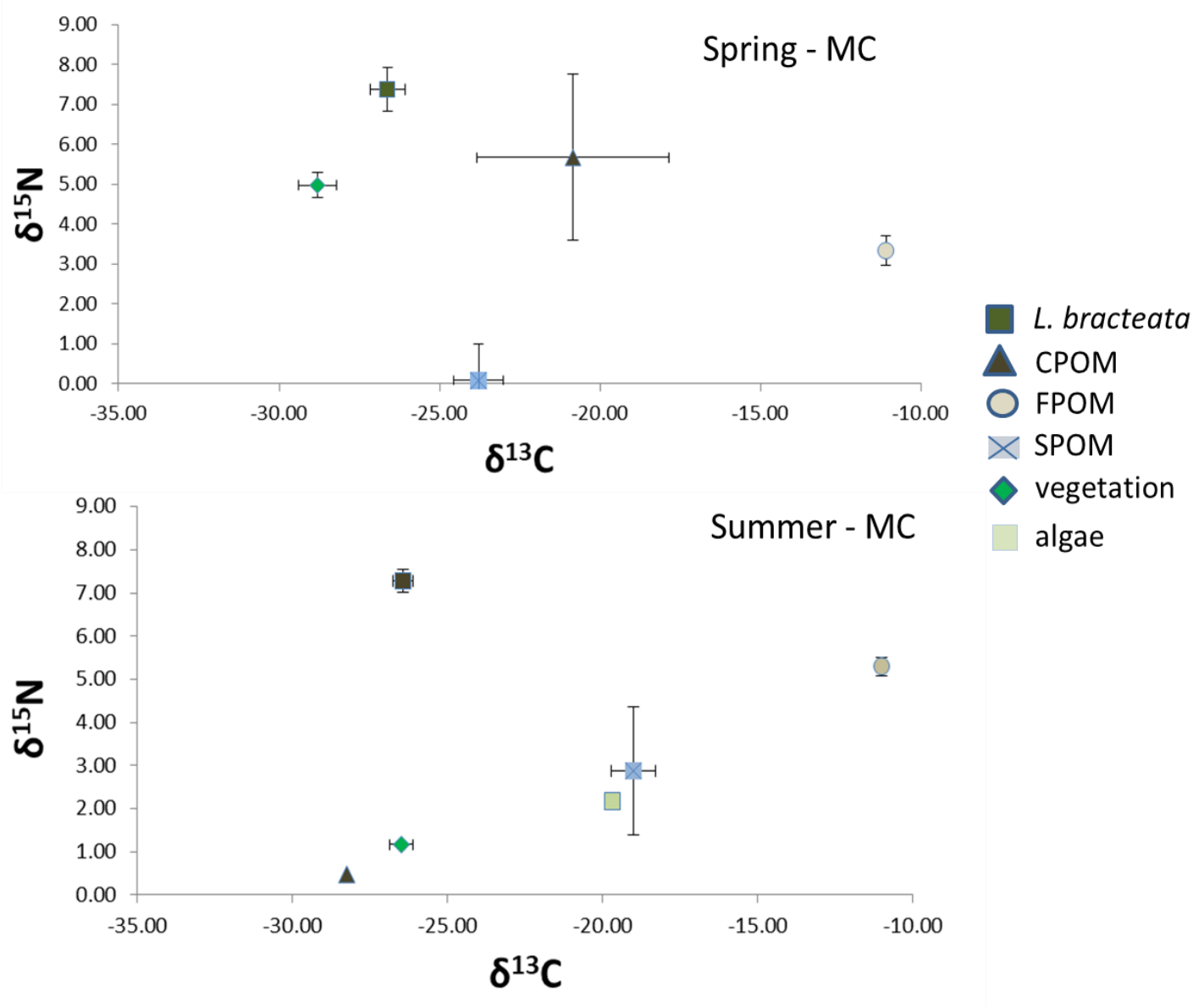

Figure 3. Stable CN isotope biplots for the Middle Colorado River basin site in April (Spring) and July (Summer) 2017. CPOM is coarse particulate organic matter (benthic detritus), FPOM is fine particulate organic matter from benthic sediments, SPOM is suspended particulate organic matter (seston), 'vegetation' is *Justicia*, and 'algae' is benthic filamentous algae.

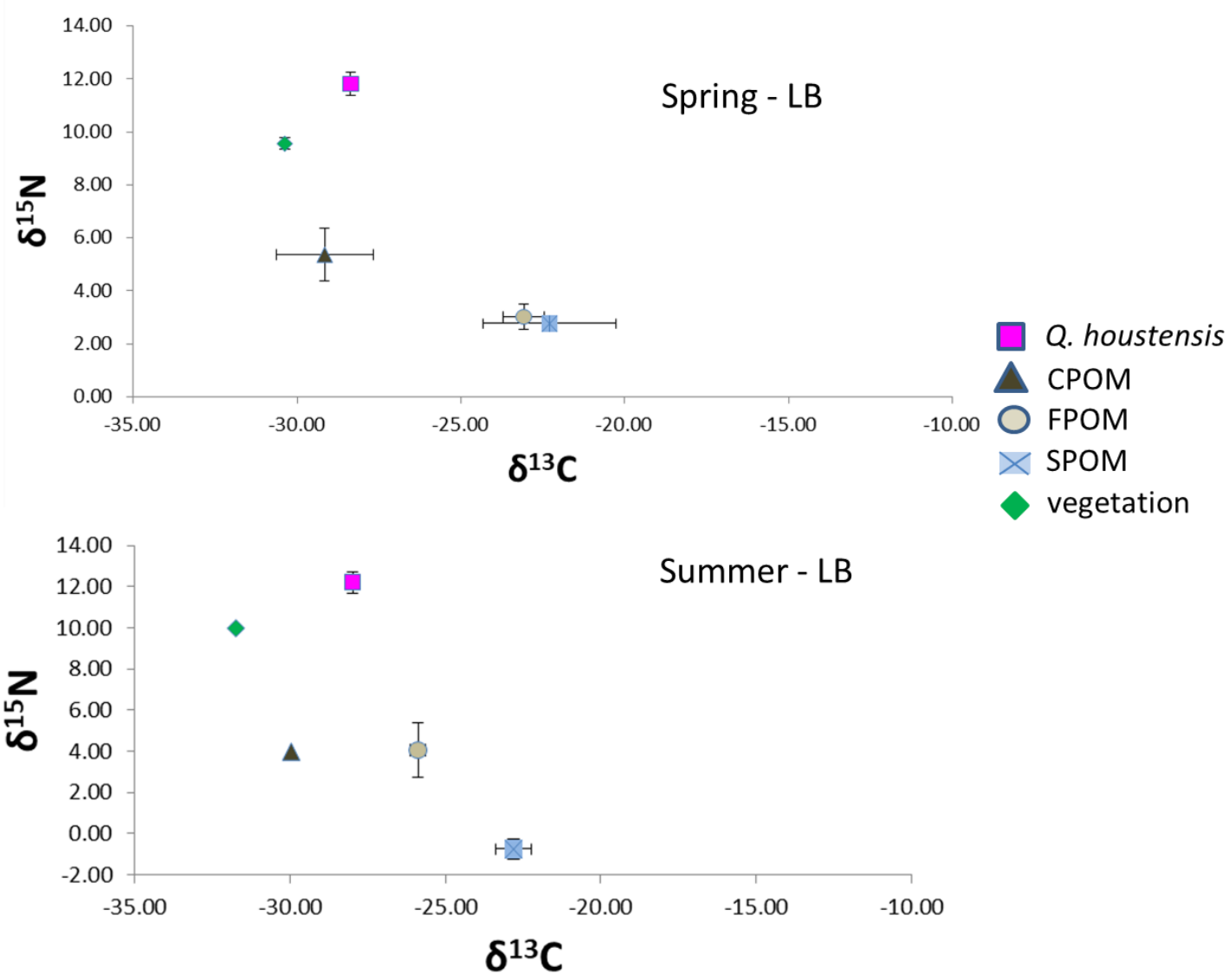

Figure 4. . Stable CN isotope biplots for the Lower Brazos River basin site in April (Spring) and July (Summer) 2017. CPOM is coarse particulate organic matter (benthic detritus), FPOM is fine particulate organic matter from benthic sediments, SPOM is suspended particulate organic matter (seston), and 'vegetation' is *Justicia*.

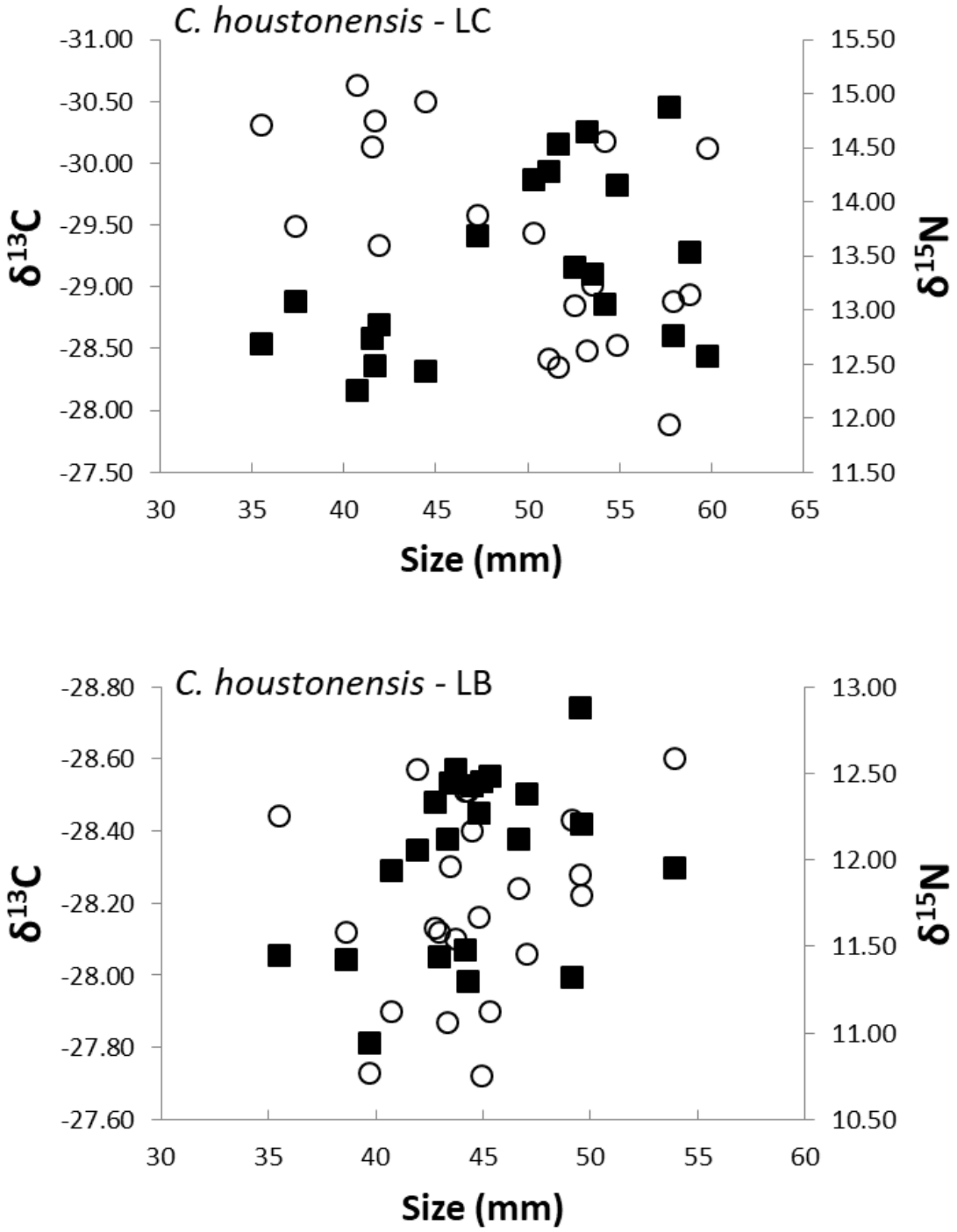

Figure 5. Relationships between stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and *C. houstonensis* size (length) in the Lower Colorado (LC) and Lower Brazos basin (LB). Open circles are $\delta^{13}C$ and filled squares are $\delta^{15}N$. Note $\delta^{13}C$ axis is inverted. See Table 7 for statistics.

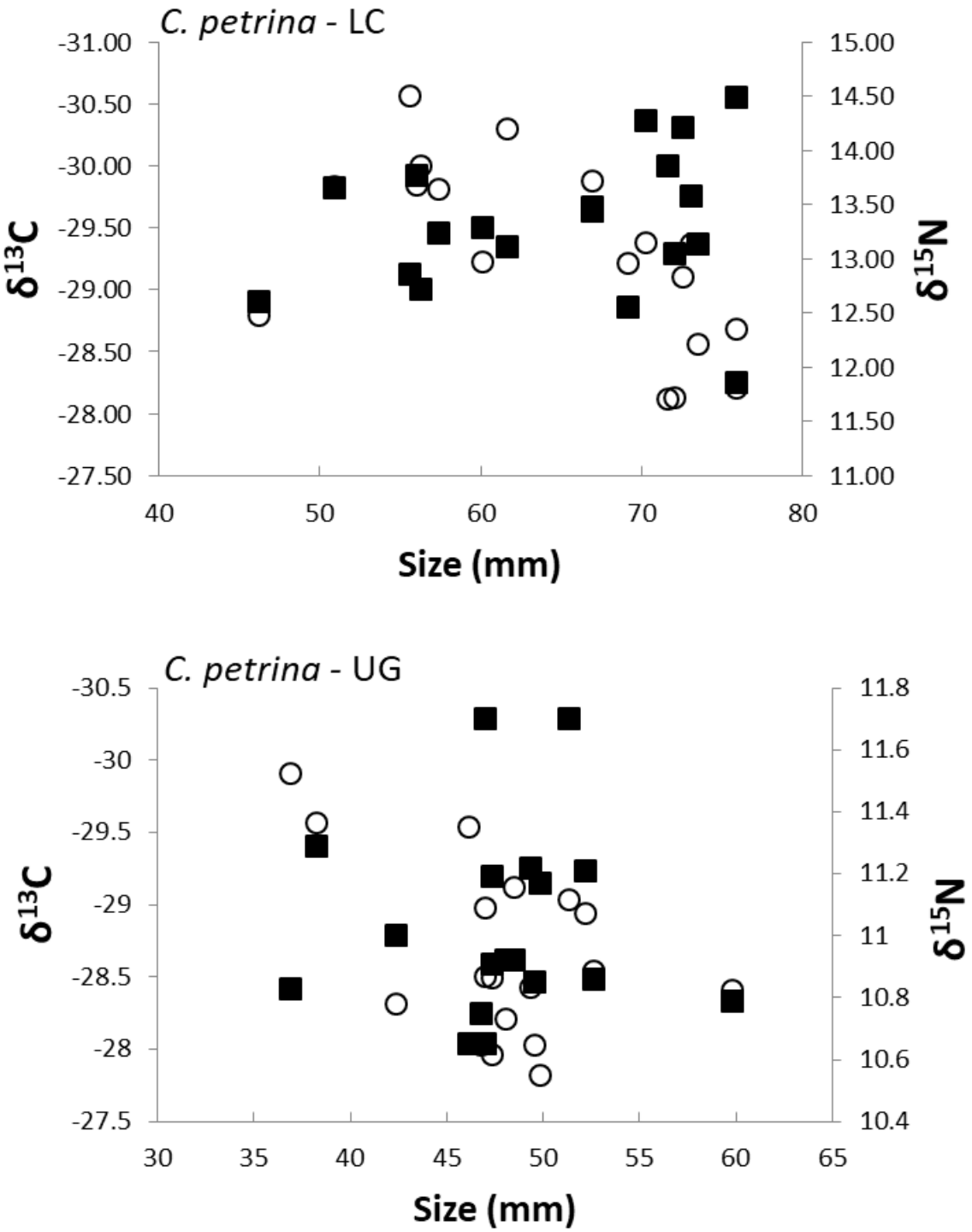

Figure 6. Relationships between stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and *C. petrina* size (length) in the Middle Colorado basin (MC) and Upper Guadalupe (UG). Open circles are $\delta^{13}C$ and filled squares are $\delta^{15}N$. Note $\delta^{13}C$ axis is inverted. See Table 7 for statistics.

