Freshwater Mussels (Unionidae): Central and West Texas
Final Report

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Executive Summary

The goal of this study was two-fold: (1) assess the taxonomic identity and phylogenetic placement of *Quadrula aurea* (Golden Orb) [Federal Candidate], *Quadrula houstonensis* (Smooth Pimpleback) [Federal Candidate], and *Quadrula petrina* (Texas Pimpleback) [Federal Candidate] through mitochondrial and nuclear DNA sequencing; and (2) provided additional information on the distribution and abundance for mussel species petitioned for listing under ESA through field surveys in portions of several major rivers in Central and West Texas. The final report is organized by 4 research tasks as per contract (see Appendix A) and submitted proposals. Below is an outline of goal-oriented tasks for this project:

Task 1 – Conduct comprehensive surveys of portions of the Brazos, Colorado, and Guadalupe River basins

Task 2 – Conduct comprehensive surveys of portions of the Rio Grande Basin

Task 3 – Develop conservation status assessment maps for 9 state-threatened mussel species in Texas

Task 4 – Delineate species boundaries, test for cryptic species, and assess phylogenetic relationships for threatened Texas mussel species in the genus Quadrula

Note the following 2 tasks were either not funded in the current contract or was a modification of the contract and added as a project deliverable:

Task 5 – Delineate species boundaries, test for cryptic species, and assess phylogenetic relationships for east Texas mussel species in the genus Fusconaia (**Not Funded**)

Task 6 – Evaluate the conservation status of Texas hornshell and other mussels in the Pecos and Devils Rivers (**Contract amendment, results combined with Task 2**).

Detailed descriptions of the research tasks and findings are found within each chapter of the report. Here, we outline and summarize project deliverables and major findings for each task.

Requirements and Key Findings

Task 1 – Conduct comprehensive surveys of portions of the Brazos, Colorado and Guadalupe River basins

- *Deliverable*: Survey at least 20 stream segments (a total of 100) across the Brazos, Colorado, Guadalupe, San Antonio and Nueces River drainages. Early in the contract period, the CPA requested San Antonio and Nueces Rivers not be surveyed due to a potential pending contract. As result, IRNR was retasked to survey the Brazos, Colorado, and Guadalupe drainages only.
- *Key Findings*: We surveyed 59, 58, and 13 sites in these drainages, respectively (total 130 surveys conducted). For each site surveyed, we reported the number of indviduals per focal species, relative abundance and demographic information.

- Species status assessments, which included a map of where populations were found, habitat assements, and comments on management, were provided as early deliverables to the CPA.

- Focal species were found at a number of locations during the course of this study. Some of these records represent new accounts while others confirmed that a given species continues to persist at a location where it was known to occur. For example, we rediscovered *F. mitchelli* within the Little River (Brazos River drainage) and confirmed its persistence at a site within the Llano River. We also found that several species only occur within a small portion of their presumptive range. For example, *Lampsilis bracteata* appears restricted to the upper reaches of major tributaries within the Colorado drainage. Similarly, *T. macrodon* appears confined to the lower reaches of the Colorado and Brazos Rivers, though we did locate live individuals of this species in several tributaries, but these populations were small, sometimes consisting of only one or two individuals.

- *Recommendations*: Our ability to locate rare species, especially those with low abundance, suggests the current survey protocol is an effective approach to sampling freshwater mussels. Future surveys in Central and West Texas should consider using similiar sampling methodologies to include standardized timed-survey effort (i.e., 5 p-h/site), search area (i.e., 150 m^2), and photo vouchers where survey field crew experience may be limited, to allow comparision among survey efforts.

Task 2 – Conduct comprehensive surveys of portions of the Rio Grande Basin

- *Deliverable*: Survey at least 30 sites using a combination of quantitative and qualitative sampling methods. It was later determined that this approach proposed by Buffalo State-SUNY was challenging in detecting and accurately assessing the status and distribution of petitioned species in the Rio Grande drainage. As a result, sampling was conducted following the same methodology used in Task 1.

- *Key Findings*: We surveyed 114, 43, and 39 sites in the Rio Grande, Pecos, and Devils Rivers (total 196 surveys conducted). For each site surveyed, we reported the number of indviduals per focal species, relative abundance and demographic information.

- Species status assessments, which included a map of where populations were found, habitat assement, and comments on management, were provided as early deliverables to the CPA, which were then shared with the workgroup.

- We also developed an occupancy-model to identify factors that influence detection and occupancy of Texas hornshell in the Rio Grande and Devils River. Occupancy analaysis across 153 sites showed that detection probabilities varied and were influenced primarily by abundance, whereas occupancy was driven by proximity to urban centers (Middle Rio Grande), cumulative number of springs located upstream of a given sample location (Lower Canyons and Devils River), presence of boulder/bedrock habitat (Middel Rio Grande and Lower Canyons) and siltation (Devils River). The results of this study were shared early with the CPA and distributed to the workgroup.

- *Potamilus metnecktayi* (Salina mucket), *Popenaias popeii* (Texas hornshell), and *Truncilla cognata* (Mexican fawnsfoot) were found at a number of locations during the course of this study. For example, we discovered the only remaining population of *P. metnecktayi* in the Rio Grande, previous survey efforts in this drainage have reported only singletons or shell material. We also rediscovered two populations of *P. popeii* (Lower Canyons of the Rio Grande and Pecos River) and confirmed its persistence within the Devis River. For *T. cognata*, we found that it was more prevalent than previously thought, though the species occurs in habitats that are susceptible to dewatering and other water related impacts.

- *Recommendations*: Similar to Task 2, we found that locating our focal species was supported by our sampling protocol and occupancy modeling results for *P. popeii*. Use of similar survey methods in sampling the Rio Grande and associated tributaries is recommended.

Task 3 – Develop conservation status assessment maps for 9 state-threatened mussel species in Texas

- *Deliverable*: Develop Conservation Status Maps for 9 state-threatened species from central and west Texas petioned for listing under the ESA.
- *Key Findings*: Presence/absence data obtained from this study, along with Texas Parks and Wildlife Department [TPWD], Texas Department of Transportation [TxDOT], Texas Commission on Environmental Quality [TCEQ], Texas Water Development Board [TWDB], and published literature, were used in updating status assessment maps. All maps were provided to the CPA earlier during the contract period and distributed to the workgroup.

Task 4 – Delineate species boundaries, test for cryptic species, and assess phylogenetic relationships for threatened Texas mussel species in the genus Quadrula

- *Deliverable*: Evaluate evolutionary relationships within and among several freshwater mussel genera that belong to the Quadrulini. Using an integrated taxonomic approach, we show that the following 12 nominal taxa investigated in this study be assigned to the genus *Cyclonaias*: *C. aurea*, *C. asperata, C. houstonensis, C. infucata, C. kleiniana, C. mortoni, C. nodulata, C. petrina, C. pustulosa, C. refulgens, C. succissa,* and *C. tuberculata.*

- *Key Findings*: We revise species-level classifications by synonymizing four taxa (*C. aurea*, *C. houstonensis, C. mortoni, and C. refulgens*) considered either species or subspecies under *Cyclonaias pustulosa* and provide evidence for a previously unrecognized species from the *Cyclonaias petrina* complex that is endemic to the Guadalupe River basin.