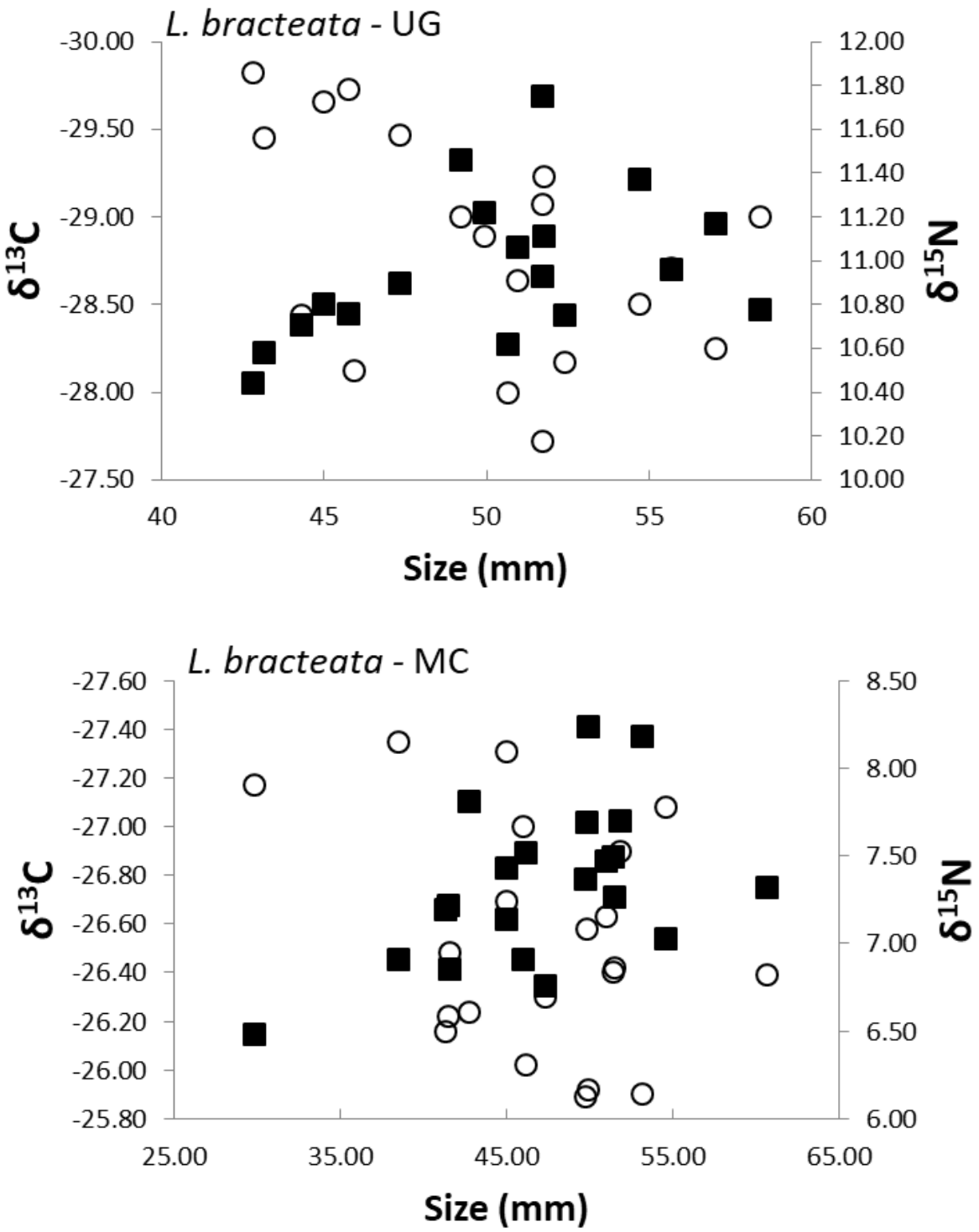

Figure 7. Relationships between stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and *L. bracteata* size (length) in the Upper Guadalupe (UG) and Middle Colorado basin (MC). Open circles are $\delta^{13}C$ and filled squares are $\delta^{15}N$. Note $\delta^{13}C$ axis is inverted. See Table 7 for statistics.

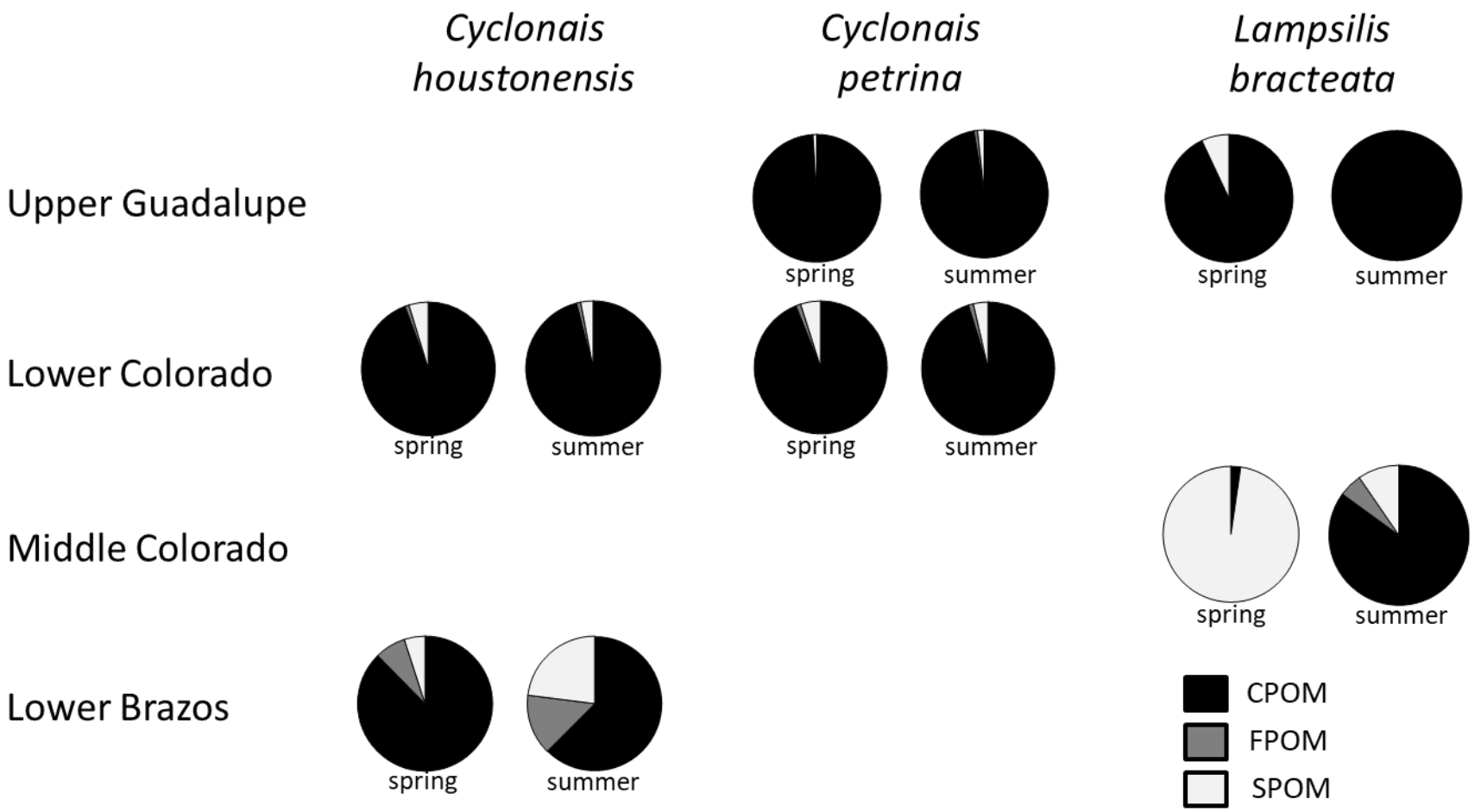

Figure 8. Estimated carbon source of *C. houstonensis, C. petrina,* and *L. bracteata* across the Upper Guadalupe, Lower Colorado, Middle Colorado, and Lower Brazos basin. Charts reflect mean estimate values of potential C sources from linear mixing models derived from IsoSource

Task 3 Environmental Flow Analysis and Modeling Update and Evaluation

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Traditional instream flow studies model changes in simple hydraulic parameters, such as depth and velocity, under varying stream discharge levels and how they influence habitat availability for target organisms, usually fishes (BIO-WEST 2008). The underlying assumptions are that target organisms select their habitat based on these parameters, and that they have the ability to move to new habitats when discharge conditions change. Freshwater mussels challenge these assumptions due to their slow locomotion and sessile nature. Compared to fishes, mussels tend to move little and occupy small habitat patches for long periods. Therefore, for a mussel to persist at a location, suitable habitat must occur across a range of flow conditions. As a result, some have suggested that modeling simple hydraulic variables such as depth and velocity are of little use in determining conservation flows for mussels, but that complex hydraulic parameters such as shear stress are better predictors of mussel abundance (Layzer and Madison 1995, Maloney et al. 2012). However, some of the same authors recognized that mussels did show a preference for particular hydraulic conditions and that depth and velocity were important factors limiting their distribution under base flow conditions (Layzer and Madison 1995). Due to the variety of factors influencing suitable mussel habitat, others have suggested that environmental flow recommendations for mussels should focus more on the

biological traits of species guilds (Gates et al. 2015), although such biological information is lacking for many species.

Previous environmental flow studies conducted on the lower Colorado River as part of the Lower Colorado River Authority (LCRA) – San Antonio Water System (SAWS) Water Project (LSWP) established 10 intensive study sites with detailed hydraulic models (Figure 1) (BIO-WEST 2008). These models were used to generate instream flow recommendations for the lower Colorado River based on fish habitat modeling and other flow dependent ecological variables. However, freshwater mussels were not considered as part of this previous instream flow assessment. Therefore, the overall goal of the current study was to evaluate availability and persistence of freshwater mussel habitat within the lower Colorado River using a variety of both traditional (depth, velocity) and complex (shear stress) hydraulic variables at existing hydraulic model sites used previously for fish habitat modeling.

To do this, the first specific objective of Task 3 was to conduct mussel surveys at previously established hydraulic model sites on the lower Colorado River to determine which sites support significant mussel populations. These sites were included in Task 1 survey work to assess the occurrence and abundance of freshwater mussels at each location. Based on the results of these surveys, sites were chosen to focus additional habitat modeling efforts, as described in the results below.

The second specific objective was to develop habitat suitability criteria for freshwater mussels within the lower Colorado River. Detailed habitat information collected as part of Task 1 survey work was summarized and used to develop initial habitat suitability criteria. Additional site-specific data collection is planned in 2018 to characterize mussel habitat associations at a finer spatial scale and refine suitability criteria.

The third specific objective of this task was to collect additional physical and hydraulic data to validate and/or update existing hydraulic models. Initially, it appeared that hydraulic model sites had changed little since development of the models and only some minor validation data would be necessary. However, a large flood event occurred in the lower Colorado River following heavy rains from Hurricane Harvey in August 2017. A visit to one of the selected model sites shortly afterwards revealed extensive changes to channel bathymetry. Therefore, current bathymetric and hydraulic data at multiple flow ranges will be necessary to update existing models to incorporate these changes. Collection of this additional physical and hydraulic data is planned in 2018, after which habitat suitability information will be combined with revised model output to evaluate the effects of varying flows on freshwater mussel habitat within the selected study sites. This complete analysis will be provided in a final report in August 2018. The analysis and results below summarize initial environmental flow work conducted in 2017 pertaining to site selection and development of initial habitat suitability criteria.

Methods

Freshwater mussel data was collected using the methodology described in detail under Task 1 surveys. Surveys were conducted at several of the previously established hydraulic model sites to evaluate the mussel communities present and choose sites for further habitat modeling. Habitat utilization data from the lower Colorado River were used to develop Habitat Suitability Criteria (HSC) for all freshwater mussels, as well as for each of the candidate species inhabiting the lower Colorado River (i.e., *Cyclonaias houstonensis*, *Cyclonaias petrina*, and *Truncilla macrodon*). For each species or combination of species, HSC were developed for depth, mean column velocity, Froude number, Reynolds number, mean substrate compaction,

FST hemisphere number, minimum bottom shear stress (MBSS; inferred from FSTs using Statzner et al. 1991), and dominant substrate. Data for depth, mean column velocity, mean substrate compaction, FST hemisphere number, and minimum bottom shear stress (inferred from FSTs using Statzner et al. 1991) were measured during surveys as describe in the Task 1 methods. Percent substrate composition taken from survey data was converted to dominant substrate for development of suitability criteria. Froude number and Reynolds number are hydraulic parameters used to evaluate turbulence. Froude number represents a ratio of inertial to gravitational forces. Reynolds number represents a ratio of inertial force to viscous force. These parameters were calculated from survey data using the following equations:

Reynolds number *(Re):* $Re = Ud/v$

where U = benthic velocity (m/s), d = water depth (m), v = kinematic viscosity of water (1.0×10^{-6} m^2/s)

Froude number *(Fn):* $Fn = U/\sqrt{gd}$

where U = benthic velocity (m/s), g = acceleration of gravity (9.8 m/s^2), d = water depth (m)

Suitability criteria for continuous variables were created using nonparametric tolerance limits (NPTL) (Bovee 1986). The tolerance limits representing the central 50% of the data were used to represent the highest utilization and given a suitability value of 1.0. The tolerance limits for the central 75% were assigned a suitability of 0.5. The tolerance limits for the central 90% of the data were given a suitability of 0.2. Lastly, the tolerance limits representing the central 95% of the data were given a suitability value of 0.1. Anything outside of the central 95% tolerance limit was considered unsuitable, and given a suitability value of 0.0. For the categorical variable of dominant substrate, suitability values were established using the Strauss Linear Index (Strauss

1979, Persinger et al. 2011). Values of this index range from -1 to 1, with larger positive values indicating selection and negative values indicating avoidance. Significant positive values were given a suitability of 1.0, non-significant positive values were given a suitability of 0.5, non-significant negative values were given a suitability of 0.2, and significant negative values were assigned a suitability of 0.0.

Results

Study Site Selection

A total of 85 person-hours (p-h) of search time was conducted at the ten intensive model sites resulting in collection of 862 mussels for an overall catch-per-unit-effort (CPUE) of 10.1 mussel/p-h (Table 1). Site-specific CPUE ranged from 0.0 – 2.5 mussels/p-h at sites between Longhorn Dam and Smithville, and from 3.2 – 47.6 mussels/p-h from La Grange downstream to Lane City. Maximum site-specific CPUE (47.6 mussels/p-h) was observed at the Altair intensive study site. Candidate species were present from La Grange downstream, with all three candidate species (i.e., *C. houstonensis*, *C. petrina*, and *T. macrodon*) being present at the Altair and Wharton study sites.

Initial Habitat Suitability Criteria

Initial HSC generated from survey data for all unionids in aggregate demonstrated highest habitat suitability at depths of 0.6 – 0.9 meters (m), mean column velocity below 0.2 m/s, Froude number below 0.05, and Reynolds numbers below 50,000 (Figure 2). Mussels were most commonly found in habitats with mean substrate compaction less than 0.075 kg/cm^2, and showed highest suitability when MBSS < 2 dyn/cm^2 and in silt and boulder substrates (Figure 3).

For *C. houstonensis*, suitability was highest at depths of 0.9 – 1.2 m, mean column velocities of 0.1 – 0.2 m/s, Froude numbers near 0.05, and Reynolds numbers of approximately

50,000 (Figure 4). They were most commonly found in habitats with mean substrate compaction from 0.025 – 0.05 kg/cm^2, in habitats with MBSS < 2 dyn/cm^2, and exhibited highest suitability in boulder substrates (Figure 5).

For *C. petrina*, suitability was highest at depths from 0.6 – 0.9 m, mean column velocities below 0.2 m/s, Froude numbers below 0.05, and at Reynolds numbers below 150,000 (Figure 6). They were most commonly found where mean substrate compaction ranged from 0.025 - 0.075 kg/cm^2, at MBSS < 2 dyn/cm^2, and showed moderate suitability (0.5) in sand, boulder, and bedrock substrates (Figure 7).

Due to insufficient sample size (n = 9), HSC were not generated for *T. macrodon*. However, data suggests highest utilization in depths of 0.6 – 0.9 m, in relatively low velocities, and in habitats with relatively low Froude and Reynolds numbers (Figure 8). They were most commonly found where substrate compaction and MBSS were relatively low, and Strauss Linear Index values for substrate were highest in silt (0.25), clay (0.09), and boulder (0.08) (Figure 9).

Discussion

Based on survey results, the Altair and La Grange study sites were chosen as the focus for additional data collection and modeling. The Altair study site exhibited the highest mussel abundance, and contained all three candidate species. Therefore, it was chosen to represent high-quality habitat conditions within the lower Colorado River. The La Grange study site was the upstream-most site that contained a candidate species (*C. houstonensis*), and it ranked third in overall CPUE. Understanding differences in hydraulic and habitat conditions between these two sites may elucidate patterns in freshwater mussel occurrence and abundance within the lower Colorado River. Additional physical and hydraulic data collection to update hydraulic models at these two sites is planned in 2018.

Initial habitat suitability criteria developed from survey data suggest that freshwater mussels in the lower Colorado River are most commonly utilizing moderate-depth low-energy habitats with silt and boulder substrates. Compared to all mussels in aggregate, *C. houstonensis* showed lower suitability in areas with 0 velocity, 0 turbulence, and 0 substrate compaction. In contrast, *C. petrina* showed broader curves for mean column velocity, Froude number, Reynolds number, and MBSS, suggesting increased utilization of high-energy environments compared to all mussels in aggregate.

Although the HSC described above provide a good starting point for evaluating habitat utilization, they are based on data collected under relatively low base flow conditions during mussel surveys, and should therefore be interpreted with caution. Given that freshwater mussels are rather sessile organisms they must occupy habitat that is acceptable at base flows, as well as high flows, in order to persist. Additional mussel habitat data collection is planned in 2018 under varying flow conditions. Once additional habitat data is available, it will be combined with updated hydraulic model output to evaluate the influence of varying flow conditions on the availability and persistence of habitat within the selected model sites.

Table 1. Results of mussel surveys at ten previously established LSWP intensive study sites on the Lower Colorado River.