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Introduction

Freshwater mussels (Family: Unionidae) play an important role in freshwater ecosystems through nutrient cycling, increasing habitat heterogeneity, and as a food source for fishes, mammals, and birds (Haag and Williams 2013). Due to their sensitivity to various environmental stressors, inability to move far from human-mediated perturbations, and reliance on certain fish species to complete their reproductive life cycle, they are one of the most imperiled taxonomic groups in North America (Williams et al. 1993). In Texas, 15 of 52 described species (29%) are listed as state threatened, of which 6 are now candidates for protection under the Endangered Species Act (ESA) (Texas Parks and Wildlife Department [TPWD] 2010; U.S. Fish and Wildlife Service [USFWS] 2001, 2011).

At its core, species conservation relies on the ability of biologists and their stakeholders to distinguish one species from another. Despite its centrality in mussel conservation, delineating species boundaries is an ever-changing and challenging endeavor, as evidenced by the number of described versus valid species (4,839 and 840, respectively; Graf and Cummings, 2007). The majority of these taxonomic uncertainties stem from our general reliance on shell characters for identification, which are often (1) lacking in diagnosable characteristics or (2) phenotypically plastic wherein morphological differences are caused by within-population variation or environmental forces. Respectively, these conchological phenomena can lead to "lumping" of morphologically indistinguishable but genetically (and often geographically) distinct animals, or the "splitting" of a morphologically variable lineage into two or more superficial entities. Consequently, some taxonomic species do not reflect biologically accurate species boundaries and can misinform listing decisions.

Geographic patterns of species distributions, combined with information on the factors that contributed to their endangerment, are necessary for developing effective conservation strategies (Burlakova et al. 2011). For freshwater mussels, specifically those occurring in Texas, distribution information is available, but only at a broad scale, and much of it is based on shell material rather than live individuals. Information on the location of mussel populations and the viability of those populations is all but non-existent, although this problem is not unique to Texas (Howells et al. 1996; Haag and Williams 2013). Studies by TPWD and more recently by university researchers over the last 5 to 10 years have begun to address these knowledge gaps, however, given the geographical size of Texas (172 million acres) combined with the fact that most of this land area is privately owned (over 140 million acres; TXGLO 2013) means that many rivers and streams have yet to be surveyed for rare or common mussel species.

For mussel species in the genus *Quadrula*, little attention has been given to understanding species boundaries. Much of the confusion can be attributed to the difficulties in distinguishing similarity between species (interspecific) and variability within species (intraspecific; Valentine and Stansbery, 1971, Neck 1982, Burlakova et al. 2011). Howells (2002) suggests, "… only biochemical genetic studies will resolve the long-standing questions about them [Texas *Quadrula*]". The inability to accurately delineate species boundaries in *Quadrula* is particularly troubling for several species of conservation concern: *Quadrula aurea* (Golden orb); *Quadrula houstonensis* (Smooth pimpleback); and *Quadrula petrina* (Texas pimpleback).

In an effort to address knowledge gaps regarding species boundaries and geographic distribution of species currently proposed by USFWS for listing under the ESA the following tasks were completed throughout the coarse of this project:

Research Tasks

Task 1. Comprehensive surveys of portions of the Brazos, Colorado and Guadalupe River basins

Task 2. Comprehensive surveys of portions of the Rio Grande Basin

Task 3. Develop conservation status assessment maps for 9 state-threatened mussel species in Texas

Task 4. Delineate species boundaries, test for cryptic species, and assess phylogenetic relationships for threatened Texas mussel species in the genus Quadrula

Report Organization

This report is organized by task (sections) and appendices. Each contract task is presented as an independent section within the report. Below is a summary of specific contract requirements derived from study proposal (Appendix A) and contract (Appendix B).

Task 1. Comprehensive surveys of portions of the Brazos, Colorado and Guadalupe River basins

To address knowledge gaps regarding the geographic distribution and conservation status of *Q. aurea*, *Q. houstonensis*, and *Q. petrina*, we surveyed selected stream segments within the Guadalupe, Colorado and Brazos River basins. Conservation Status Maps (see Task 3) were developed to identify streams segments within these basins where survey information for these species is either dated or not available and where additional information could help inform the listing process. For this task, the research questions we sought to answer were: (1) Do our target species occur in selected stream segments? (2) What is their abundance? (3) Do other threatened species also occur in these stream segments and, if so, what is their abundance?

To answer these questions, we surveyed at least 30 stream segments across the three river basins in Central Texas, though in the Guadalupe only 13 sites were sampled due to persistent high flows in 2015 and 2016. Within each segment, timed, qualitative mussel surveys were performed for a minimum of 5 person-hours (p-h) per site to evaluate occupancy and relative abundance of our target species. The timed search sampling method is ideal for detecting rare species and provides a more cost-effective means of estimating relative abundance (Vaughn et al. 1997). Data derived from these surveys were used to provide a total species list per site, relative abundance, and catch-per-unit effort for our target species.

Task 2. Comprehensive surveys of portions of the Rio Grande Basin

Unionid bivalves of the Rio Grande basin represent a unique assemblage distinct from the rest of Texas (Neck, 1982; Neck and Metcalf, 1988; Burlakova et al., 2011). Recent surveys of portions of the Rio Grande have documented many of the species historically reported from this river (Karatayev et al., 2012). However, live individuals have not been found for *Quadrula conchiana* and *Fusconaia mitchelli*, which means these species were either too rare to detect or are now extirpated from the Texas part of the Rio Grande basin. Additionally, among the three Rio Grande's endemic species (*Potamilus metnecktayi, Popenaias popeii,* and *Truncilla cognata*), only *P. popeii* is known to occur in significant numbers, but only from a few localities. Live individuals for both *P. metnecktayi* and *T. cognata* have been observed in the Rio Grande, but most of these collections were comprised of a handful of individuals from a few sampling locations.

In an effort to address knowledge gaps regarding the distribution of *P. metnecktayi*, *P. popeii*, and *T. cognata*, we conducted detailed surveys throughout the Rio Grande drainage in Texas. For this task, the questions we sought to answer were: (1) Do our target species occur in selected stream segments? (2) If so, what is their abundance? (3) Do other threatened species also occur in these stream segments and, if so, what is their abundance?

To answer these questions, we surveyed portions of the Rio Grande, Pecos River and Devils River following the same methodology described for Task 1. Data obtained during these surveys were used to provide a total species list per site, abundance, and catch-per-unit effort for our target species.

Task 3. Develop conservation status assessment maps for 9 state-threatened mussel species in Texas

In Texas, range maps are available for the 15 state-listed mussel species (Howells 2010), but the spatial scale of those maps are broad that it limits their use for supporting status assessments. We addressed this problem by developing conservation status assessment maps for 9 state-listed species. Conservation status assessment maps are a way to efficiently determine the status of a given species and have been used in conservation assessments by U.S. Fish and Wildlife Service for rare aquatic species. Generally, conservation maps are suitable for coarse-level assessments and are generated using occurrence data mapped at a watershed scale using GIS.

To develop these maps, we used the Conservation Status Map package provided by the Georgia Department of Natural Resources (http://www.georgiawildlife.com/conservation_status_assessment_maps). Occurrence data were obtained from state agencies (e.g., Texas Parks and Wildlife Department [TPWD], Texas Department of Transportation [TxDOT], Texas Commission on Environmental Quality [TCEQ], Texas Water Development Board [TWDB]), universities (e.g., University of Texas at Tyler, Texas A&M), museums (in state and out-of-state), published literature, and other known sources.