LSWP Site	Search Time (person-hours)	Mussel Abundance	CPUE (mussels/p-h)	Number of Candidate Species Present
Longhorn Dam	6	0	0.0	0
Utley	11	28	2.5	0
Bastrop	6	0	0.0	0
Smithville - US	6	0	0.0	0
Smithville - DS	6	0	0.0	0
La Grange	11	74	6.7	1
Columbus	10	88	8.8	2
Altair	13	619	47.6	3
Wharton	11	35	3.2	3
Lane City	5	18	3.6	2
	85	862	10.1	

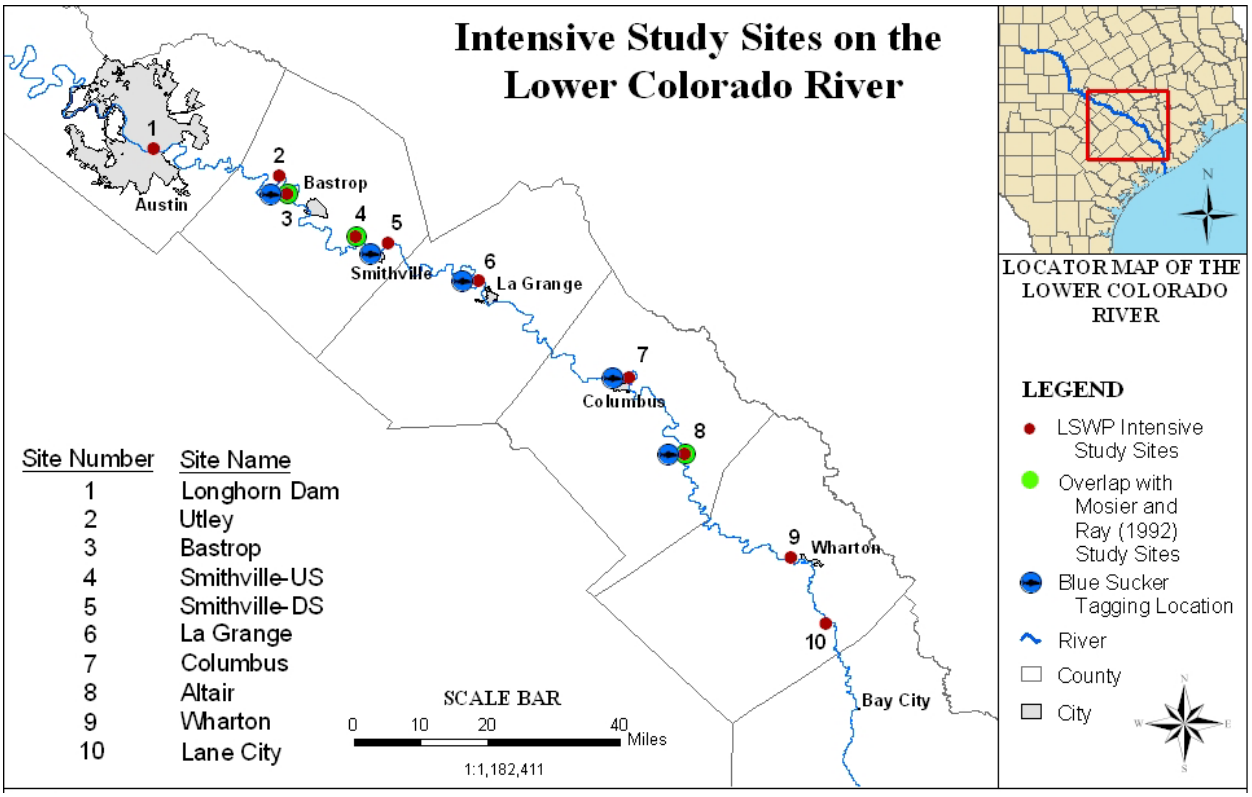

Figure 1. LSWP intensive study sites with existing hydraulic models.

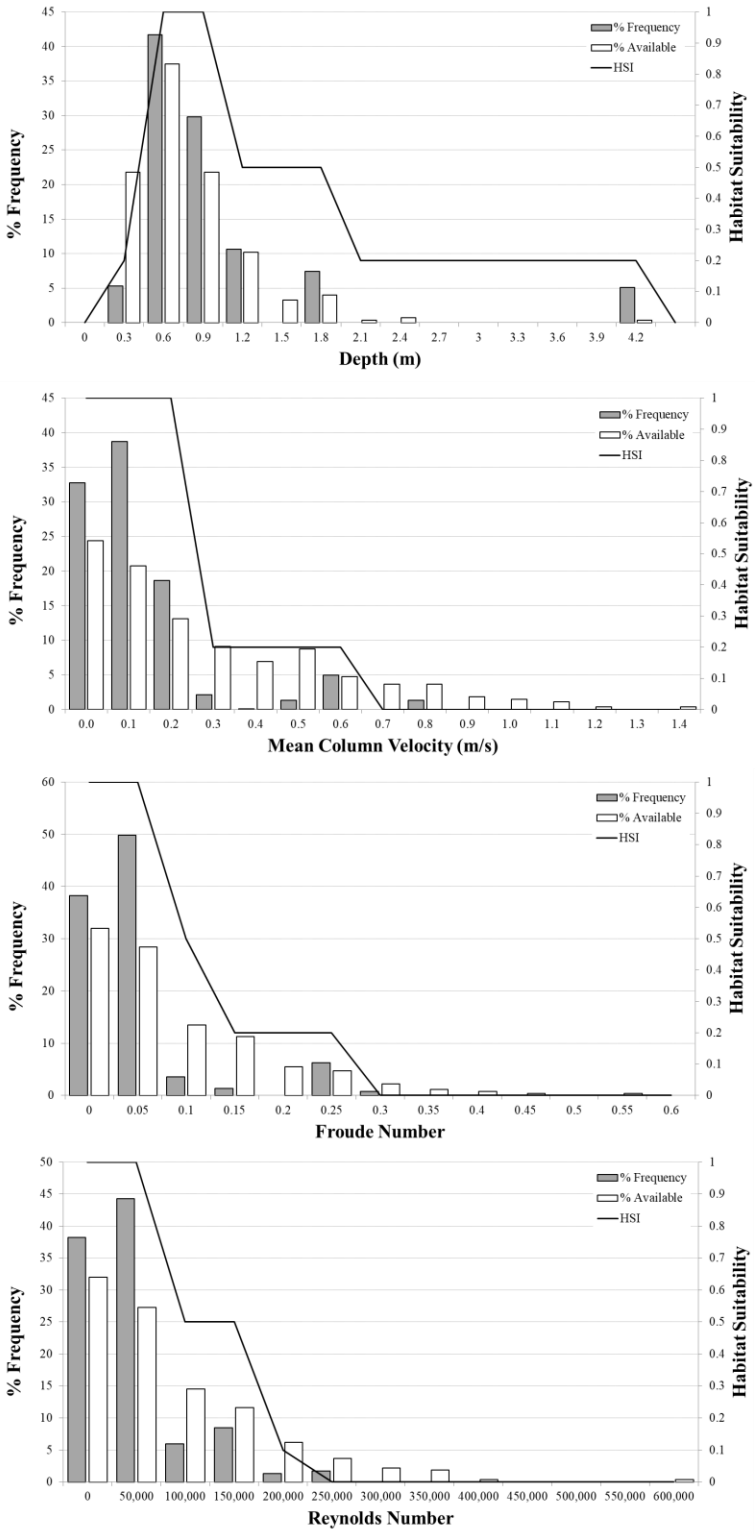

Figure 2. Percent frequency of occurrence (gray bars), percent frequency of habitats sampled (white bars), and habitat suitability values (black line) for lower Colorado River unionids (n = 2327) in relation to depth (m), mean column velocity (m/s), Froude number, and Reynolds number.

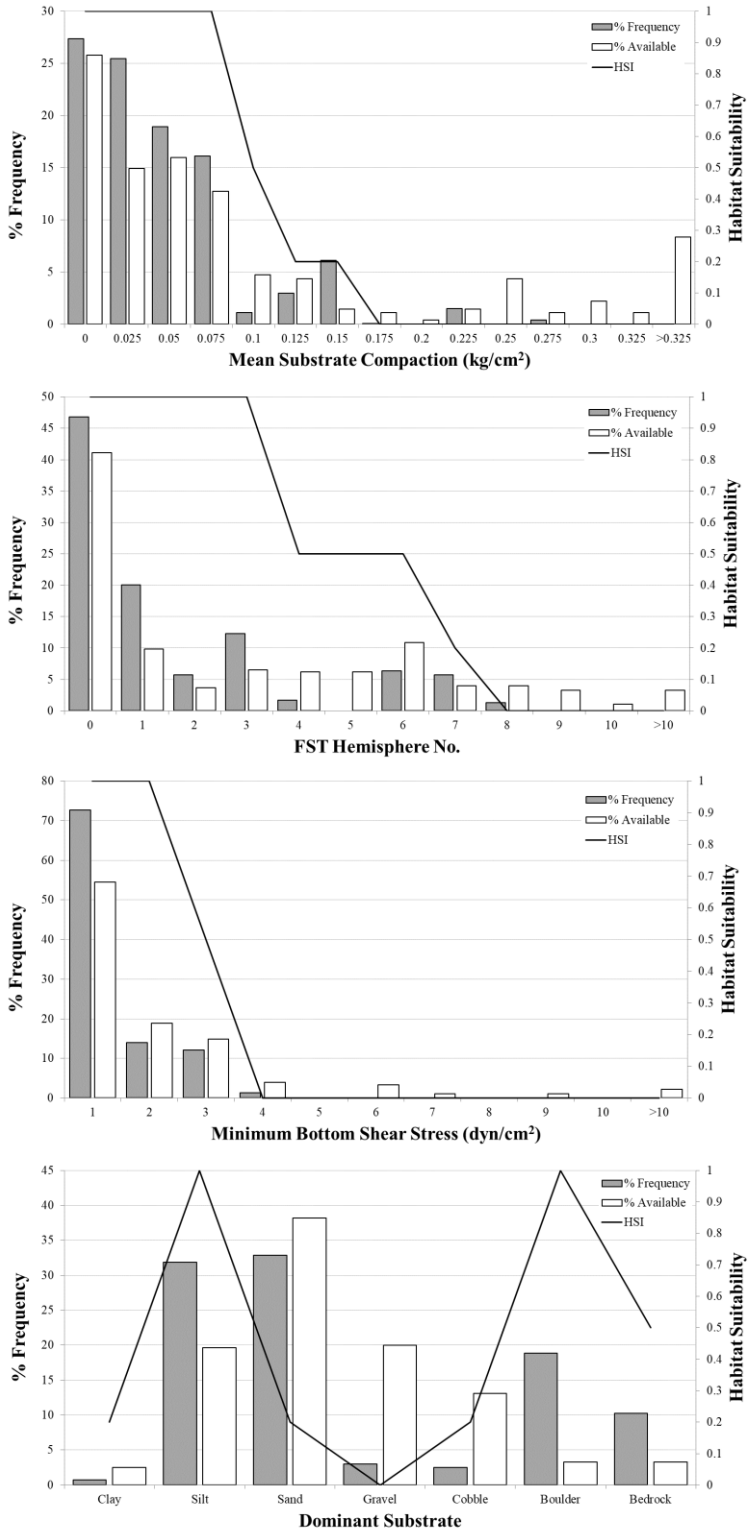

Figure 3. Percent frequency of occurrence (gray bars), percent frequency of habitats sampled (white bars), and habitat suitability values (black line) for lower Colorado River unionids (n = 2327) in relation to mean substrate compaction (kg/cm^2), FST hemisphere number, minimum bottom shear stress (dyn/cm^2), and dominant substrate.

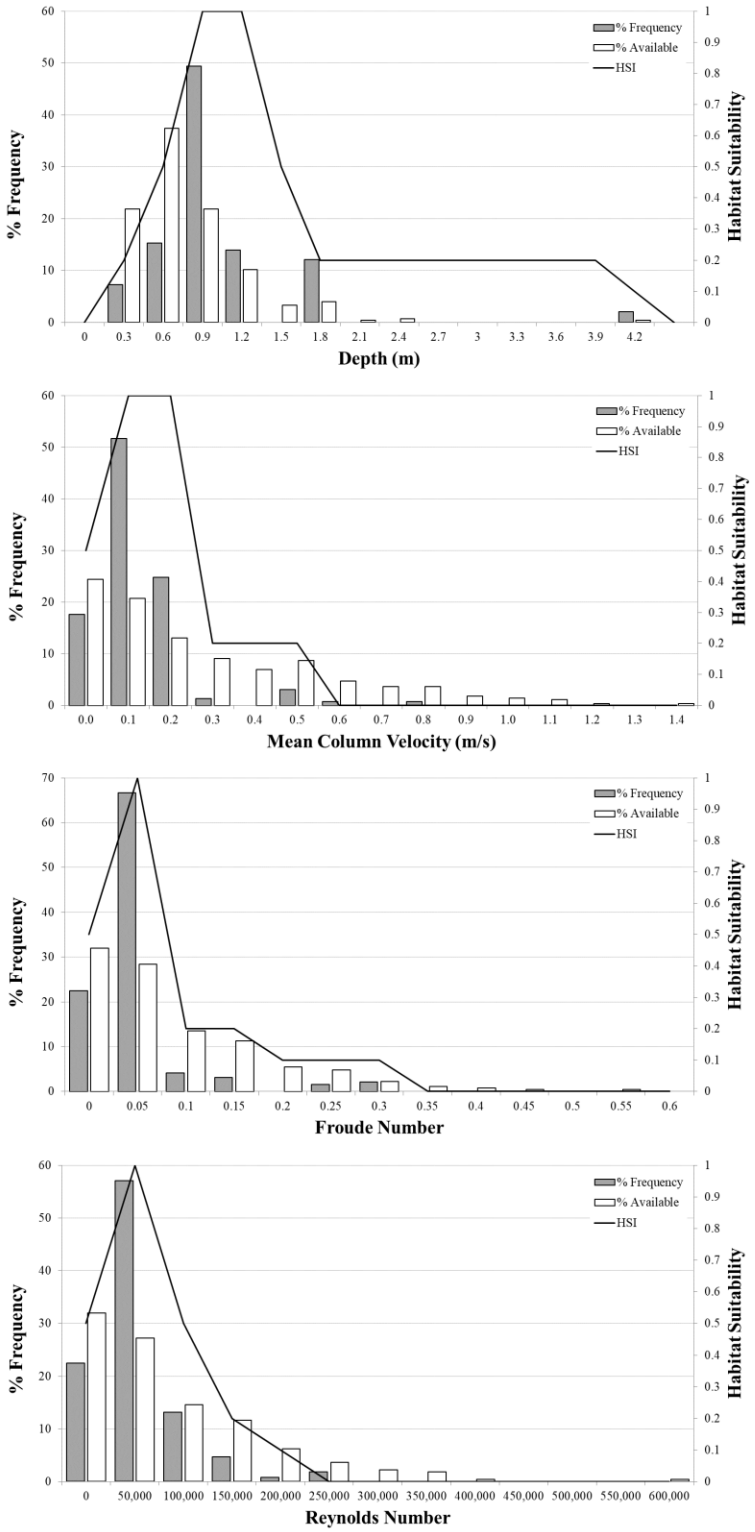

Figure 4. Percent frequency of occurrence (gray bars), percent frequency of habitats sampled (white bars), and habitat suitability values (black line) for lower Colorado River *C. houstonensis* (n = 387) in relation to depth (m), mean column velocity (m/s), Froude number, and Reynolds number.

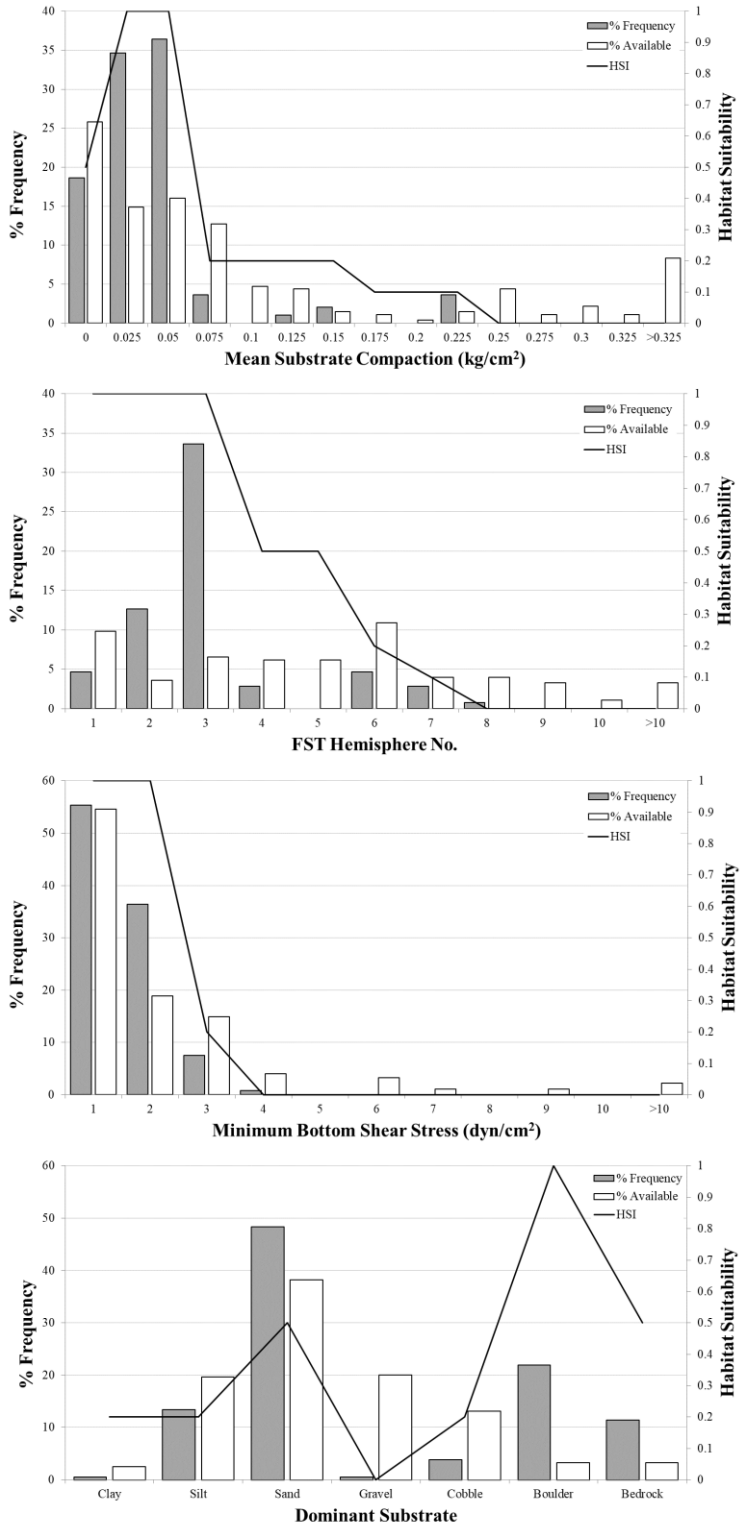

Figure 5. Percent frequency of occurrence (gray bars), percent frequency of habitats sampled (white bars), and habitat suitability values (black line) for lower Colorado River *C. houstonensis* (n = 387) in relation to mean substrate compaction (kg/cm^2), FST hemisphere number, minimum bottom shear stress (dyn/cm^2), and dominant substrate.

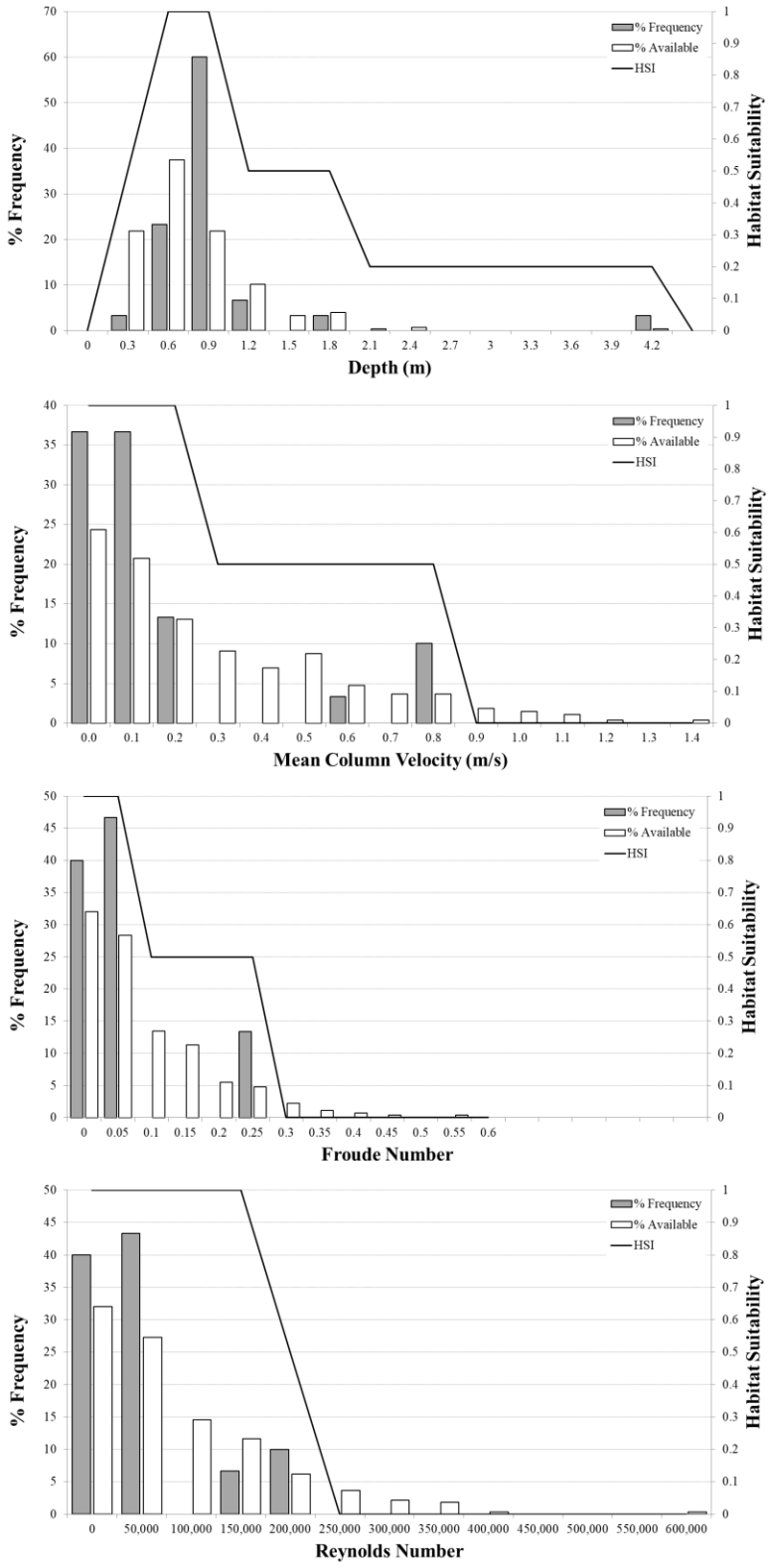

Figure 6. Percent frequency of occurrence (gray bars), percent frequency of habitats sampled (white bars), and habitat suitability values (black line) for lower Colorado River *C. petrina* (n = 30) in relation to depth (m), mean column velocity (m/s), Froude number, and Reynolds number.

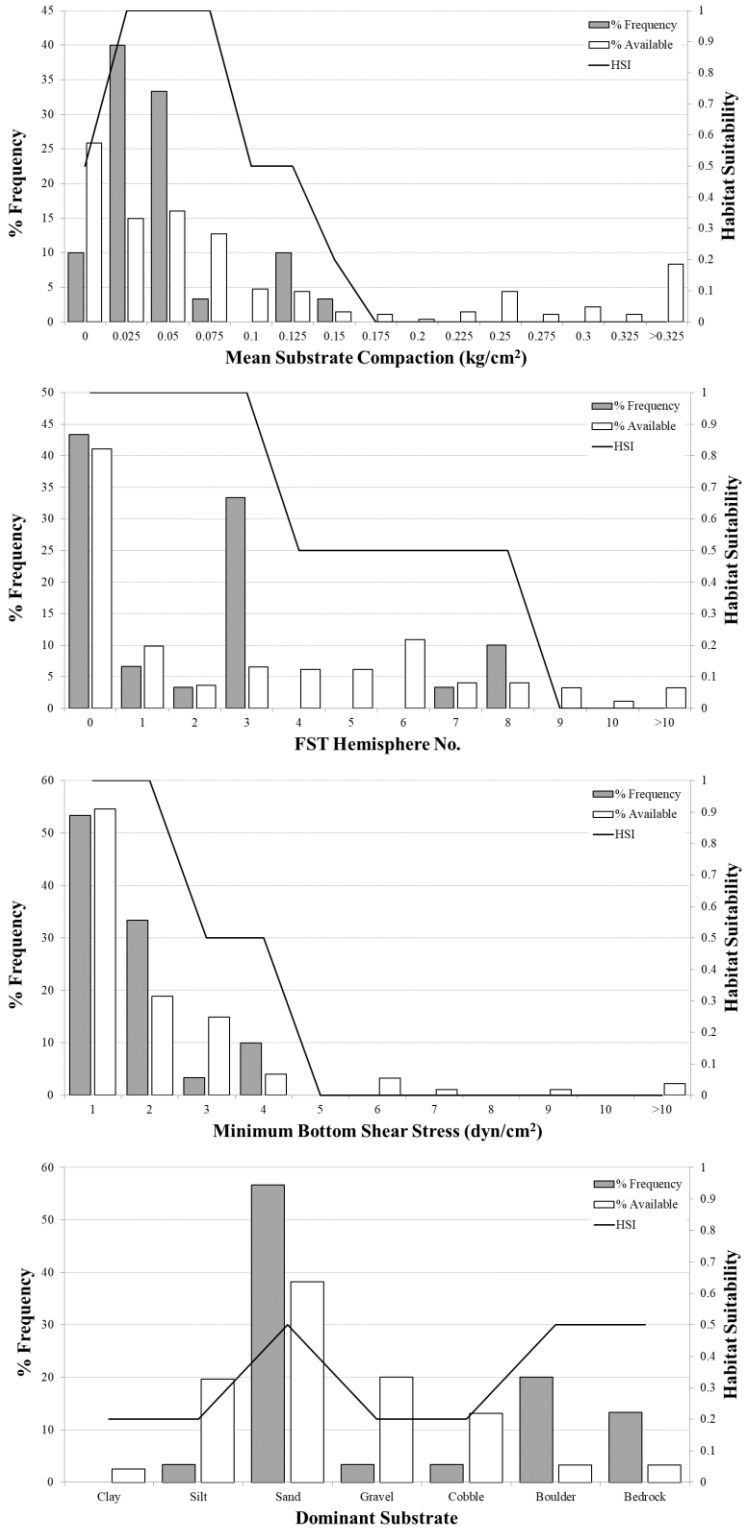

Figure 7. Percent frequency of occurrence (gray bars), percent frequency of habitats sampled (white bars), and habitat suitability values (black line) for lower Colorado River *C. petrina* (n = 30) in relation to mean substrate compaction (kg/cm^2), FST hemisphere number, minimum bottom shear stress (dyn/cm^2), and dominant substrate.

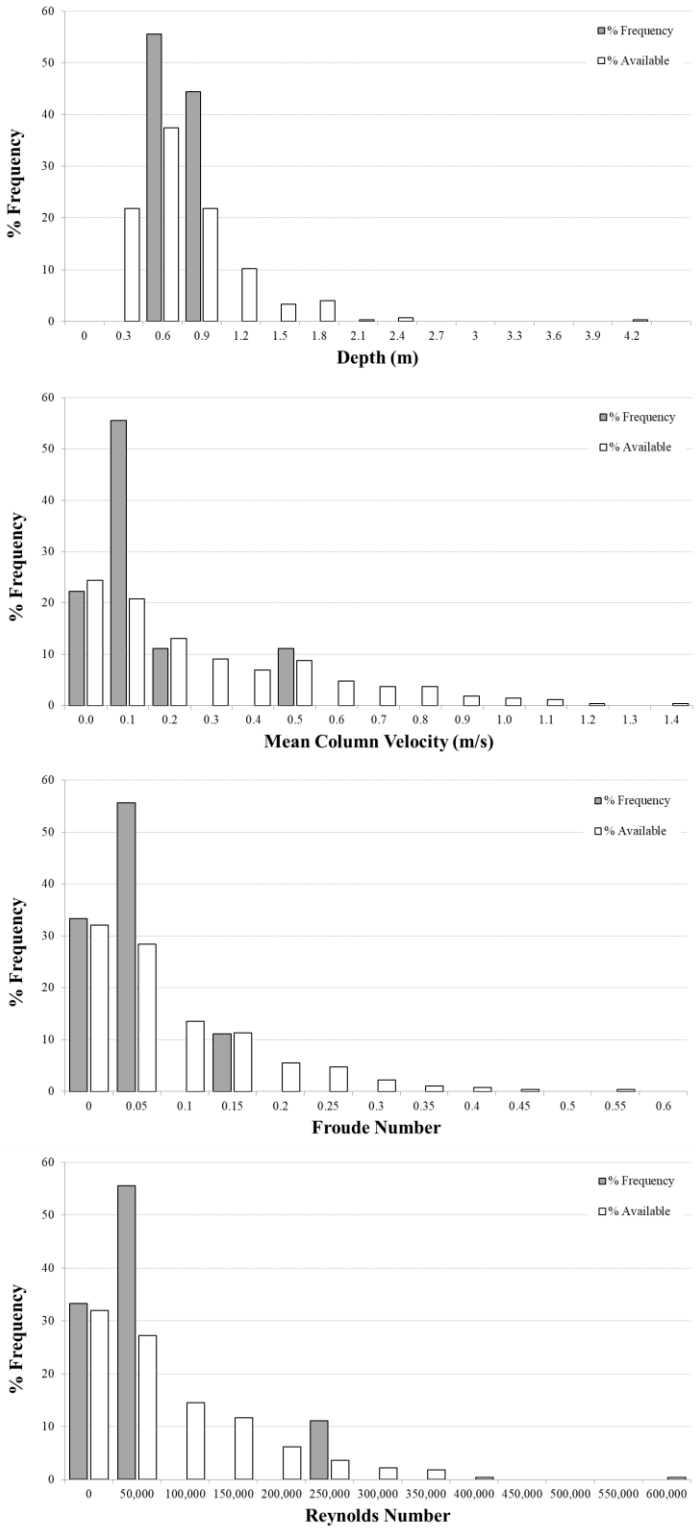

Figure 8. Percent frequency of occurrence (gray bars) and percent frequency of habitats sampled (white bars) for lower Colorado River *T. macrodon* (n = 9) in relation to depth (m), mean column velocity (m/s), Froude number, and Reynolds number. HSC were not generated for *T. macrodon* due to insufficient sample size.

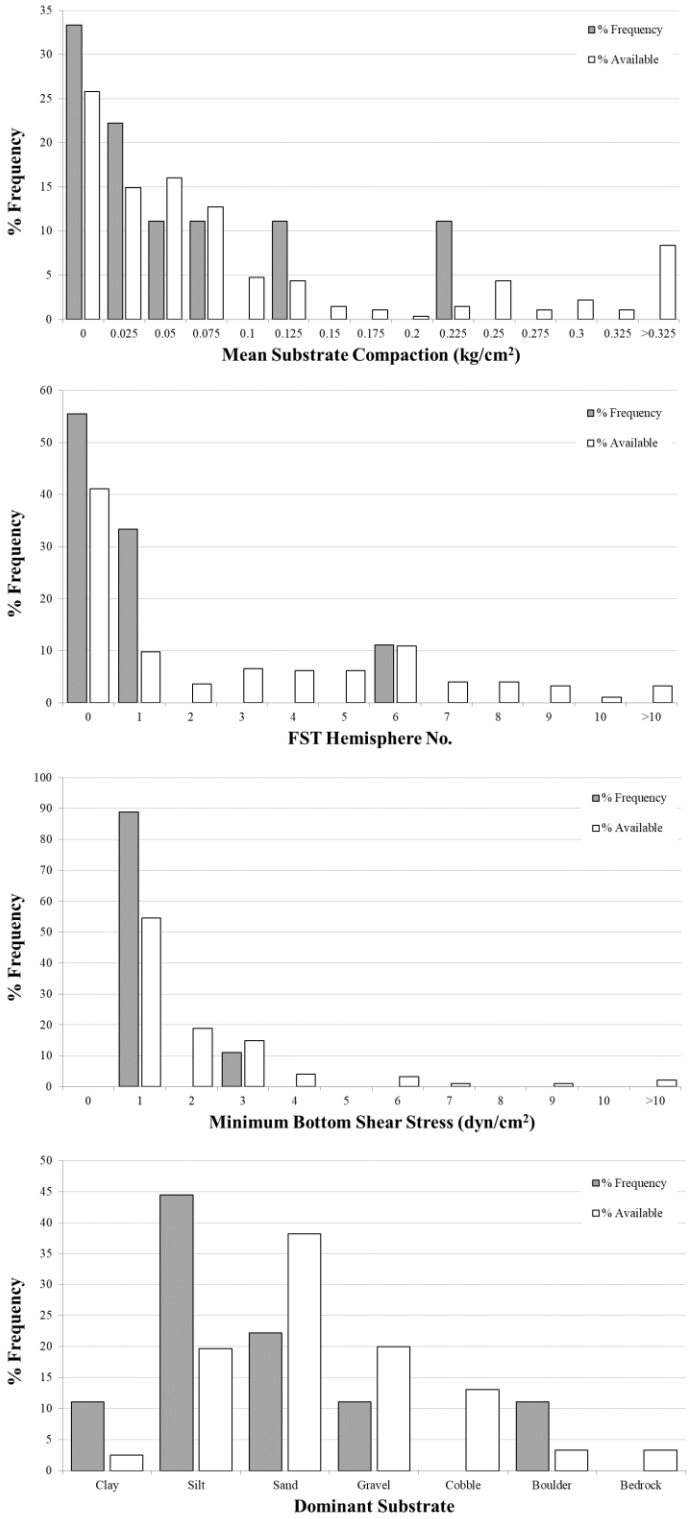

Figure 9. Percent frequency of occurrence (gray bars) and percent frequency of habitats sampled (white bars) for lower Colorado River *T. macrodon* (n = 9) in relation to mean substrate compaction (kg/cm^2), FST hemisphere number, minimum bottom shear stress (dyn/cm^2), and dominant substrate. HSC were not generated for *T. macrodon* due to insufficient sample size.

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Task 4: Freshwater mussel mark-capture assessment

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When surveying freshwater mussels with conventional methods, certain factors have the potential to affect sampling efficiency such as habitat, substrate particle size, water temperature, turbidity, and shell length (Strayer & Smith 2003; Wisniewski et al. 2013). Freshwater mussels can also remain endobenthic for extended periods of time, resulting in reduced capture efficiency (Strayer & Smith 2003). Decreased sampling efficiency may result in incomplete detection, which could bias survey results (Wisniewski et al. 2013). This is particularly problematic when trying to estimate population parameters. To account for incomplete detection and improve population demographic calculations, recent studies have utilized mark-recapture methodologies which survey the same location multiple times (Strayer & Smith 2003; Meador et al. 2013; Wisniewski et al. 2013; Inoue et al. 2014).

Robust mark-recapture study designs are considered beneficial to prevent biased population estimates, including population size, capture rates, and survival (Strayer & Smith 2003). A robust design incorporates two types of sampling intervals, primary and secondary. The primary sampling period is associated with open-population models (e.g., annual, seasonal), wherein the assumptions of recruitment, mortality, immigration, and emigration are relaxed. The secondary sampling period is associated with closed-population models, and are conducted as

multiple sampling events occurring within the primary sampling period. These secondary sampling periods operate under the assumption that recruitment, mortality, immigration, and emigration are not occurring during these relatively shorter time periods. The number of secondary sampling events and the intervals between them vary depending on the goals of the study (Meador et al. 2011, Inoue et al. 2014).

In addition to the estimation of population demographics, mark-recapture study designs can also be useful for the assessment of ancillary life history characteristics. The comprehensive individual monitoring involved during field studies can provide valuable data on both mussel movement and rates of growth.

A variety of methods have been used to tag mussels during mark-recapture studies, most commonly numbered shellfish tags. Compared to the use of traditional shellfish tags, Passive Integrated Transponder (PIT) tags can greatly increase recapture success. Kurth et al. (2007) found that PIT tagged mussel recapture rates ranged from 72% - 80%, while recapture rates for visual searches ranged 30% - 47%. In addition, currently available PIT tag readers that record GPS coordinates along with each detection (BioMark HPR Plus) enhance the ability to monitor the horizontal movement of freshwater mussels at an individual level.

As outlined above, mark-recapture studies in combination with PIT tagging can provide considerable data on population parameters, growth, and movement of freshwater mussels. This information is generally lacking for most mussel species, particularly rare species, and is beneficial for accurate conservation assessments. In the Colorado River basin of Texas, five species of freshwater mussels (i.e., *Cyclonaias houstonensis*, *Cyclonaias petrina*, *Lampsilis bracteata*, *Truncilla macrodon*, and *Fusconaia mitchelli*) are currently being evaluated for endangered species listing by the United States Fish and Wildlife Service (USFWS). The goals

of this study were to use mark-recapture methodologies in combination with PIT tag technology to gain insight into population parameters and movement of these species, and how they relate to river discharge. Specific objectives were to: (1) quantify population demographics (i.e., abundance, survival, immigration) of candidate mussels in relation to river discharge and flow events, (2) assess candidate mussel movement and its relationship to flow, and (3) to evaluate baseline growth rates for candidate species. Although additional data collection is planned to fully evaluate these objectives, this report presents a summary of 2017 results and analysis conducted to date.