Task 4. Delineate species boundaries, test for cryptic species, and assess phylogenetic relationships for threatened Texas mussel species in the genus Quadrula

For this task the research questions we pose are as follows: (1) are *Quadrula aurea*, *Quadrula houstonensis*, and *Quadrula petrina* valid species?, (2) Do any populations or sets of populations represent morphologically cryptic species?, and (3) What are the phylogenetic relationships between these species and other *Quadrula* found in Texas?

We answered these questions using an integrative taxonomic approach that considers newly generated data from three molecular markers (mitochondrial and nuclear) and available data regarding the morphological variation and geographic distributions of each species. The molecular dataset utilized existing DNA sequences from our UnioBarcode database, an endeavor led by scientists from the Southeast Ecological Science Center-USGS in Gainesville, Florida, along with sequences published on NCBI and those obtained from specimens collected through additional field sampling. For the latter, we collected several live individuals (5-10) from sites along the periphery of each species' geographic range and from drainages within their range where we have no or few reference sequences. To ensure accurate identification of all specimens collected for genetic analysis, we sequenced the mitochondrial COI gene using standard extraction and sequencing protocols.

To delineate species boundaries, test for cryptic species, and assess the phylogenetic relationships for *Q. aurea*, *Q. houstonensis*, and *Q. petrina* in Central, and West Texas, we built a comprehensive DNA sequence data from three molecular markers, including two regions of the mitochondrial genome (COI: 657 nucleotides; ND1: 811 nucleotides) and one region of the nuclear genome (ITS: 619 nucleotides). These markers were chosen because they are routinely used for systematic studies involving freshwater mussels (e.g., Campbell and Lydeard 2012) and represent two independently evolving genomes. Genetic variation within and between species was calculated using pairwise genetic distances and analyzed using haplotype networks. Maximum Likelihood and Bayesian Inference statistical methods (e.g., RAxML and MrBayes) were used to reconstruct the phylogenies of all three species and bootstrapping and posterior probabilities will be used to test the strength of the resulting topologies (i.e., groupings). Molecular-based species delimitation methods were implemented using several Bayesian and Maximum Likelihood approaches. All genetic material was processed and analyzed at the Southeast Ecological Science Center-USGS.

Task 1: Comprehensive surveys of Brazos, Colorado and Guadalupe River basins

Distribution and Habitat Use for *Fusconaia mitchelli* (False Spike)

Section Summary

The goal of this study was to determine the status, distribution, and mesohabitat associations for *Fusconaia mitchelli* (false spike), a candidate for protection under the Endangered Species Act, in the Brazos, Colorado, and Guadalupe River drainages. We used recent and historical data to inform a sampling program within the range of *F. mitchelli.* In total, we surveyed 130 sites across the Brazos, Colorado, and Guadalupe River drainages, and found 31 live *F. mitchelli* at 8 of the 130 (6%) sites. The majority of individuals were found in the Brazos River drainage (*n*=29) at 6 of the 59 (10%) sites. We surveyed three tributaries of the Brazos River: Brushy Creek, the San Gabriel River, and Little River. In Brushy Creek, we found 5 individuals at 1 of 30 (3%) sites near the confluence with the San Gabriel River. Below Granger Lake, 2 individuals were found at 2 of 20 (10%) sites in the San Gabriel River, and 22 individuals were found at 3 of 9 (33%) sites in the Little River. *Fusconaia mitchelli* was found primarily in riffle habitats within the Brazos River system with live individuals found in 42% of riffle habitats (5/12 sites) and a single individual found in a backwater. We also observed reproductively active females (gravid, i.e., gills containing a brood of either developing eggs or viable larvae) in each river surveyed during this time period, as well as sub-adults indicating that recruitment is occurring within the Brazos River system. In the Colorado River basin, we found 1 live individual in a pool habitat out of 58 sites surveyed on the Llano River. This individual was a sub-adult and thus not reproductively active at the time, though this suggests that recruitment is occurring to some degree. No live individuals were found in the San Saba or Pedernales rivers (Colorado River basin). In the Guadalupe River, we found 1 adult individual at 1 of 13 (8%) sites surveyed. No riffles were sampled in the Guadalupe, which indicates that *Fusconaia mitchelli* occurs at low densities in non-riffle habitats.

Introduction

Fusconaia mitchelli, false spike, is known historically from the Brazos, Colorado, and Guadalupe River drainages of Texas (Pfeiffer et al. 2015). The type specimen was collected from the Guadalupe River in Victoria County by Simpson (Dall 1896). The range of *F. mitchelli* was previously thought to extend into the Rio Grande as well, based on synonymy with *Sphenonaias taumilapana* (Frierson 1927, Strecker 1931). However, Pfeiffer et al. (2015) argued that because *S. taumilapana* is morphologically different from *F. mitchelli* from Central Texas, it likely represents a distinct species. Others have also suspected that *S. taumilapana* and *F. mitchelli* are separate species (Simpson 1914, Metcalf 1982, Howells et al. 1997). Interestingly, no records of either *S. taumilapana* or *F. mitchelli* exist from the Nueces River system, the basin between the Central Texas drainages and the Rio Grande (Johnson 1999).

In the Brazos River basin, historic records of *F. mitchelli* occur in the Little River system and the Brazos River. Specimens were collected from the Leon River (a tributary of the Little River) in Bell County by A. L. Fitzpatrick (BU-MMC_MO31544-A-B, BU-MMC_MO31545-A-B) and in Coryell County by J. K. Strecker (BU-MMC_MO33131-A-B, BU-MMC_MO33132-A-B) (Strecker 1931). In the Brazos River a specimen of *F. mitchelli* was collected by J.A. Singley from State Highway 21 at the boundary of Brazos and Burleson counties (FLMNH_ 270511). In 1980, a recently dead specimen was found on the Lampasas River (a tributary of the Little River) in Bell County by Joseph Bergman (TX0084; R. G. Howells database).

In the Colorado River basin, Simpson (1914) reported *F. mitchelli* collected by B. H. Wright from the San Saba River in Menard County (USNM_158756). Strecker (1931) reported additional collections made from the San Saba River in Menard County by Smith and A. L. Fitzpatrick (BU-MMC_MO33127-A-B, BU-MMC_MO33128-A-B, BU-MMC_MO33129-A-B,BU-MMC_MO33130-A-B, ANSP_113945). Gwyn collected a specimen from Santa Anna, TX (Colorado River drainage) in Coleman County (USNM_131645) and from Sulphur Springs, TX (San Saba County) on the Colorado River (USNM_131644). A. L. Fitzpatrick collected a specimen from the Llano River in Mason County (USNM_363911). In 1972 specimens were collected by H. D. Stansbery from the Llano River in Mason County (OSUM_1153), Kimble County (OSUM_34622), and Llano County (OSUM_34626). In 1974, C. M. Mather collected recently dead specimens from the Llano River near Castell in Llano County (CMM 1772, 2237; R. G. Howells database) and near Hedwigs Hill in Mason County (CMM 1774; R. G. Howells database). Howells (1994) collected a weathered specimen of *F. mitchelli* from the Pedernales River in Blanco County.

In the Guadalupe River, Strecker (1931) reported specimens of *F. mitchelli* collected near New Braunfels in Comal County by A. L. Fitzpatrick (BU-MMC_MO33135-A-B), near Kerrville in Kerr County by J.D. Mitchell (USNM_ 464620), in Kendall County by J.K. Strecker (BU-MMC_MO33144-A-B), and in Victoria County by J.D. Mitchell (BU-MMCMO33133-A-B, BU-MMC_MO33134-A-B). Wurtz (1950) reported a specimen of *F. mitchelli* from the Guadalupe River near Seguin in Guadalupe County between Routes 123 and 90 (ANSP_185974). In 1974 C. M. Mather collected a single recently dead specimen from the Guadalupe River in Kendall County (R.G. Howells database). A single subfossil valve of *F. mitchelli* has been reported from Salado Creek (San Antonio River basin); however, the weathered condition of the valve made identification difficult, thus the presence of *F. mitchelli* in the San Antonio River basin was unverified (Howells 2002, Pfeiffer et al. 2015).

Strecker (1931) originally recorded *F. mitchelli* as common wherever it was found; however, beginning in the early 1970s *F. mitchelli* was listed as rare throughout its range (Stansbery 1971). Williams et al. (1993) identified *F. mitchelli* as threatened while NatureServe (2012) ranked *F. mitchelli* as possibly extinct. This species is currently listed as state threatened by the Texas Parks and Wildlife Department (TPWD 2010) and is under review for listing through the U.S. Endangered Species Act (USFWS 2011). As of Howells (2010), no living populations had been documented in over 30 years, though two recently dead valves had been found in the San Marcos River (Gonzales County) in 2000. As such, *F. mitchelli* was thought to have been extinct until the discovery of 7 live individuals from the Guadalupe River, near Gonzales TX (Gonzales County) in 2011 (Randklev et al. 2012). Since then, live individuals for this species have been reported from the lower Guadalupe River (DeWitt, Gonzales, and Victoria counties), San Saba River (San Saba County), Llano River (Mason County), and the San Gabriel River (Milam and Williamson counties) (Randklev et al. 2013).

Fusconaia mitchelli is considered a valid species (Pfeiffer et al. 2015); however, little is known about its life history or reproductive requirements (Howells 2010). Like other freshwater mussel species, it is likely an obligate ectoparasite on one or more host-fish species, and its congeners appear to be short-term brooders that are host specialists (Haag 2012). Based on recent observations it appears that adults have an affinity for flowing-water and occur in gravel and cobble substrates in riffle and run mesohabitats (Howells 2010, Sowards et al. 2013). Despite these observations, habitat associations for adults remain untested and for juveniles, undescribed.

The objectives of this study were to assess the distribution, abundance, and habitat use for *F. mitchelli* in the Brazos, Colorado, and Guadalupe drainages. The resulting survey information was then used to develop Conservation Status Assessment Maps for this species within the Brazos, Colorado, and Guadalupe drainages**.**

Methods

Study Area:

The Brazos River begins near the Texas-New Mexico border and flows southeast into the Gulf of Mexico at Freeport, Texas, with an approximate length of 1900 km and draining a total of 118,000 km^2 (Kammerer 1990). The portion of the basin located in Central Texas has a humid subtropical climate and averages between 800 and 1000 mm precipitation per year (BBEST 2012). The Little River, San Gabriel River, and Brushy Creek, are all part of the Little River system, a tributary of the Brazos River, which drains the Edwards Plateau and Blackland Prairie regions of Central Texas (Rose and Echelle 1981). The Little River system transitions from high gradient streams in the upper San Gabriel watershed to low gradient streams in the lower watershed which is characterized by clay and fine sediments, woody debris, and generally slow currents (Labay 2010). The land use types within the lower Little River drainage area include urban areas and agricultural land (Labay 2010). The Little River is formed by the confluence of the Leon and Lampasas rivers in Bell County and flows for 258 km, draining an area of approximately 12,485 km^2 before emptying into the Brazos River in Milam County (Rose and Echelle 1981). The San Gabriel River begins in Georgetown where the North and South Forks converge, and flows east for roughly 80 km through Williamson and Milam counties until its confluence with the Little River (Belisle and Josselet 1977). The San Gabriel is impounded in Williamson County by Granger Lake, a 1619 hectare reservoir used primarily for flood control (Mcalister et al. 2013). Brushy Creek originates in Williamson County and flows east through Milam County for 111 km before emptying into the San Gabriel River near Rockdale (Belisle and Josselet 1977).

The Colorado River of Texas originates in northeastern Dawson County and is the largest river that is contained entirely within Texas, with an approximate length of 1,040 km and draining a total area of nearly 100,000 km^2 (Huser 2000). The San Saba, Llano, and Pedernales rivers are three of the major tributaries of the Colorado River and all are spring-fed rivers, originating in the Edwards Plateau region (Higgins 2009). The San Saba River begins in Schleicher County where the North Valley Prong and Middle Valley Prong San Saba converge near Fort McKavett. The San Saba River flows for 225 km passing through Menard, Mason, McCulloch, and San Saba counties until its confluence with the Colorado River (Belisle and Josselet 1977). The Llano River originates in Kimble County where the North Llano and South Llano rivers converge in Junction, TX. The Llano River flows for 161 km through Mason and Llano counties emptying into Lake Lyndon B. Johnson, an impoundment on the Colorado River. The Pedernales River originates in Kimble County and flows approximately 170 km through Gillespie, Blanco, Hays, and Travis counties, emptying into Lake Travis, an impoundment on the Colorado River (Perkin et al. 2010).

The Guadalupe River originates in Kerr County, Texas, and is one of few major rivers contained entirely within Texas (Huser 2000). With an approximate length of 402 kilometers, and draining 15,539 km^2, this spring fed river originates in the Edwards Plateau region and flows through Kerr, Kendall, Comal, Guadalupe, Gonzales, Dewitt, Victoria, and Calhoun counties before emptying first into the Guadalupe Bay, then the San Antonio Bay, and ultimately the Gulf of Mexico (Huser 2000). The major tributaries of the Guadalupe River are the Blanco-San Marcos and the San Antonio rivers. The Guadalupe River has 10 main stem impoundments in its upper reaches with Canyon Lake in Comal County as the largest upstream impoundment followed by Lake McQueeney in Guadalupe County (Huser 2000, Roach et al. 2014). Many small dams are located on the San Marcos and Guadalupe rivers, the most downstream located just below their confluence near the city of Gonzales.

Sampling Methods:

Survey sites within the Brazos drainages were selected using a random sampling design. We delineated lengths of the river between bridge crossings that could be accessed by canoe into 1 km segments with the following strata: 1) river kilometer and 2) mesohabitat: (banks, backwater, mid-channel, riffles, and pools). The exact location and habitats were identified prior to field sampling using aerial imagery. Specifically, at each river segment, habitats within the entire length of the river extending to the next access point were identified and numbered. Then a random number generator was used to randomly select the habitat type to be sampled in each 1 km segment. For locations where specific habitats could not be identified using satellite imagery

(e.g., riffles), we modified our sampling design by surveying the nearest habitat encountered for that target habitat type to a randomly selected point. In total, 30 sites in Brushy Creek, 20 sites in the San Gabriel River, and 9 sites in the Little River were selected for sampling.
Survey sites in the Colorado drainages were selected using a random sampling design with the following strata: 1) upstream or downstream of a bridge crossing, 2) linear distance from the bridge; and 3) mesohabitat: (banks, backwater, mid-channel, riffles, and pools). The exact location and habitats were identified prior to field sampling using aerial imagery. Specifically, at each bridge crossing, discrete habitats within the length of the river extending up to 2 river kilometers in either up or downstream direction from each bridge were identified and numbered. Then a random number generator was used to randomly select the linear distance from the bridge and the habitat type to be sampled at that location. For locations where specific habitats could not be identified using satellite imagery (e.g., riffles), we modified our sampling design by surveying the first habitat encountered for that target habitat type. In total, 20 sites in the Llano and 19 sites in each the Pedernales and San Saba were selected for sampling. Selected sites included sites that had both been sampled during previous efforts and sites that had not been sampled previously.