Methods

Site Selection

We selected a site within both the Lower Colorado River (Colorado County) and Middle Colorado River (San Saba County) based on results of Task 1 survey work and where candidate species were known to occur within the Colorado River basin (Figure 1). We delineated each site into 300 m^2 areas and used a GPS unit to mark the boundaries of each area so that we could accurately return to the same location. The Middle Colorado River Site had an average depth of 0.31 m, mean column velocity of 0.25 m/s, mean benthic velocity of 0.03 m/s, and substrate consisted of silt (8%), sand (12%), gravel (25%), cobble (35%), and boulder (20%). The Lower Colorado River Site had an average depth of 0.43 m, a mean column velocity of 0.17 m/s, a mean benthic velocity of 0.33 m/s, and substrate at this site consisted of sand (15%), gravel (10%), cobble (5%), and bedrock (70%).

Study Design

We implemented a robust mark-recapture design to account for temporal variation in population dynamics and imperfect detection among sampling events, with primary periods separated by season. In the Lower Colorado River, we initially tagged mussels in March 2017 and conducted three primary period recapture surveys (i.e, Spring [April], Summer [August], Fall [November]). In the Middle Colorado, we initially tagged mussels in June 2017, and conducted two primary period recapture surveys (i.e, Summer [August-September], Fall [November]). Within each primary period, we conducted three secondary period surveys. Each secondary period survey was separated by at least 24 hours but not more than 72 hours to allow for individual mussels to reacclimate following handling but not violate closed population model assumptions.

During the initial tagging event at each site, mussels were collected via visual and tactile surveys using a two-pass depletion method within the 300 m^2 sampling area. All live mussels collected were tagged with laminated vinyl shellfish tags (Floy®), and all live candidate species collected also received a Passive Integrated Transponder (PIT) tag (Biomark®). For adhesion of shellfish and PIT tags, we used a cyanoacrylic glue (Loctite Gel Control Super Glue). The PIT tags were encapsulated with this adhesive to prevent tag damage and increase retention. After glue application, all tag types were sprayed with non-toxic accelerant to expedite curing time and decrease mussels' time out of water. The use of cyanoacrylate adhesives is beneficial when PIT tagging a high volume of mussels, due to the decrease in handling time. Furthermore, tag retention with cyanoacrylates has not been shown to differ compared to other adhesive types (e.g., epoxy, dental cement; Ashton et al. 2017).

During each sample event, we conducted a minimum of two survey passes at each site. We performed the first pass using the Biomark reader to locate PIT tagged individuals. The

second pass was conducted using visual and tactile methods to capture mussels. Unmarked individuals collected were identified to species, measured (length, width, height), sexed (if applicable), and tagged. In addition, water quality (i.e., temperature, DO, conductivity, pH), water depth, and current velocity were measured during each sampling event. Percent substrate composition was visually estimated based on the standard Wentworth scale for particle size.

Mark-Recapture Data Analysis

To investigate changes in population dynamics of the candidate species at both sites, we conducted all data analyses in R 3.3.2, package 'Rcapture', a program that utilizes loglinear models to estimate demographic parameters for mark-recapture study designs, including closed population, open population, and robust design models. Package 'Rcapture' is beneficial for these types of analyses because it offers multiple options for modeling capture probabilities to best account for potential capture heterogeneity for closed population models or within primary periods of a robust design. Having the ability to select the model of best fit for each primary period improves the demographic parameter estimates for robust design models (Baillargeon & Rivest 2007).

Prior to fitting a robust design model, closed population models were conducted separately to identify which model type was most appropriate for each primary period. In addition, an open population model was performed to identify if it was appropriate for the robust design model. After the selected models were deemed appropriate, we ran robust design models for each candidate species where sufficient data were available. The model of best fit for closed, open, and robust design models were selected based on Rivest and Daigle (2004) and Baillargeon and Rivest (2007).

Candidate Species Growth Evaluation

As part of the mark-recapture study, mussel size data was recorded for all native unionids captured. Shell length (mm) at recapture from multiple recapture events was used to evaluate growth of candidate species. Lower Colorado River Site growth estimates are based on four sampling events occurring in March, April, August, and November, respectively. Middle Colorado River Site growth estimates are based on three sampling events occurring in June, August-September, and November. For some species and time intervals, the repeated individual length measurements required for growth estimates were not available. For both sites and all candidate species, growth rate estimates from all seasons were standardized by month (i.e., mm/month).

Movement Analysis

Movements of *C. houstonensis*, *C. petrina*, and *T. macrodon* were assessed using mark-recapture data collected from primary and secondary sampling using the Biomark reader. Movement calculations were derived from the final known location of species of concern from previous primary sampling event and the first known location from the subsequent primary sampling event. Last known location was subtracted from first known location and converted to meters using a formula derived from the Law of Cosines. Average (± SD) movement was calculated between all primary periods. In the lower Colorado River, movement was calculated for the periods of March to April, April to August, and August to November. In the middle Colorado River, movement was calculated for the periods of June to August and August to November.

Results

Lower Colorado River Site: Colorado County

During initial sampling in March, a total of 160 candidate species were captured: 103 *C. houstonensis* (64%), 48 *C. petrina* (30%), and 9 *T. macrodon* (5.6%). Across all subsequent primary and secondary sampling periods there were a total of 380 recaptures, of which, 259 were *C. houstonensis* (68%), 119 were *C. petrina* (31%), and 2 were *T. macrodon* (0.5%). Recaptures varied between primary sampling periods, with 177 recaptures in April, 132 recaptures in August, and 12 recaptures in November (Table 1). Numbers of *T. macrodon* collected (N=16) were insufficient for mark-recapture modeling. Preliminary modeling results of 2017 data are presented below for *C. houstonensis* and *C. petrina*.

Cyclonaias houstonensis

Capture probability of *C. houstonensis* in the lower Colorado River site using visual and tactile searches ranged from 0.47 ± 0.06 (SE) in Period 2 to 0.52 ± 0.19 in Period 3 (Table 3). We observed a decline in survivorship between periods, which decreased from 0.88 ± 0.13 (SE) between Period 1 and 2, to 0.04 ± 0.03 (SE) between Periods 3 and 4. Abundance estimates were 401.9 ± 22.10 (SE) within Period 1, 351.6 ± 47.60 (SE) within Period 2, and decreased sharply to 19.9 ± 7.40 (SE) within Period 3. Number of new arrivals (immigrants) ranged from 0.0 ± 0.00 between Period 1 and Period 2 to 6.2 ± 4.80 between Period 2 and Period 3.

Capture probability of *C. houstonensis* in the lower Colorado River was much higher with the PIT reader, and ranged from 0.87 ± .13 (SE) to 0.95 ± 0.01 (SE) (Table 3). PIT tag information was valuable in monitoring movement of individual mussels between primary periods. However, each mussel detected with the PIT reader was not collected to confirm if the

individual was alive, and therefore, abundance and survival estimates from this data have less confidence until PIT tag individuals are confirmed alive via tactile searches.

Cyclonaias petrina

Capture probability of *C. petrina* at the lower Colorado River site ranged from 0.54 ± 0.33 (SE) in Period 3 to 0.58 ± 0.09 in Period 1 (Table 3). We observed a substantial decline in survivorship between periods, which decreased from 0.79 ± 0.15 (SE) between Period 1 and 2, to 0.04 ± 0.04 (SE) between Periods 3 and 4. Abundance estimates were 127.4. ± 21.30 within Period 1, 127.8 ± 21.10 in Period 2, and decreased sharply to 7.5 ± 5.30 (SE) within Period 3. Number of new arrivals (immigrants) ranged from 26.7 ± 20.70 (SE) between Period 1 and 2, to 2.5 ± 3.20 between Period 2 and 3.

Capture probability of *C. petrina* in the lower Colorado River was much higher with the PIT reader, and ranged from 0.82 ± 0.14 (SE) to 0.94 ± 0.02 (SE) (Table 3). However, each mussel detected with the PIT reader was not collected to confirm if the individual was alive, and therefore, abundance and survival estimates from this data have less confidence until PIT tag individuals are confirmed alive via tactile searches.

Movement

A total of 386 individual movements were calculated for *C. houstonensis* (N = 259), *C. petrina* (N = 113), and *T. macrodon* (N = 14). Movements ranged between 0 m and 20 m for *C. houstonensis* and *C. petrina* and between 1 m and 20 m for *T. macrodon* (Figure 4). Between primary sampling periods, *C. houstonensis* average movement (± 1 SD) ranged from 3.9 (± 2.9) m between March and April (N = 88) to 12.4 (± 5) m between August and November (N = 6), *C.*

petrina average movement ranged from 3.9 (± 2.9) m between March and April (N = 42) to 7.6 (± 5.4) m between April and August (N = 64), and *T. macrodon* average movement ranged from 6.6 (± 5) m between March and April (N = 7) to 10.2 (± 6.2) m between August and November (N = 7).

Middle Colorado River Site: San Saba County

During initial sampling in June, a total of 123 candidate species were captured: 9 *C. houstonensis* (7.3%) and 114 *C. petrina* (92.7%). Across both subsequent primary and secondary sampling periods, there was a total of 255 recaptures, of which, 238 were *C. petrina* (93.3%) and 17 were *C. houstonensis* (6.7%). Recaptures did not vary much between primary sampling events with 128 recaptures in August, and 127 recaptures in November (Table 2). Numbers of *T. macrodon* collected (N = 1) were insufficient for mark-recapture modeling and growth analysis. Preliminary modeling results of 2017 data are presented below for *C. houstonensis* and *C. petrina*.

Robust design models require data from three or more primary periods, and only two recapture events were conducted at the Middle Colorado River Site in 2017. Therefore, for the Middle Colorado River Site, we estimated abundance within primary periods using closed population models based on recapture data from visual/tactile searches. Robust-design mark-recapture models will be conducted after additional sampling events occur.

Estimated abundance of *C. houstonensis* ranged from 7.0 ± 0.00 (SE) within Period 1 to 4.90 ± 1.60 (SE) within Period 2 (Table 4). Estimated abundance of *C. petrina* ranged from 490.2 ± 47.50 (SE) within Period 1 to 254.8 ± 4.2 (SE) within Period 2.

Movement

A total of 265 individual movements were calculated for *C. houstonensis* (N = 11) and *C. petrina* (N = 254), and movements ranged between 0 m and 24 m for both species (Figure 5). Between primary sampling periods, *C. houstonensis* average movement ranged from 5 (± 0.8) m between August and November (N = 4) to 9.4 (± 6.4) m between June and August (N = 7), and *C. petrina* average movement ranged from 4.2 (± 2.6) m between August and November (N = 151) to 7.8 (± 3.9) m between June and August (N = 103).

Mussel Growth Rates

Lower Colorado River

At the Lower Colorado River Site, lengths for *C. houstonensis* ranged from 24 – 79 mm, lengths of *C. petrina* ranged from 32 – 85 mm, and lengths of *T. macrodon* ranged from 32 – 58 mm. Across all candidate species, observed monthly growth ranged from 0 to 12 mm. The highest mean monthly growth rate observed was for *C. petrina* between April and August (2.250 mm/month) (Table 5). The lowest mean monthly growth rate observed was for *C. houstonensis* during the same time period (1.289 mm/month).

Middle Colorado River

At the Middle Colorado River Site, lengths for *C. houstonensis* ranged from 36 – 84 mm, lengths of *C. petrina* ranged from 30– 94 mm, and only one *T. macrodon* (14 mm) was collected. Across all candidate species, observed monthly growth ranged from 0.400 to 0.754 mm. The highest mean monthly growth rate observed was for *C. petrina* between April and August (0.754

mm/month) (Table 6). The lowest mean monthly growth rate observed was for *C. houstonensis* between June and August (0.400 mm/month).

Discussion

Preliminary results of robust design mark-recapture models at the Lower Colorado River Site suggest population sizes of approximately 350 – 400 *C. houstonensis* and approximately 127 *C. petrina* from within our 300 m^2 sampling area during April and August sampling events. A large flood event occurred in the lower Colorado River resulting from intense rains from Hurricane Harvey in late August and September 2017 (Figure 2). The study site was extensively scoured and large bathymetric changes were noted in the river following this event. Population estimates for both species at the Lower Colorado River Site decreased sharply. Mark-recapture models estimated approximately 20 *C. houstonensis* and 7.5 *C. petrina* during the November sampling event. Additional sampling to evaluate recovery following this event will be important in understanding the impacts of such flood events on mussel communities in the Lower Colorado River.

As expected, PIT reader capture probabilities were relatively high (0.82 – 0.95) when compared to capture probabilities from visual/tactile surveys (0.47 – 0.58). However, not every individual detected by the PIT reader was captured and removed from the substrate to assess survival, so data from visual and tactile surveys were more appropriate for estimating population parameters. Georeferenced detections from the PIT reader were used to evaluate movement of candidate species. Target species moved between 0 and 20 meters in the lower Colorado River and between 0 and 24 meters in the middle Colorado River among all primary sampling events.

Preliminary results of mark-recapture analysis at the Middle Colorado River Site suggest population sizes of approximately 5 – 7 *C. houstonensis* and 254 – 490 *C. petrina* within our 300 m^2 sampling area. Although insufficient data exists to run robust design models and analyze changes among primary periods, no substantial changes in mussel abundance were observed by surveyors in 2017. The Middle Colorado River was influenced less by flooding in 2017 (Figure 3), and overall abundance of mussels appeared to be relatively stable between events based on field observations. As more data becomes available from additional sampling, robust design models will be analyzed to evaluate changes among primary periods.

Preliminary estimates of mean monthly growth of candidate species were generally higher for *C. petrina* than *C. houstonensis*, although limited data is available for *C. houstonensis* from the Middle Colorado River Site. Observed mean monthly growth rates were lower at the Middle Colorado River Site than at the Lower Colorado River Site. This may be influenced by a wide variety of environmental factors that have yet to be evaluated. Additionally, this may be a result of differences in size structure of the population. The Middle Colorado River Site was dominated by large individuals, which are assumed to have slower growth rates, than smaller individuals.

Table 1. Results of mark-recapture primary sampling periods at the Lower Colorado River Site.

	March	April		August		November	
Species	**Initial Captures**	**New Captures**	**Recaptures**	**New Captures**	**Recaptures**	**New Captures**	**Recaptures**
C. houstonensis	103	128	115	62	135	6	9
C. petrina	48	39	61	31	55	2	3
T. macrodon	9	6	1	1	1	0	0
Total	160	173	177	94	191	8	12

Table 2. Results of mark-recapture primary sampling periods at the Middle Colorado River Site.

	June	August		November	
Species	**Initial Captures**	**New Captures**	**Recaptures**	**New Captures**	**Recaptures**
C. houstonensis	9	2	10	1	7
C. petrina	114	212	118	68	120
T. macrodon	0	1	0	0	0
Total	123	215	128	69	127

Table 3. Results of Rcapture robust design model in the Lower Colorado River.

	Visual/Tactile Search Efforts		**PIT Tag Reader**	
Model Fit	*C. houstonensis*	*C. petrina*	*C. houstonensis*	*C. petrina*
Deviance	124.87	76.76	157.80	107.68
Df	496	496	494	495
AIC	298.09	196.48	353.76	247.48
Model Parameters				
Capture Probabilities				
Period 1	0.49 ± 0.03	0.58 ± 0.09	0.90 ± 0.03	0.91 ± 0.06
Period 2	0.47 ± 0.06	0.54 ± 0.08	0.95 ± 0.01	0.94 ± 0.02
Period 3	0.52 ± 0.19	0.54 ± 0.33	0.87 ± 0.13	0.82 ± 0.14
Survival Probabilities				
Period 1 - Period 2	0.88 ± 0.13	0.79 ± 0.15	0.99 ± 0.02	0.87 ± 0.05
Period 2 - Period 3	0.04 ± 0.03	0.04 ± 0.04	0.03 ± 0.01	0.08 ± 0.03
Abundance Estimates				
Period 1	401.9 ± 22.10	127.4 ± 21.30	204.4 ± 7.60	88.3 ± 4.90
Period 2	351.6 ± 47.60	127.8 ± 21.10	292.1 ± 5.50	108.3 ± 3.60
Period 3	19.9 ± 7.40	7.5 ± 5.30	11.4 ± 2.10	9.8 ± 2.20
Number of New Arrivals Estimates				
Period 1 - Period 2	0.0 ± 0.00	26.7 ± 20.70	91.5 ± 12.50	31.0 ± 7.30
Period 2 - Period 3	6.2 ± 4.80	2.5 ± 3.20	3.3 ± 2.00	1.0 ± 1.20

Table 4. Results of Rcapture closed population models for each primary period in the Middle Colorado River.

	Visual/Tactile Search Efforts	
Model Fit	*C. houstonensis*	*C. petrina*
Period 1		
Deviance	2.96	0.77
Df	2	2
AIC	24.19	46.95
Period 2		
Deviance	5.14	3.77
Df	3	2
AIC	19.75	50.12
Abundance Estimates		
Period 1	7.0 ± 0.00	490.2 ± 47.50
Period 2	4.9 ± 1.60	254.8 ± 4.20

Table 5. Candidate species average monthly growth rate estimates for the Lower Colorado River mark-recapture study site.

	Average Monthly Growth (mm ± SE)		
Species	**March – April (n)**	**April – August (n)**	**Total**
Cyclonaias houstonensis	1.429±0.235 (98)	1.289±0.438 (45)	1.359
Cyclonaias petrina	1.878±0.474 (41)	2.250± 0.396 (16)	2.064
Truncilla macrodon	1.500±1.147 (6)	N/A	1.500
Total	1.602	1.770	**1.641**

Average growth estimated between mark-recapture periods was standardized on a monthly rate.

Table 6. Candidate species average monthly growth rate estimates for the Middle Colorado River mark-recapture study site.

	Average Monthly Growth (mm ± SE)		
Species	**June – August (n)**	**August – November (n)**	**Total**
Cyclonaias houstonensis	0.400±0.125 (5)	N/A	0.400
Cyclonaias petrina	0.643±0.133 (43)	0.754±0.121 (61)	0.609
Truncilla macrodon	N/A	N/A	N/A
Total	0.522	0.754	**0.405**

Average growth estimated between mark-recapture periods was standardized on a monthly rate.