Survey sites within the Guadalupe River were selected using a random sampling design. We delineated lengths of the river between Cuero and Victoria, TX into 10 km reaches then randomly chose reaches to survey. Within each reach, sites were selected with the following strata: 1) river kilometer and 2) mesohabitat: banks, backwater, mid-channel, riffles, front of point bars, behind point bars, and pools. The exact location and habitats were identified prior to field sampling using aerial imagery. Specifically, a river segment was chosen at random, then a habitat type was chosen at random and assigned to each selected segment. For locations where specific habitat types were not correctly identified by aerial imagery, we modified our sampling design by surveying the nearest correct habitat encountered within the selected segment. In total, 3 sites in DeWitt County and 10 sites in Victoria County were selected for sampling as a result of higher than normal flow conditions during 2015.

Qualitative surveys using the timed search method were performed in each selected mesohabitat type in all three basins (Brazos, Colorado, and Guadalupe). The timed search method provides a more effective means of detecting rare species than quantitative sampling methodologies (Vaughn et al. 1997). At each site (i.e., mesohabitat type), we confined the search boundaries to the specific habitat type, ensuring that the search area did not exceed 150 m^2, though in some cases within the Colorado basin the search area included multiple mesohabitat types (e.g., pool-run or riffle-run habitats). Each site was surveyed tactilely and visually for a total of 4 person-hours (p-h). However, because we are interested in the amount of effort needed to detect *F. mitchelli* (which will be important for designing long-term monitoring programs), we divided the total search time into 4, 1 p-h intervals. At the end of each search interval, surveyors combined all live specimens into a mesh bag, which was kept submerged in water until completion of the survey. During each interval, surveyors were spread out in the search area and every effort was made to search all available microhabitats. Following completion of the survey, all live mussels from each time period were identified to species, counted, measured, checked for gravidity, and then returned back to the river into the appropriate habitat.

Quantitative surveys using the quadrat search method were performed in each selected mesohabitat type in the Guadalupe River. Additional quantitative surveys were conducted in the Little and San Gabriel rivers to determine if mussel densities and abundance were sufficient to justify sampling quantitatively. The quadrat search method provides a more effective means of detecting sub-adults of a species and thus more accurate demography in areas with high abundance (Vaughn et al. 1997). At each site (i.e., mesohabitat type), we confined the search boundaries to the specific habitat type, ensuring that the search area did not exceed 150 m^2. Each site was surveyed first quantitatively (if appropriate), then qualitatively. The 150 m^2 search area was subdivided into a square meter grid and 20 points were selected within the grid using a random number generator. At each randomly selected point, quadrats were sampled by excavating sediment up to 15 cm in depth using a modified Surber sampler with a 0.25 m^2 search area. Sediment was sieved through 3.175 mm mesh screen, and all live specimens from each quadrat were placed into individuals mesh bags, which was kept submerged in water until completion of the survey. Following completion of the survey, all live mussels from each quadrat were identified to species, counted, measured, checked for gravidity, and then returned back to the river into the appropriate habitat.

Scatter plots of relative abundance (CPUE: number of individuals/total person-hours) and density (Density: mean number of individuals/0.25m^2) vs. river kilometer (RKM) were used to examine the effect of stream position on total mussel and *F. mitchelli* abundance in each river. Boxplots and length-frequency histograms were developed for *F. mitchelli* to assess demographic patterns and population structuring within populations. Generally, multimodal size class distribution may indicate recruitment, whereas truncated distributions (absence of a particular age class, large, or small individuals) may indicate a lack of recent recruitment or a localized extinction event. Bar graphs were also used to visually represent presence of *F. mitchelli* by mesohabitat type (i.e., riffle, mid-channel, pool, bank, and backwater).

Results/Discussion

Brazos River Basin

A total of 236 person-hours were spent surveying 59 sites located in Brushy Creek and the San Gabriel and Little Rivers of the Brazos River drainage (Figure 1). A total of 29 live individuals of *F. mitchelli* were found, occurring at 1 of 30 (3%) sites in Brushy Creek (n = 5 individuals), 2 of 20 (10%) sites in the San Gabriel River (n = 2 individuals), and 3 of 9 (33%) sites in the Little River (n = 29 individuals). Relative abundance ranged from 0 to 4 mussels/person-hour across all drainages, with the highest average occurring in the Little River (0.61 ± 3.12 mussels/person-hour; mean ± SD) while the San Gabriel River and Brushy Creek had similar relative abundances (0.03 ± 1.16 mussels/person-hour and 0.04 ± 0.90 mussels/person-hour respectively; mean ± SD) (Table 1). These results indicate that among these sites, the Little River contains the highest abundance of *F. mitchelli*; the single site with the highest abundance of *F. mitchelli* (n = 23 individuals) also occurred in the Little River. Across all three rivers, an average of 0.49 ± 2.24 live individuals of *F. mitchelli* was found per site (mean ± SD).

In Brushy Creek, previous surveys occurred in the upper portion of the Brushy Creek watershed, west of Interstate 35 in Williamson County, and found no live individuals (Johnson and Groce

2011, Wilkins et al. 2011). Our survey efforts focused on the lower portion of Brushy Creek in Williamson County east of Interstate 35 and detected 5 live individuals at one riffle site near the confluence with the San Gabriel River (Figure 2). This represents the only live individuals found to date in Brushy Creek. Our findings indicate that *F. mitchelli* is present in Brushy Creek in low densities. At the site where *F. mitchelli* was present, individuals of several size classes were present, including the only sub-adult individual found in our surveys in the Brazos River system (see Figure 3 for a comparison within the Brazos River system). In addition, we observed three individuals from this creek at the time of sampling (early July) with swollen and colored gills, evidence of active brooding. At that time the gills contained both developing eggs and immature glochidia.

In the San Gabriel River, previous relocation efforts from 2012 and 2013 reported 3 live individuals from the lower portion of the San Gabriel River in Milam County (Randklev et al. 2013). In the present study, we observed 2 individuals during qualitative timed searches from 2 of 20 sites surveyed. Both sites where *F. mitchelli* was found were riffle habitats and are located just below Granger Lake dam (Figure 4). No live individuals of *F. mitchelli* were found during the quantitative quadrat sampling (Figure 5). Our results combined with those from recent relocation efforts indicate that *F. mitchelli* occurs in low densities in the San Gabriel River (Randklev et al. 2013). Both of the individuals sampled from the San Gabriel River were over 60 mm in length, suggesting that they represent a population that is not recruiting, however, both individuals were observed to have swollen and colored gills, containing both developing eggs and immature glochidia (early July).