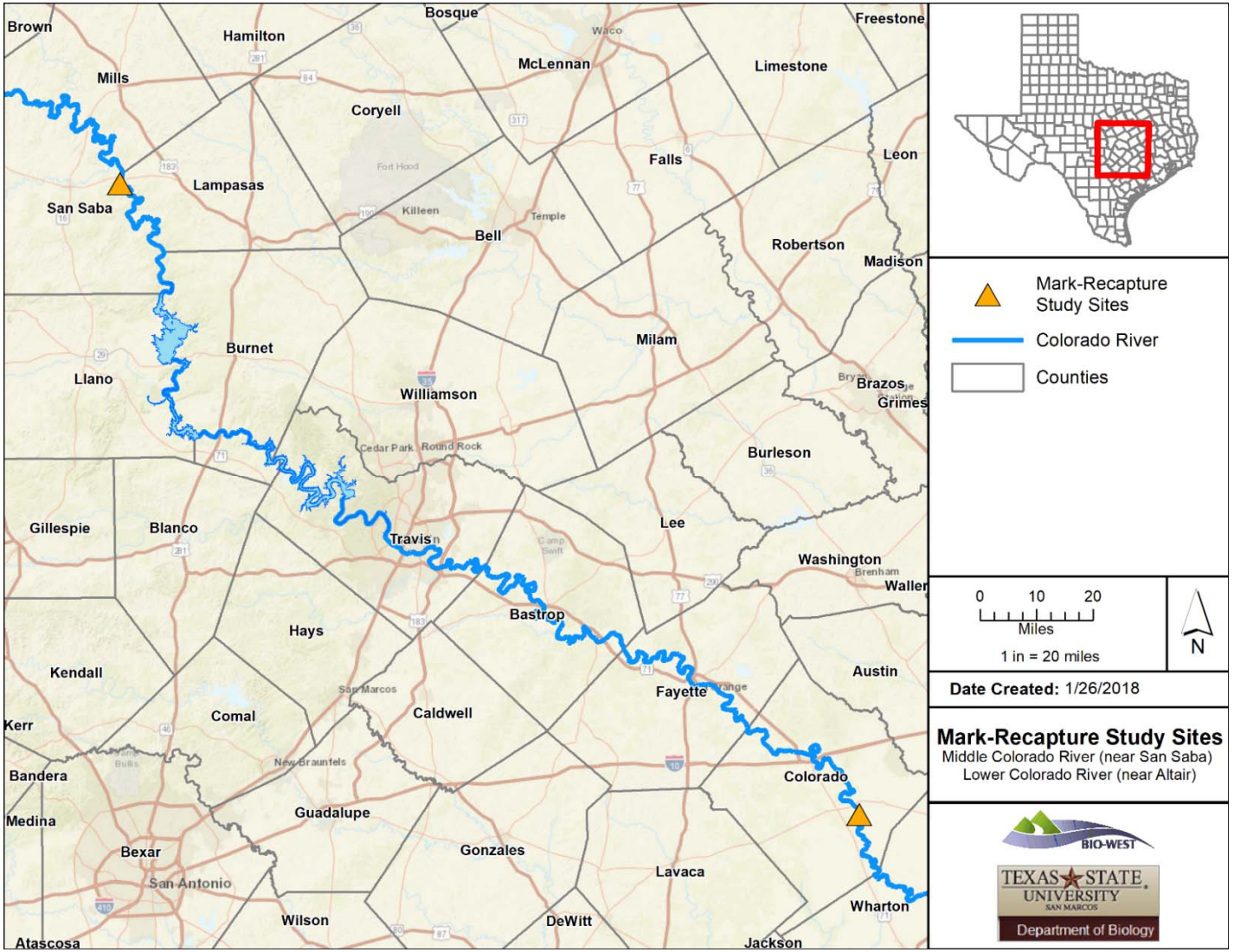

Figure 1. Map of mark-recapture study sites in the Colorado River basin, Texas.

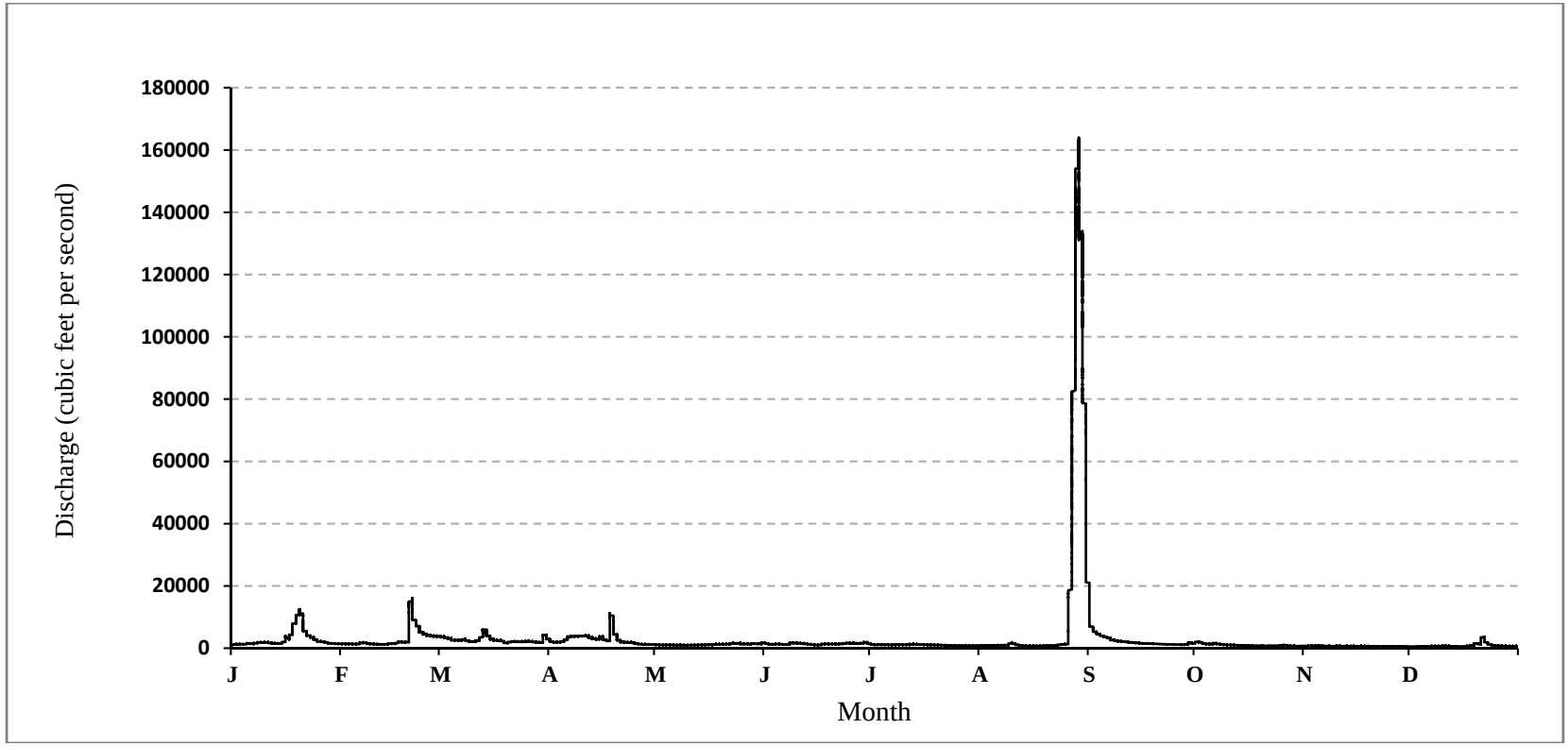

Figure 2. Discharge from the USGS gage on the Lower Colorado River at Columbus (USGS gage# 08161000) during 2017.

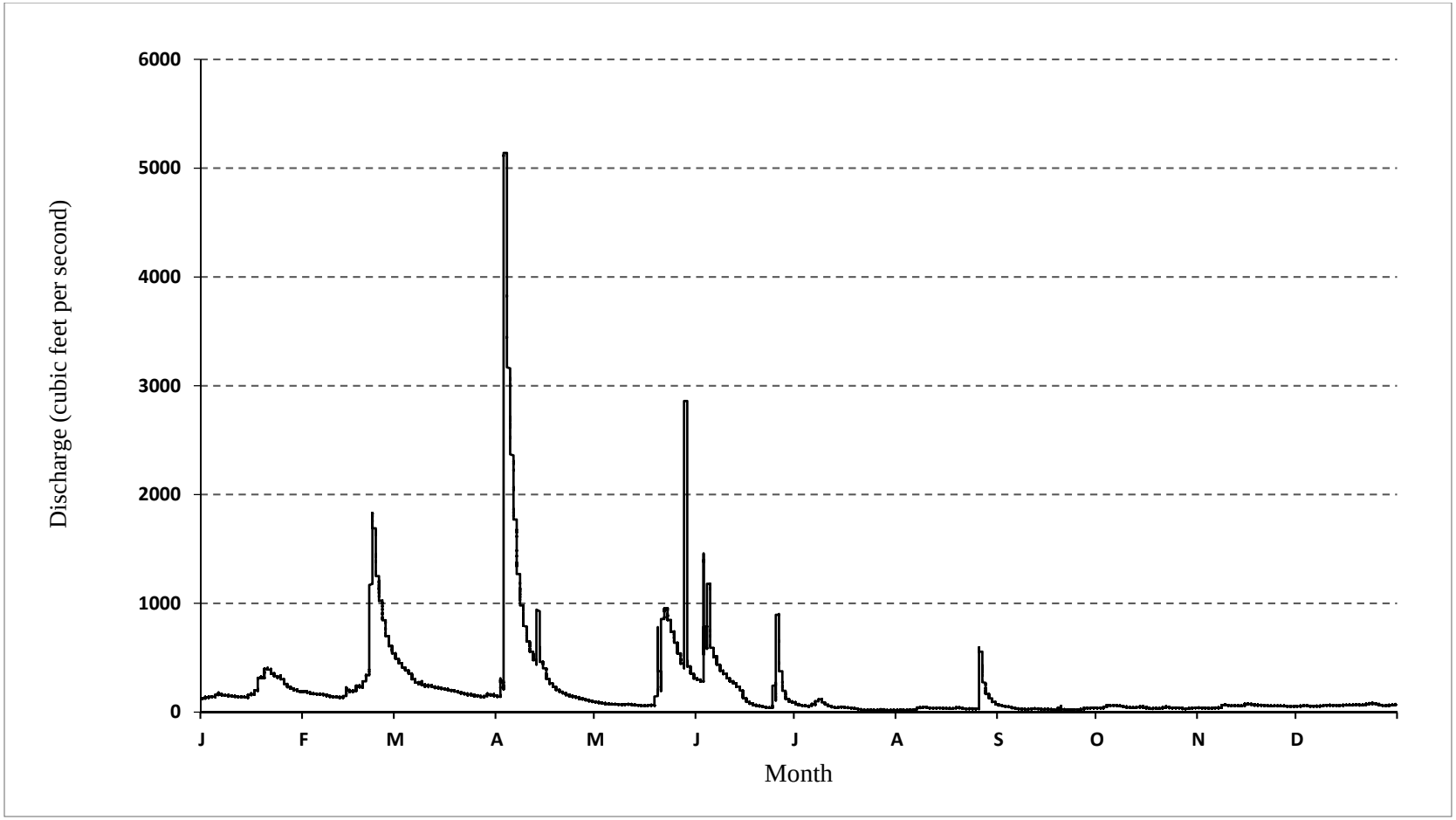

Figure 3. Discharge at the USGS gage on the Colorado River near San Saba (USGS gage# 08147000) during 2017.

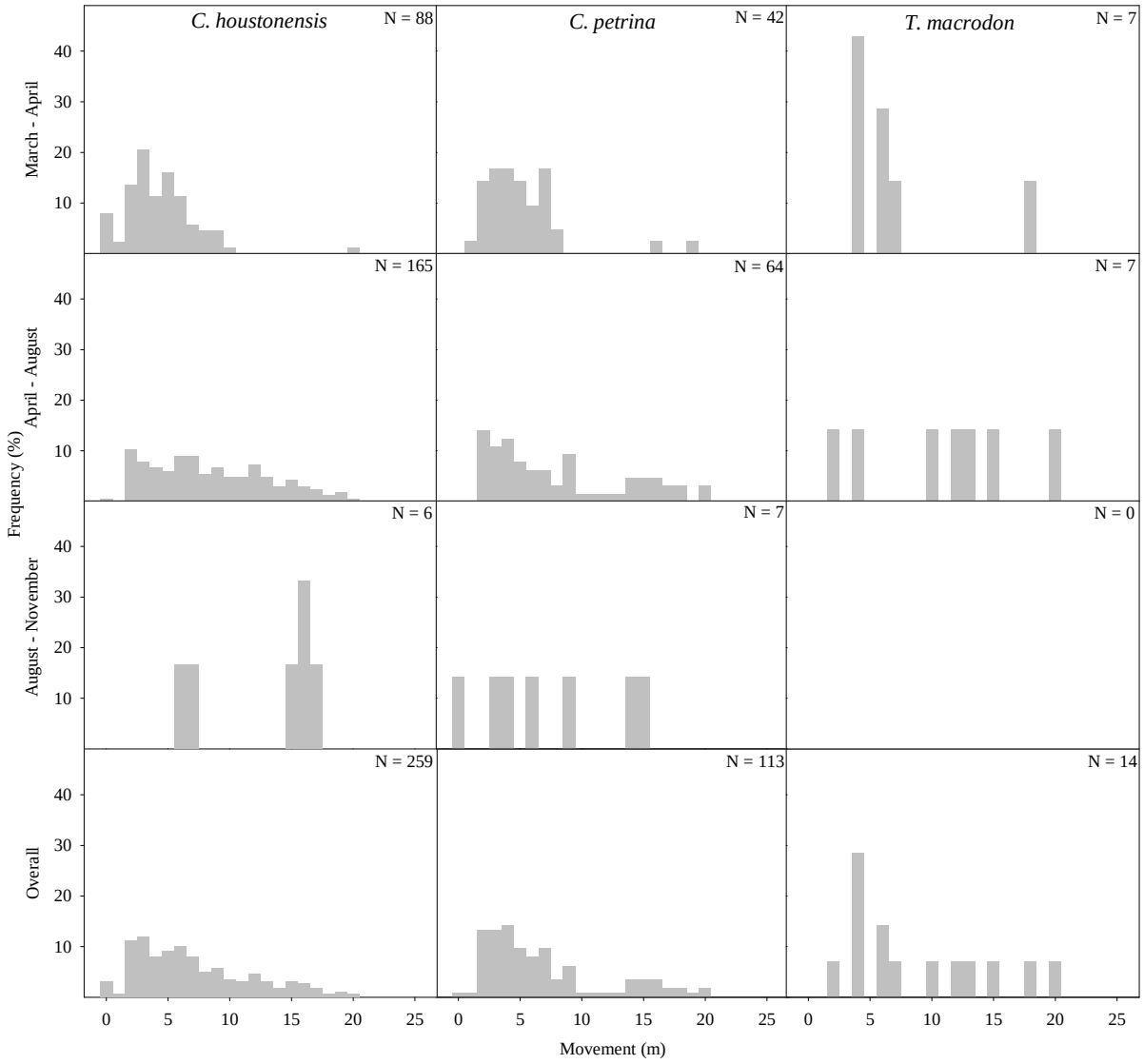

Figure 4. Percent frequency of mussels by distance (m) by time period and overall by species from the Lower Colorado River site in 2017.

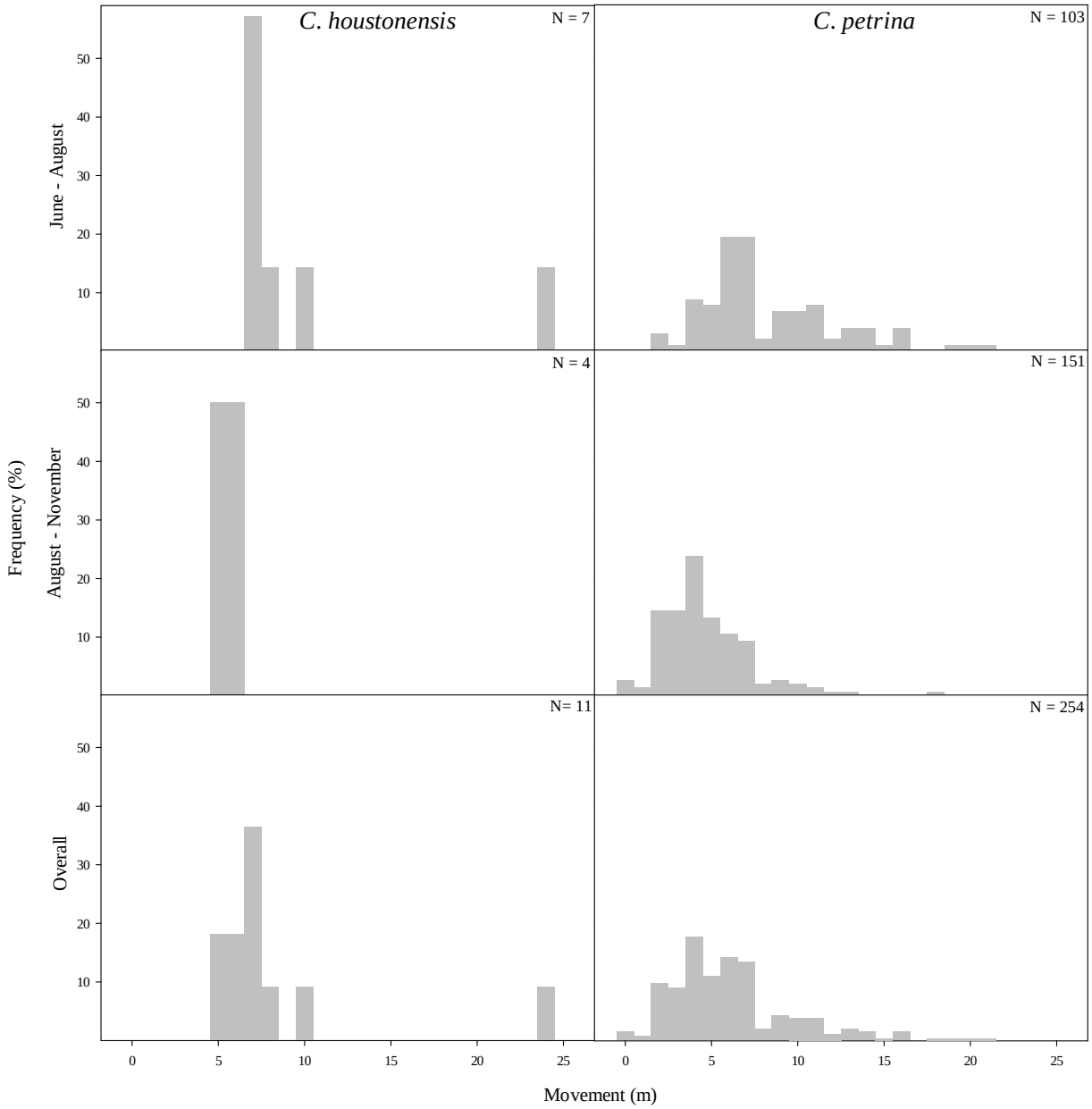

Figure 5. Percent frequency of mussels by distance (m) by time period and overall by species from the middle Colorado River site in 2017.

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Task 5: Captive Propagation
Task 6: Captive Rearing

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San Marcos Aquatic Resources Center

Task 5: Captive Propagation

Collection

In March 2017, San Marcos Aquatic Resources Center (SMARC) staff collected two gravid Texas fatmucket mussels as part of collection efforts for desiccation and long-term holding of adults. These mussels were brought to the SMARC and held in recirculating systems containing coarse sand substrate (Figure 1). These mussels were fed a 2:1 mixture of Shellfish Diet 1800 and Nannochloropsis 3600 (Reed Mariculture Inc. Campbell, CA) daily at a target concentration of 300,000 cells/mL. Feeding concentrations were verified using a Beckman Coulter Multisizer 4e (Figure 2).

Captive Propagation of Juveniles

SMARC staff inoculated Green sunfish (*Lepomis cyanellus*), Bluegill (*Lepomis macrochirus*), and Blacktail shiner (*Cyprinella venusta*) with glochidia from two gill water tubes of a gravid Texas fatmucket (*Lampsilis bracteata*) to determine the most efficient host fish. After 28 days, 21 juveniles transformed on Green sunfish, seven juveniles transformed on Bluegill, and zero juveniles transformed on

Blacktail shiner. Based on these results, 25 Green Sunfish were inoculated with the remaining glochidia from the previous trial and an additional gravid Texas fatmucket. All host fish were held in a modified flow-through Aquatic Habitats (AHAB) system at 21°C (Figure 3). After 35 days, 1533 live glochidia transformed into juveniles. All juveniles were then held in a pulsed flow-through system equipped with an automatic feeder (Figure 4). A 2:1 mixture of Shellfish Diet 1800 and Nannochloropsis 3600 (Reed Mariculture Inc. Campbell, CA) was delivered to each individual holding container hourly with a target concentration of 30,000 cells/mL. Substrate was changed weekly in beakers and growth rates were assessed. All juveniles measured approximately 300 µm upon transformation. Three weeks post transformation, juveniles averaged 450 µm (Figure 5) in length. However, within six weeks, growth rates stopped and an effective mortality rate of 100% was observed. Based on these results, several changes will be implemented before propagation efforts in 2018 begin. Among these are using filtered pond water instead of well water, increasing holding temperatures to 25°C, and modifying the delivery system of feed in pulsed flow-through systems.

Task 6: Long-term Captive Rearing and Holding

Mussels collected in March and April 2017 were housed in indoor recirculating systems containing coarse sand substrate (Figure 6). Survival rates of both Texas pimpleback and Smooth pimpleback have been 100%. These mussels are fed daily, the same mixture listed above. Beginning in Spring 2018, all long term holding will be moved to floating baskets, filled with coarse sand substrate, in outdoor ponds. All Texas fatmuckets collected for long-term rearing in this task were used as organisms in the lethal desiccation trials.

Inks Dam National Fish Hatchery

Increasing concern over the status of native freshwater mussels in the Lower Colorado River watershed has prompted efforts to evaluate the viability of freshwater mussel propagation in a hatchery setting. Inks Dan National Fish Hatchery (IDNFH) is currently holding on station Texas pimpleback (*Cyclonaias petrina*), Smooth pimpleback (*Cyclonaias houstonensis*) and Texas fatmucket (*Lampsilis bracteata*) for research and development of a propagation program (Figure 7). In early 2017 IDNFH staff completed an outdoor flow-through aquaculture system with six independent 5-ft. circular tanks for holding adult fresh water mussels (Figure 8). Since that time, multiple indoor and outdoor systems have been planned and brought into operation.

Task 5: Captive Propagation

Collection

IDNFH collection efforts of adult mussels for captive propagation began in April, 2017 with a trip to the Llano River at FM1871 with San Marcos Aquatic Resource Center (SMARC) staff. Texas pimpleback and Texas fatmucket were collected in sufficient numbers for compliance with state collection permits. Four mussels of each species were collected and brought to IDNFH for holding in the outdoor flow-through system. The functionality of the system had previously been tested using locally abundant Giant floaters (*Pyganodon grandis*). IDNFH and SMARC staff next accompanied BIO-WEST, Inc. staff for collection on the Lower Colorado River Hwy 90 crossing near Altair, TX. This trip yielded plentiful numbers of Texas pimpleback and Smooth pimpleback. Twelve of each species were brought on station, separated by species and held in the outdoor flow-through system. Additional collection trips to multiple sites on the Llano River were made but yielded low numbers for collection. IDNFH staff returned to the Llano River at FM1871 for two additional collections and sampled different reaches of the river during each trip. During these collection trips, only Texas pimplebacks were brought back to the

hatchery. Most recently, five gravid Texas fatmucket females were brought on station from a dewatering operation on a reservoir of the Llano River near Llano, TX.

Captive Propagation of Juveniles

IDNFH hatchery staff, with the assistance of SMARC staff, infested Bluegill sunfish (*Lepomis macrochirus*) and Green sunfish (*Lepomis cyanellus*) using glochidia from two gravid Texas fatmuckets on June 20, 2017. After infestation, host-fish were held in Z-Habitat type rearing units until metamorphosis and drop-off occurred 10-14 days post infestation (Figure 9). Staff estimated 2,300 metamorphosed juveniles were collected. Due to the time consuming nature of microscopic enumeration of newly metamorphosed juveniles, an additional Nikon microscope was purchased to increase future propagation efficiency.

Juvenile mussels were placed into a staff designed flow-through rearing system with sand substrate (Figure 10). The rearing system utilized water from Inks Lake filtered to 50 µm by the facilities filtration system. No supplemental feed was offered. The juvenile mussels experienced good growth over the first month, increasing in size from approximately 250 µm to 1000 µm. Anoxic influent water (dissolved oxygen < 0.5 mg/L) from the hatchery water source (Inks Lake) was experienced in early August, 2017 and led to near total mortality of the juvenile mussels.

One survivor was found in the system on September 27, 2017 and measured 2.8 mm in length (Figure 11). Since September, 2017, this individual has shown steady growth (over 8 mm by November, 2017) and is presumably meeting nutritional requirements for survival on filtered lake water.

Additionally, in mid-December, 2017, while modifying the juvenile rearing system, a second juvenile Texas fatmucket from the June, 2017 infestation was discovered on the filter screen of the system. This individual may have been living on the filter screen before anoxic conditions led to the mortality event for the June, 2017 cohort. If this was the case, its larger size may have been the result of more favorable environmental conditions during the anoxic event. The continued growth and survival of

these mussels lends credence to the belief that freshwater mussels can be cultured at IDNFH without the addition of supplemental feed (Figure 12). To mitigate the impact of future anoxic events, IDNFH staff are currently modifying the original juvenile rearing system. The new system(s) will have increased rearing capacity and features that allow for supplemental aeration should the need arise.

As previously mentioned, five gravid female Texas fatmuckets were brought to IDNFH from a de-watered reservoir near Llano, TX. The mussels were placed in newly constructed indoor raceway style tanks with flow-through lake water (Figure 13). Mussels from prior collection trips had already been brought indoors and placed in the new holding system. While in the indoor system, the five newly collected females began to actively display their lures. Two females were transferred to the Z-habitat system where water temperature could be controlled via an in-line heater. Water temperature was gradually increased from 18ºC to 24ºC which resulted in an increase in lure display frequency. Because a December infestation was not planned, only 26 Green sunfish were available on station, most of which had previously been infested in June of that year. All 26 host-fish were infested on December 8, 2017 with glochidia from a single gravid female. The glochidia were found to be 99% viable with an estimated number of 117,400 (Figure 14). After infestation of all 26 Green sunfish, unattached glochidia were enumerated and estimated at 59,200. Assuming equal distribution among host-fish, this yielded an attachment rate of approximately 2,200 glochidia per fish. Recovery of metamorphosed juveniles was low compared to the June, 2017 host-fish infestation. Staff believe the low number of metamorphosed juveniles was due to the reuse of the Green sunfish, which may have developed immunity to infestation. Only 550 juveniles were recovered from the December, 2017 host-fish infestation. Furthermore, survival of the metamorphosed juveniles has been low. Because host-fish infestation occurred during a winter month when primary production in the facilities source water would be relatively low, and juvenile mussels were maintained at a temperature (24ºC) indicative of spring or summer, metabolic requirements for growth and survival may have out-paced available food supply.

Task 6: Long-term Captive Rearing and Holding

The new indoor raceway system is functionally similar to the outdoor system in that they both utilize flow-through design and supplemental aeration. However, the new system is much shallower and allows for observation without handling. This system will be especially useful for observation of short term brooders (*Cyclonaias petrina* and *C. houstonensis*) and collection of glochidia if conglutinates from gravid females are released. The outdoor circular tank system will be utilized for holding different fish species in quarantine for future host-fish identification studies.

Once juvenile mussels reach a size to be contained in floating baskets, they will be moved to ponds for grow-out. In November, 2017, two floating docks were installed at IDNFH to assist with the investigation of pond mussel culture (Figure 15). Floating baskets fitted with wire mesh and sand substrate will be tethered to the floating docks. After the spring 2018 collection of glochidia from captive wild caught adult mussels, juvenile mussels will be moved to floating baskets as individual size and circumstances allow.

Mussel Inventory at IDNFH

Freshwater mussel inventory at IDNFH is shown in Table 1.

Table 1. January, 2018 freshwater mussel inventory at IDNFH.

Species	Scientific name	Adults held on station	Drop-offs produced	Juveniles held alive on station
Texas Fatmucket	*Lampsilis bracteata*	12	2850	2
Texas Pimpleback	*Cyclonaias petrina*	23	-	-
Smooth Pimpleback	*Cyclonaias houstonensis*	9	-	-

UVALDE NATIONAL FISH HATCHERY

Construction

In May, as an effort to assist in mussel conservation and conduct future studies (e.g. on propagation and rearing techniques) on the Central Texas federal candidate and petitioned fresh water mussel species, a Beckman Multisizer 4E Coulter Particle Analyzer was purchased. The Multisizer would be used to automatically count algal densities for feeding studies and the culture of the federal candidate freshwater mussels that are currently on station. The particle analyzer will save personnel on labor and time in measuring algal densities fed to both young and adult mussels. The analyzer is also capable of quantifying, mammalian cells, bacteria, yeast, spheroids, and large protein and cell aggregates. This instrument has data overlay capability for detecting and analyzing complex samples over a wide variety of particle sizes from 0.2 µm-1600 µm in diameter from sample volumes as small as 5 ml. On July 10th, a Beckman Coulter technician trained staff members from the UNFH how to measure samples and use some of the standard operating procedures (SOPs).

In July, Pat Duncan guided Lead Fish Biologist Valentin Cantu and Fish Biologist Greg Cottingham in constructing a flow-through system for mussels held in separate containers by species and number at the facility's existing Quarantine Building (Figure 16). All effluents from the system's holding mussels flow through a chlorinated sand filter and then on dry ground (Figure 17) to guard against the release of organisms into outside waters. During FY 2017, staff members maintained and monitored the chlorine levels and sand in the system to maximize the efficacy and efficiency of the system.

In August, Pat Duncan had made plans and guided lead Fish Biologists Valentin Cantu and Fish Biologist Greg Cottingham to begin to construct an algal feeding system that is driven by a peristaltic pump. By September 19th, the mussel systems were equipped with peristaltic pumps to provide continuous feeding of concentrated algal solutions. A Tecniplast stand-alone Zeb-Hab rack system with a complete recirculating system was installed on September 11th at UNFH (Figure 18). The system will provide additional facilities for holding mussels and host fish for future glochidia production and later

juvenile rearing and culture trials. The Z-Hab is a computerized tank system capable of automatically monitoring temperature, pH, and specific conductivity and setting off alarms if water parameters exceed upper or lower limits. A Z-Hab technician trained UNFH staff members on how to use and maintain the Z-Hab system on September 12th. Data may be uploaded from the system for data analysis. The system is designed with specialized containers for holding aquatic species and a drum filter that automatically removes debris from the system. The system will be used for mussels in refugia and for propagation. Tecniplast personnel are scheduled to install a chiller for system completion.

On October 31st, UNFH staff worked with USFWS IT personnel to authorize software to begin installing a state of the art microscope that will be used to conduct mussel research. The microscope is an Olympus BX43 Phase Contrast Upright Custom System uses unique software (Cellsens) that will save personnel on labor and time on live acquisition, live measuring, annotation, time lapse, and fluorescence overlay. The unique software has a process manager for several types of experiments and images that can be readily shared with other Cellsens users without having to convert the image format or lose all functionality on the analysis side. An Olympus representative installed the microscope in the laboratory of the UNFH and trained staff members on microscope operation.

Collection

Freshwater mussel collection near Comfort, Texas was scheduled on April 12th, but was cancelled due to inclement weather.

On August 3rd, Lead Biologist Valentin Cantu, Fish Biologist Greg Cottingham, and Student Intern Brittany Germain met BIO-WEST, Inc. at the Colorado River, Columbus, Texas (Figure 19) to collect the candidate species for the UNFH refugia. UNFH team members were transported to shallow collection sites by boat. Mussels were collected from the Colorado River with the assistance of BIO-WEST, Inc. staff members.

The river water from the Colorado River was warm (31.40ºC) and turbid (visibility < 1 inch). Most mussels were collected by hand from substrates rich in mud, sand, and detritus over coble and bedrock. A total of 30 Smooth Pimpleback (*Cyclonaias houstonensis*), 15 Texas Pimpleback (*C. petrina*), and two Texas Fawnsfoot (*Truncilla macrodon*) mussels were collected. Mussels were carefully packed into 40-quart ice coolers with ice packs and burlap bags and transported to the UNFH (Figure 20). Standard hatchery protocols were followed to prevent the transfer of aquatic nuisance species and disease. Upon arrival to the UNFH Quarantine Building, mussels were carefully acclimated to their tank systems and specimens were monitored daily.

Culture

In August, the Project Leader Pat Duncan began training the Lead biologist Valentin Cantu and Fish Biologist Greg Cottingham the art of feeding a special algal diet to three species of federal candidate and petitioned freshwater mussels including the Texas Fawnsfoot (*Truncilla macrodon*), smooth pimpleback (*Cyclonaias houstonensis*), and Texas pimpleback (*C. petrina*) that were brought on station in early August. After the freshwater mussels were brought on station, staff members routinely fed mussels, maintained water flows, cleaned screens of debris, replaced air stones, and checked for mortalities. Mussels were maintained in flow-through systems throughout culture period.

Figure 1: Texas fatmuckets held in indoor recirculating system.

Figure 2: Beckman Coulter Multisizer 4e used to size and quantify particle levels in water.

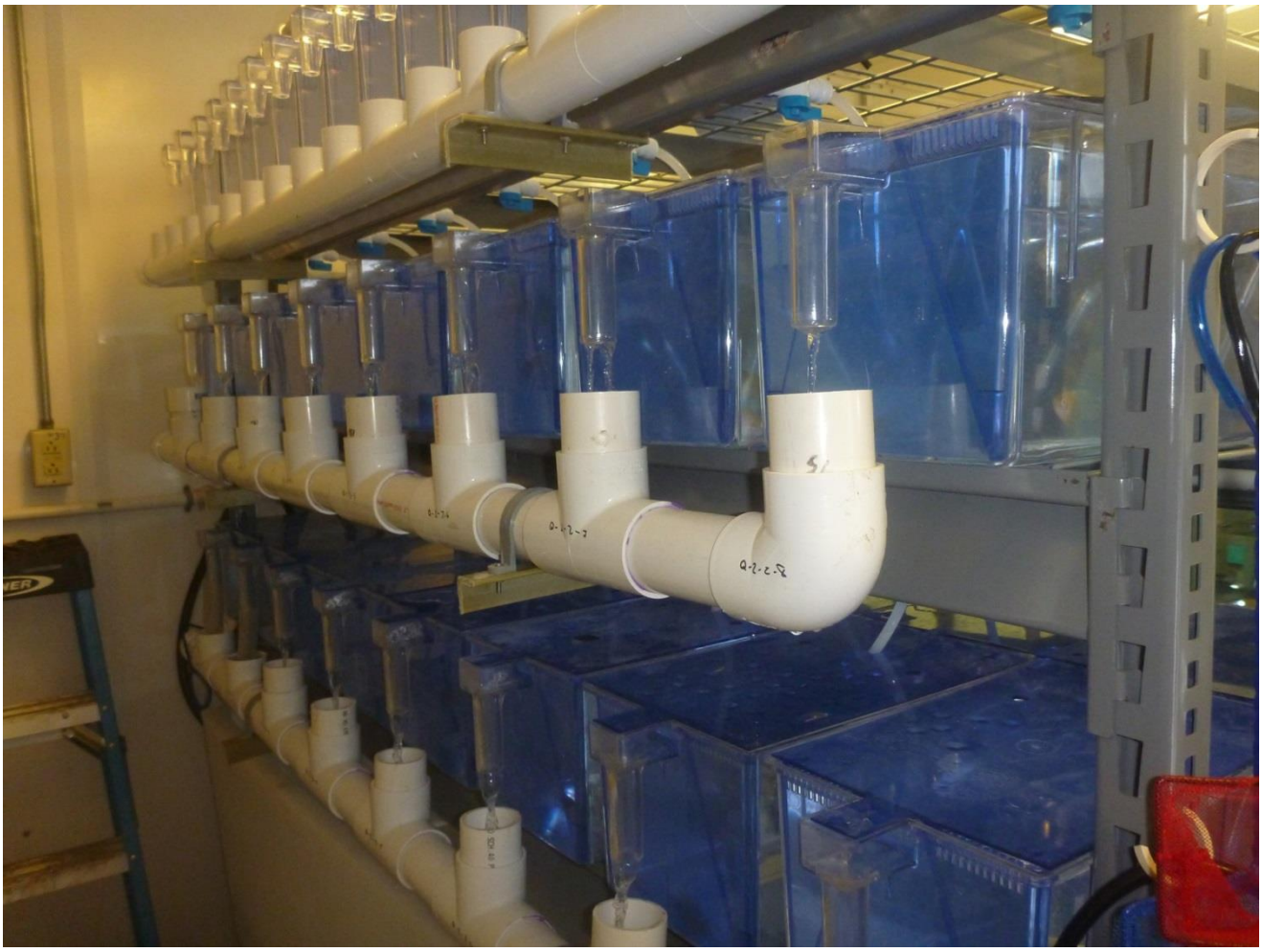

Figure 3: Flow-through AHAB system used to house host fish.