In the Little River, no previous surveys have been conducted to our knowledge. In the present study, we observed 29 live individuals from 3 of 9 sites surveyed during qualitative (n =22) and quantitative (n = 7) surveys. These individuals represent the only live collection of *F. mitchelli* found to date in the Little River. With only 9 sites occurring within a small segment of the total river length, it is not possible to discuss trends in distribution within this river; however, when the rivers are combined, a trend of increasing abundance towards the Brazos mainstem, or with increasing stream order is evident (Figures 6 and 7). Our results indicate that *F. mitchelli* is more abundant within the Little River than its tributaries. As in the other rivers, the majority of individuals (n = 21) were found in riffle habitats, with the exception of a singleton found in a backwater (Figure 8). The shell lengths of individuals found in the Little River approximate a normal distribution (Figure 3) and suggest that the population contains various age groups and recruitment has occurred recently. Median shell length for this population was 53 mm and minimum and maximum shell lengths were 42 mm and 75 mm, respectively (Figure 9). In addition, we observed several individuals from this river at the time of sampling (late April and July) with swollen and colored gills, which indicated active brooding. At that time the gills contained both developing eggs and immature glochidia.

Colorado River Basin

A total of 232 person-hours were spent surveying 58 sites located in the San Saba, Llano, and Pedernales rivers of the Colorado River drainage (Figure 10). One live individual of *F. mitchelli* was found in the Llano River at Site 20, which was the site furthest upstream from the confluence with the Colorado River (Table 2). Previous surveys collected three live individuals from the San Saba River and one live individual from the Llano River (Randklev et al. 2013, Sowards et al. 2013). The *F. mitchelli* sampled in the Llano was found in a pool habitat and was 37 mm long, suggesting sub-adult status and thus not reproductively active at the time.

Guadalupe River Basin

A total of 52 person hours were spent surveying 13 sites in the Guadalupe River (Figure 11). One live individual of *F. mitchelli* was found at one of the 13 sites (8%) sampled in this river (Table 3). The *F. mitchelli* sampled in the Guadalupe was found in a pool habitat and was 51 mm long. Previous surveys of 52 sites conducted by our group in 2014 on the Guadalupe River between Gonzales and Cuero, TX, found 651 *F. mitchelli,* including sub-adult individuals. An Indicator Species Analysis was performed on the 2014 dataset and from this analysis (IV = 0.911, p-value = 0.001, and frequency = 22). We concluded that this species occurs primarily in riffle habitat (Randklev unpublished data). Based on our surveys to date in Central Texas, it appears that *F. mitchelli* is more abundant in the Guadalupe River than other rivers sampled.

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Table 1. Locality and collection information for mussel survey sites in the Brazos River drainage. CPUE = total number of *F. mitchelli* encountered at each site during qualitative sampling divided by the number of person hours (4) searched at each site. Density = mean ± SE number of *F. mitchelli* encountered during quantitative sampling of twenty 0.25 m^2 quadrats at each site. Habitat key: BW = backwater, P = pool, R = riffle, B = Bank, MC = Mid-Channel. Sites are orderd upstream to downstream in each river.

Site/ Reach	Habitat	Locality	County	Date of collection	Live	CPUE	Density	Sub Adult	Effort		Area (m^2)
									Hours	Quadrats	
1A	B	Brushy Creek	Williamson	07/13/15	0	0	n/a	-	4	0	150
2A	B	Brushy Creek	Williamson	07/14/15	0	0	n/a	-	4	0	150
3A	R	Brushy Creek	Williamson	07/16/15	0	0	n/a	-	4	0	150
4A	MC	Brushy Creek	Williamson	07/13/15	0	0	n/a	-	4	0	150
5A	BW	Brushy Creek	Williamson	07/16/15	0	0	n/a	-	4	0	150
6A	P	Brushy Creek	Williamson	07/14/15	0	0	n/a	-	4	0	150
7A	MC	Brushy Creek	Williamson	07/14/15	0	0	n/a	-	4	0	150
8A	R	Brushy Creek	Williamson	07/16/15	0	0	n/a	-	4	0	150
9A	BW	Brushy Creek	Williamson	07/16/15	0	0	n/a	-	4	0	150
10A	P	Brushy Creek	Williamson	07/14/15	0	0	n/a	-	4	0	150
11B	R	Brushy Creek	Williamson	07/15/15	0	0	n/a	-	4	0	150
12B	B	Brushy Creek	Williamson	07/15/15	0	0	n/a	-	4	0	150
13B	R	Brushy Creek	Williamson	07/15/15	0	0	n/a	-	4	0	150
14B	MC	Brushy Creek	Williamson	07/15/15	0	0	n/a	-	4	0	150
15B	P	Brushy Creek	Williamson	07/15/15	0	0	n/a	-	4	0	150
16B	MC	Brushy Creek	Milam	07/16/15	0	0	n/a	-	4	0	150
17B	P	Brushy Creek	Milam	07/16/15	0	0	n/a	-	4	0	150
18B	BW	Brushy Creek	Milam	07/16/15	0	0	n/a	-	4	0	150
19B	B	Brushy Creek	Milam	07/15/15	0	0	n/a	-	4	0	150
20B	BW	Brushy Creek	Milam	07/15/15	0	0	n/a	-	4	0	150
21C	P	Brushy Creek	Milam	07/10/15	0	0	n/a	-	4	0	150
22C	BW	Brushy Creek	Milam	07/07/15	0	0	n/a	-	4	0	150
23C	B	Brushy Creek	Milam	07/07/15	0	0	n/a	-	4	0	150
24C	BW	Brushy Creek	Milam	07/06/15	0	0	n/a	-	4	0	100
25C	R	Brushy Creek	Milam	07/07/15	5	1.25	n/a	Y	4	0	150
26C	MC	Brushy Creek	Milam	07/10/15	0	0	n/a	-	4	0	150
27C	P	Brushy Creek	Milam	07/07/15	0	0	n/a	-	4	0	150
28C	MC	Brushy Creek	Milam	07/07/15	0	0	n/a	-	4	0	150
29C	R	Brushy Creek	Milam	07/10/15	0	0	n/a	-	4	0	150
30C	BH	Brushy Creek	Milam	07/06/15	0	0	n/a	-	4	0	100
31F	R	Little River	Milam	04/30/15	5	1.25	0	N	4	20	104
32F	B	Little River	Milam	04/30/15	0	0	0	-	4	20	100
33F	MC	Little River	Milam	04/30/15	0	0	0	-	4	20	100
34F	BH	Little River	Milam	04/30/15	0	0	0	-	4	20	100
35F	BW	Little River	Milam	04/28/15	1	0.25	0	N	4	20	105
36F	R	Little River	Milam	04/28/15	23	4	0.35±0.74	N	4	20	100
37F	BW	Little River	Milam	04/29/15	0	0	0	-	4	20	140
38F	MC	Little River	Milam	04/29/15	0	0	0	-	4	20	100
39F	P	Little River	Milam	04/29/15	0	0	0	-	4	20	100
40D	BW	San Gabriel	Williamson	07/09/15	0	0	n/a	-	4	0	150
41D	R	San Gabriel	Williamson	07/09/15	1	0.25	n/a	N	4	0	150
42D	B	San Gabriel	Williamson	07/08/15	0	0	n/a	-	4	0	150

Table 1. Continued.

Site/ Reach	Habitat	Locality	County	Date of collection	Live	CPUE	Density	Sub Adult	Effort		Area (m^2)
									Hours	Quadrats	
43D	B	San Gabriel	Williamson	07/08/15	0	0	n/a	-	4	0	150
44D	R	San Gabriel	Williamson	07/09/15	1	0.25	n/a	N	4	0	150
45D	P	San Gabriel	Williamson	07/09/15	0	0	n/a	-	4	0	150
46D	BW	San Gabriel	Williamson	07/08/15	0	0	n/a	-	4	0	150
47D	MC	San Gabriel	Williamson	07/08/15	0	0	n/a	-	4	0	150
48D	P	San Gabriel	Williamson	07/09/15	0	0	n/a	-	4	0	150
49D	MC	San Gabriel	Williamson	07/08/15	0	0	n/a	-	4	0	150
50E	B	San Gabriel	Milam	04/23/15	0	0	0	-	4	20	100
51E	BW	San Gabriel	Milam	04/27/15	0	0	0	-	4	20	100
52E	P	San Gabriel	Milam	04/20/15	0	0	0	-	4	20	90
53E	MC	San Gabriel	Milam	04/20/15	0	0	0	-	4	20	100
54E	BW	San Gabriel	Milam	04/22/15	0	0	0	-	4	20	90
55E	P	San Gabriel	Milam	04/27/15	0	0	0	-	4	20	100
56E	B	San Gabriel	Milam	04/21/15	0	0	0	-	4	20	90
57E	R	San Gabriel	Milam	04/21/15	0	0	0	-	4	20	100
58E	R	San Gabriel	Milam	04/22/15	0	0	0	-	4	20	100
59E	MC	San Gabriel	Milam	04/23/15	0	0	0	-	4	20	90

Table 2. Locality and collection information for mussel survey sites in the Colorado River drainage. CPUE = total number of *F. mitchelli* encountered at each site divided by the number of person hours (4) searched at each site. Habitat key: BW = backwater, P = pool, R = riffle, B = Bank, MC = Mid-Channel, PR = Pool/Run combined, RR = Riffle/Run combined, All = site encompassed multiple habitat types. Sites are ordered upstream to downstream in each river.

Site Reach	Habitat	Locality	County	Date of collection	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
1A	B	San Saba	Menard	8/5/2015	0	0	-	4	150
2A	P	San Saba	Menard	8/5/2015	0	0	-	4	150
3A	P	San Saba	Menard	8/5/2015	0	0	-	4	150
4A	B	San Saba	Menard	8/3/2015	0	0	-	4	150
5A	P	San Saba	Menard	8/4/2015	0	0	-	4	150
6A	P	San Saba	Menard	8/4/2015	0	0	-	4	150
7A	BW	San Saba	Menard	8/4/2015	0	0	-	4	150
8A	B	San Saba	Menard	8/4/2015	0	0	-	4	150
9A	B	San Saba	Menard	8/5/2015	0	0	-	4	150
10A	P	San Saba	Menard	8/5/2015	0	0	-	4	150
11B	R	San Saba	Menard	8/6/2015	0	0	-	4	150
12B	B	San Saba	Menard	8/6/2015	0	0	-	4	150
13B	BW	San Saba	Menard	8/6/2015	0	0	-	4	150
14B	P	San Saba	Menard	8/6/2015	0	0	-	4	150
15B	B	San Saba	Menard	8/6/2015	0	0	-	4	150
16C	P	San Saba	McCullogh	8/7/2015	0	0	-	4	150
17C	P	San Saba	McCullogh	8/7/2015	0	0	-	4	150
18C	P	San Saba	McCullogh	8/7/2015	0	0	-	4	150
19C	P	San Saba	McCullogh	8/7/2015	0	0	-	4	150
20D	P	Llano	Mason	7/30/2015	1	0.25	N	4	150
21D	PR	Llano	Mason	7/30/2015	0	0	-	4	150
22D	PR	Llano	Mason	7/30/2015	0	0	-	4	150
23D	PR	Llano	Mason	7/30/2015	0	0	-	4	150
24D	B	Llano	Mason	7/30/2015	0	0	-	4	150
25D	PR	Llano	Mason	7/29/2015	0	0	-	4	150
26D	B	Llano	Mason	7/29/2015	0	0	-	4	150
27E	P	Llano	Mason	7/31/2015	0	0	-	4	150
28E	B	Llano	Llano	7/29/2015	0	0	-	4	150
29E	P	Llano	Llano	7/29/2015	0	0	-	4	150
30E	B	Llano	Llano	7/29/2015	0	0	-	4	150
31E	P	Llano	Llano	7/29/2015	0	0	-	4	150
32F	P	Llano	Llano	7/28/2015	0	0	-	4	150
33F	B	Llano	Llano	7/28/2015	0	0	-	4	150
34F	B	Llano	Llano	7/28/2015	0	0	-	4	150
35F	PR	Llano	Llano	7/28/2015	0	0	-	4	150
36F	P	Llano	Llano	7/27/2015	0	0	-	4	150
37F	B	Llano	Llano	7/28/2015	0	0	-	4	150
38F	PR	Llano	Llano	7/27/2015	0	0	-	4	150
39F	B	Llano	Llano	7/27/2015	0	0	-	4	150
40G	P	Live Oak Creek	Gillespie	6/24/2015	0	0	-	4	150
41G	P	Live Oak Creek	Gillespie	6/24/2015	0	0	-	4	150
42G	All	Live Oak Creek	Gillespie	6/23/2015	0	0	-	4	150
43G	All	Live Oak Creek	Gillespie	6/23/2015	0	0	-	4	150

Table 2. Continued.

Site	Habitat	Locality	County	Date of collection	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
44G	B	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
45G	P	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
46G	B	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
47G	B	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
48G	BW	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
49H	MC	Pedernales	Gillespie	6/23/2015	0	0	-	4	150
50H	B	Pedernales	Gillespie	6/23/2015	0	0	-	4	150
51H	RR	Pedernales	Blanco	6/23/2015	0	0	-	4	150
52H	B	Pedernales	Blanco	6/23/2015	0	0	-	4	150
53H	BW	Pedernales	Blanco	6/23/2015	0	0	-	4	150
54I	P	Pedernales	Blanco	6/22/2015	0	0	-	4	150
55I	RR	Pedernales	Blanco	6/22/2015	0	0	-	4	150
56I	P	Flat Creek	Blanco	6/25/2015	0	0	-	4	150
57I	BW	Pedernales	Travis	6/25/2015	0	0	-	4	150
58I	B	Pedernales	Travis	6/25/2015	0	0	-	4	150

Table 3. Locality and collection information for mussel survey sites in the Guadalupe River drainage. CPUE = total number of *F. mitchelli* encountered at each site during qualitative sampling divided by the number of person hours (4) searched at each site. Density = mean ± SE number of *F. mitchelli* encountered during quantitative sampling of twenty 0.25 m^2 quadrats at each site. Habitat key: BW = backwater, P = pool, R = riffle, BH = Bank, FPB = front of point bar, BPB = behind point bar, MC = Mid-Channel. Sites are ordered upstream to downstream.