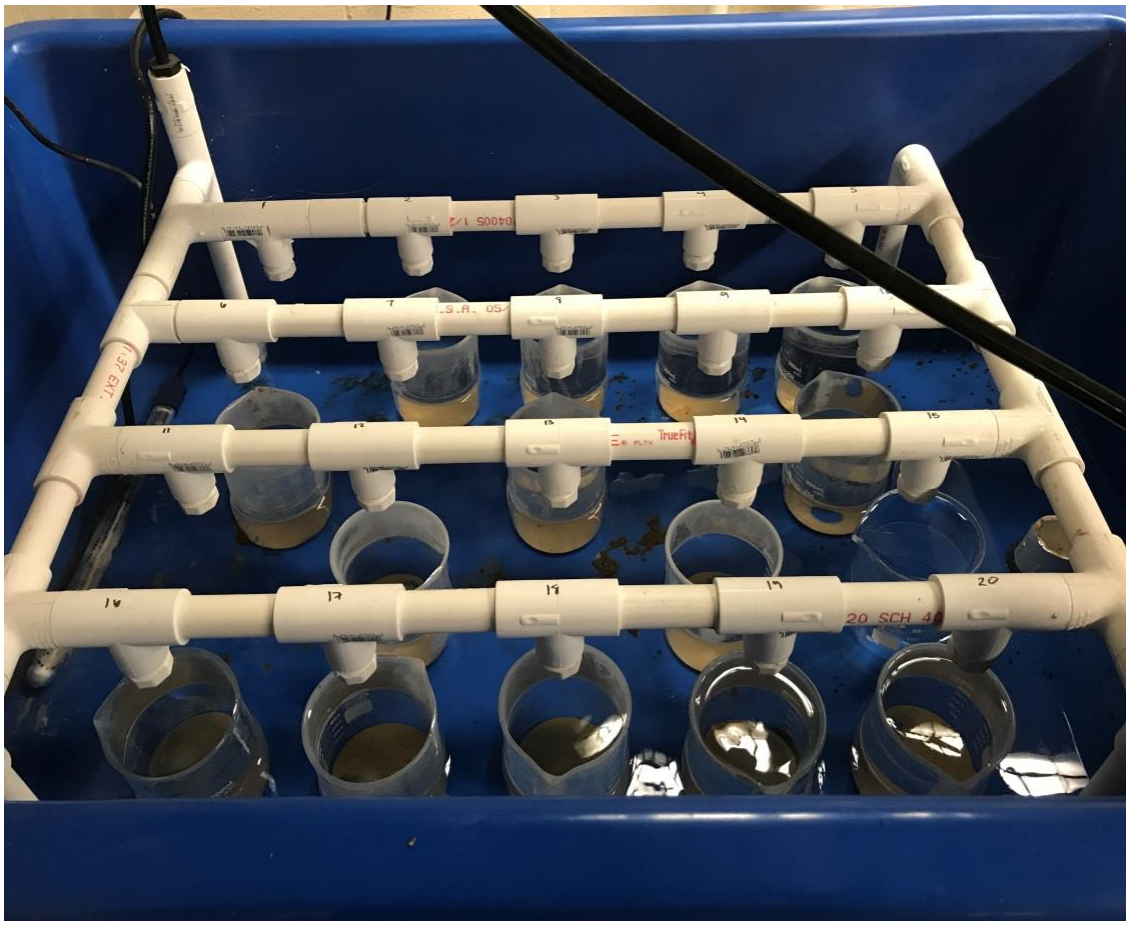

Figure 4: Pulsed flow-through system used to house juveniles. Each beaker is capable of housing approximately 100 juveniles.

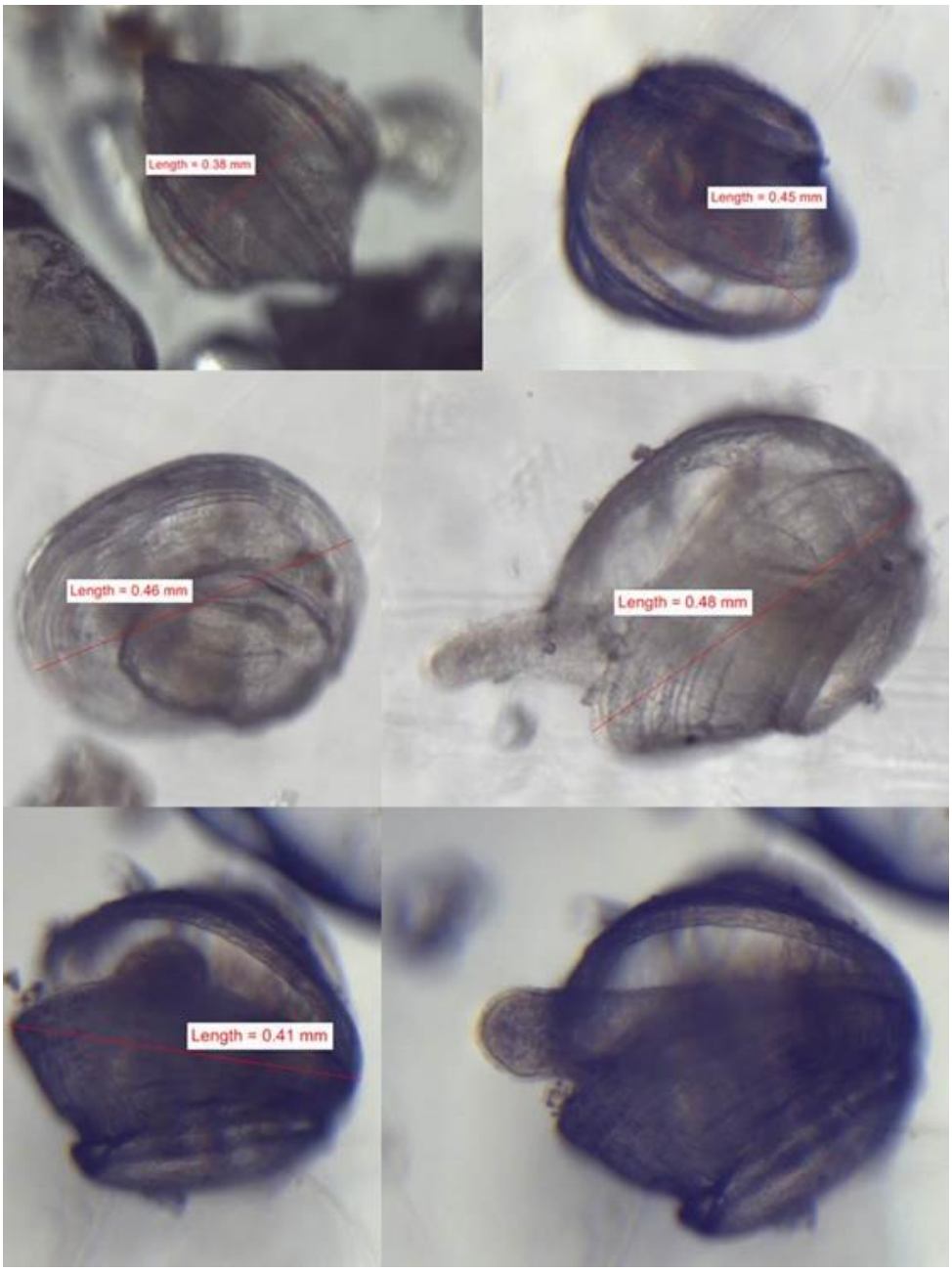

Figure 5: Juvenile Texas fatmuckets cultured at the SMARC.

Figure 6: Indoor recirculating systems used for long term holding of adult mussels in 2017.

Figure 7. Freshwater mussels collected from the Colorado River watershed for use in propagation at IDNFH. From left to right: Texas fatmucket (*Lampsilis bracteata*), Texas pimpleback (*Cyclonaias petrina*) and Smooth pimpleback (*Cyclonaias houstonensis*).

Figure 8. Outdoor flow-through freshwater mussel holding tanks at IDNFH.

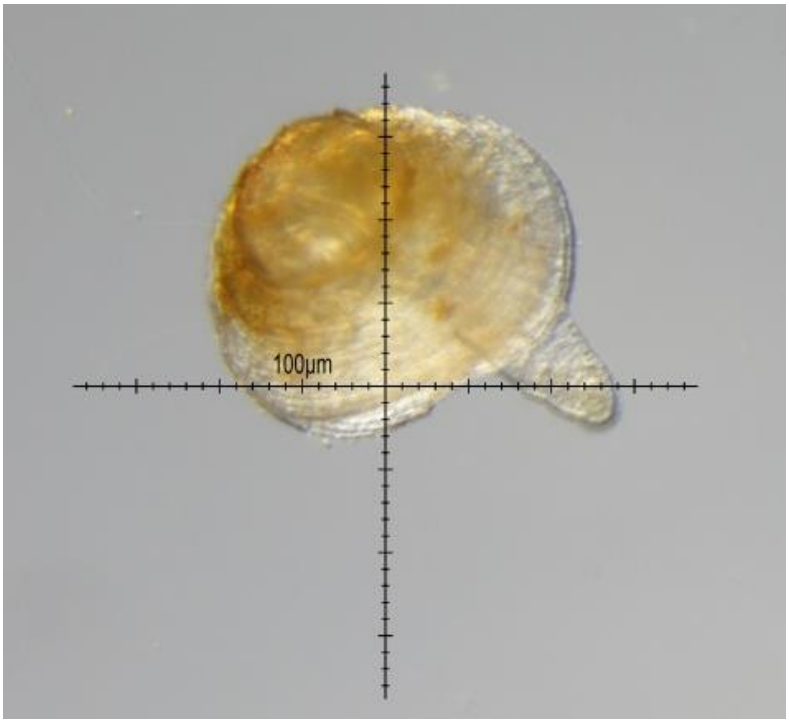

Figure 9. Newly metamorphosed juvenile Texas fatmucket (*Lampsilis bracteata*) at IDNFH.

Figure 10. Flow-through juvenile mussel rearing system at IDNFH.

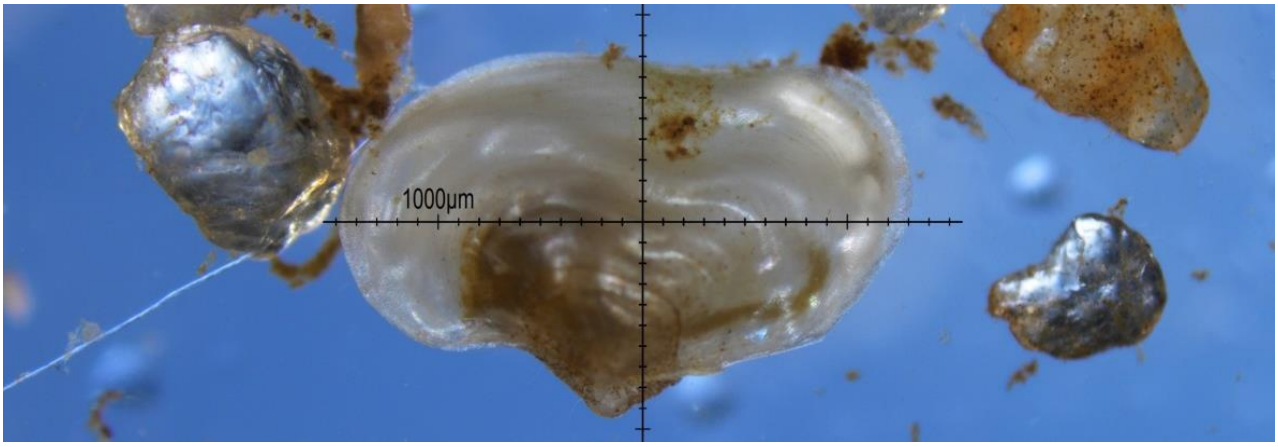

Figure 11. Juvenile Texas fatmucket at IDNFH 100-d post metamorphosis.

Figure 12. Juvenile Texas fatmuckets (*Lampsilis bracteata*) six months post metamorphosis.

Figure 13. Indoor flow-through raceway system for holding adult freshwater mussels at Inks Dam National Fish Hatchery

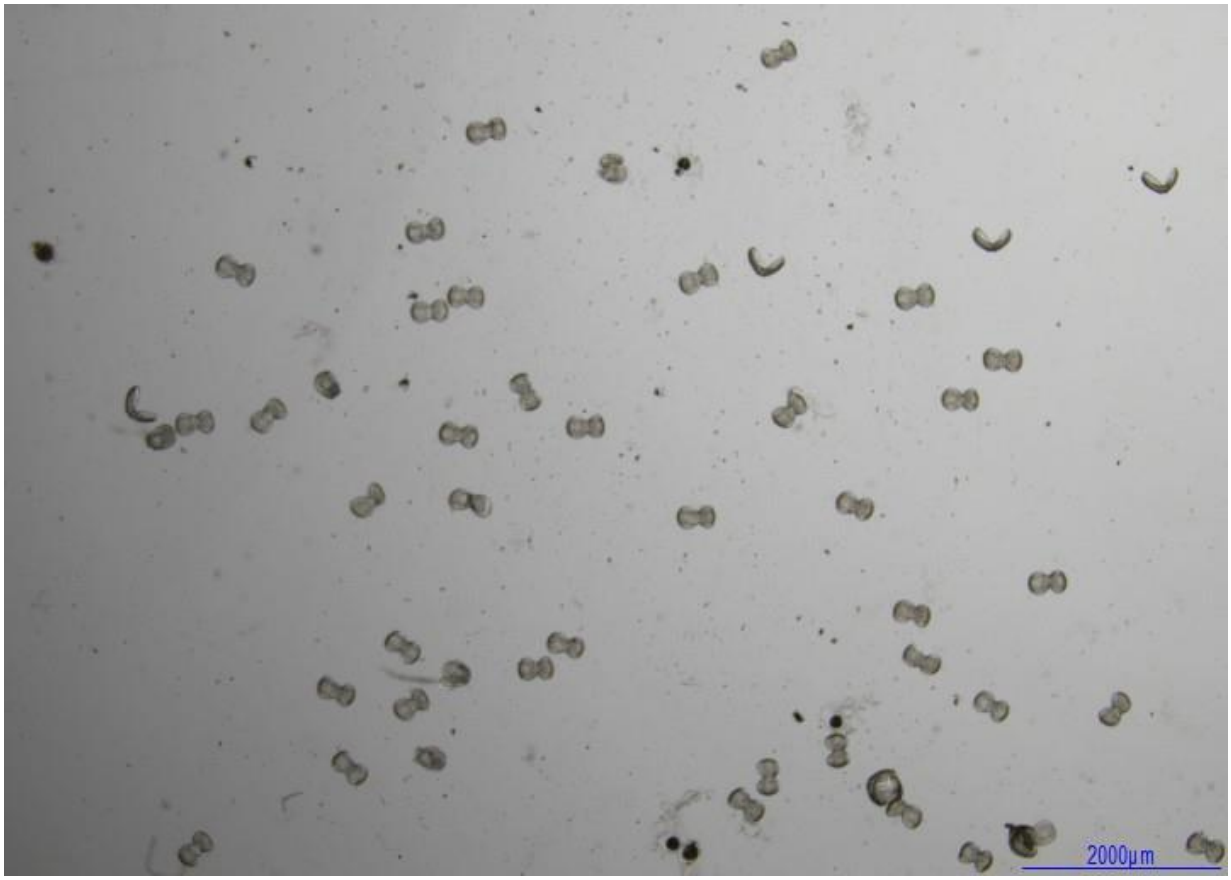

Figure 14. Glochidia extracted from Texas fatmucket (*Lampsilis bracteata*) on December 8, 2017 at Inks Dam National Fish Hatchery

Figure 15. Floating dock at Inks Dam National Fish Hatchery

Figure 16. The freshwater mussel tank system that holds Central Texas federal candidate and petitioned freshwater mussel species at the Uvalde National Fish Hatchery. Photo credit: USFWS.

Figure 17. A chlorinated sand filter system at The Uvalde National Fish Hatchery Quarantine Building. Photo credit: USFWS.

Figure 18. A Z-Hab technician training Uvalde National Fish Hatchery staff members how to use and maintain a state of the art Z-Hab system. Photo credit: USFWS.

Figure 19. The Colorado River in Columbus, Texas near where mussels were collected (photograph above). Uvalde National Fish Hatchery's Lead Biologist Valentin Cantu and Fish Biologist Greg Cottingham searching for freshwater mussels at the Colorado River (photograph below).

Figure 20. Adult mussels collected from the Colorado River packed in an ice cooler with wetted burlap bags for transport to the Uvalde National Fish Hatchery.