Site/ Reach	Habitat	Locality	County	Date of collection	Live	CPUE	Density	Sub Adult	Effort		Area (m^2)
									Hours	Quadrats	
1A	P	Guadalupe	DeWitt	8/27/2015	1	0.25	0	N	4	20	150
2A	P	Guadalupe	DeWitt	8/27/2015	0	0	0	-	4	20	150
3A	BW	Guadalupe	DeWitt	8/27/2015	0	0	0	-	4	20	150
4B	BH	Guadalupe	Victoria	8/19/2015	0	0	0	-	4	20	150
5B	R	Guadalupe	Victoria	8/25/2015	0	0	0	-	4	20	150
6B	BW	Guadalupe	Victoria	8/19/2015	0	0	0	-	4	20	150
7B	FPB	Guadalupe	Victoria	8/19/2015	0	0	0	-	4	20	150
8B	P	Guadalupe	Victoria	8/20/2015	0	0	0	-	4	20	150
9B	BH	Guadalupe	Victoria	8/18/2015	0	0	0	-	4	20	150
10B	FPB	Guadalupe	Victoria	8/18/2015	0	0	0	-	4	20	150
11B	BPB	Guadalupe	Victoria	8/25/2015	0	0	0	-	4	20	150
12B	BPB	Guadalupe	Victoria	8/25/2015	0	0	0	-	4	20	150
13B	BW	Guadalupe	Victoria	3/9/2015	0	0	0	-	4	20	150

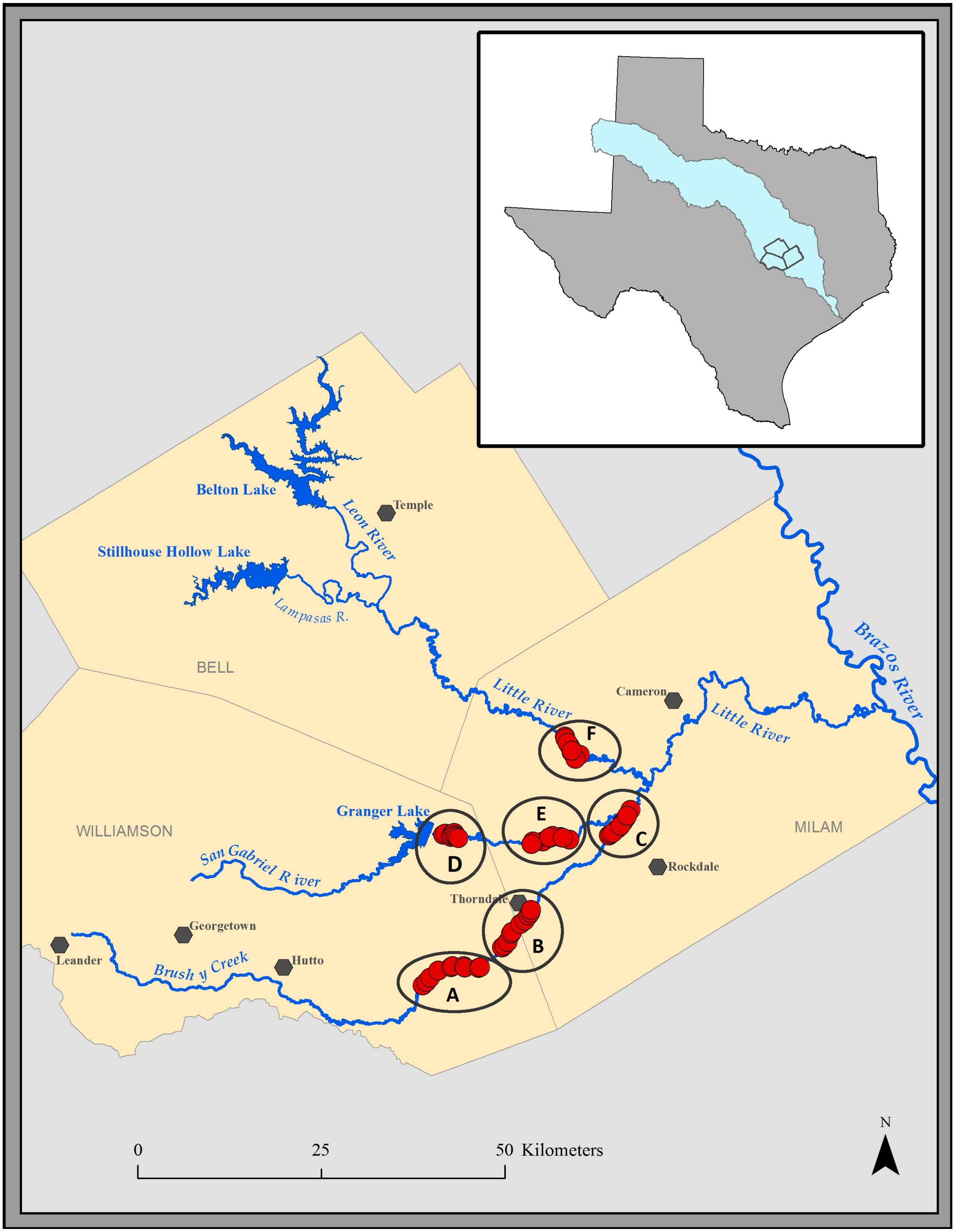

Figure 1. Map of Brazos drainage study area. Shaded circles denote sampling locations. Reaches are indicated by letter and correspond to Table 1.

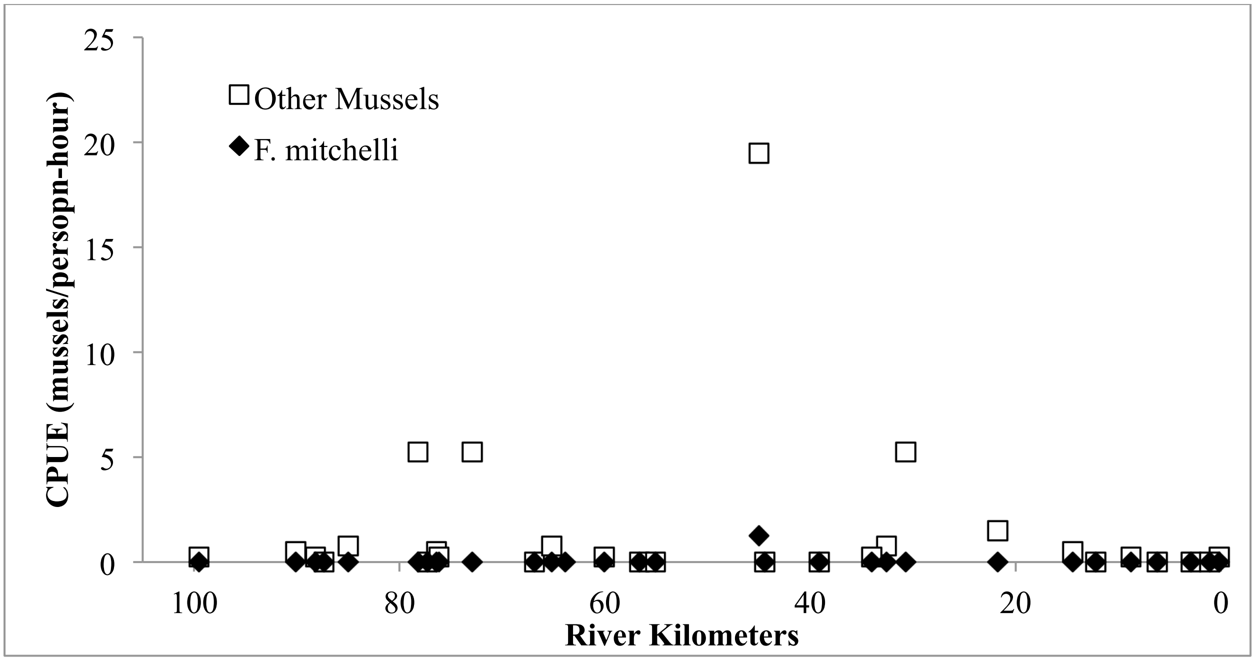

Figure 2. Relative abundance of *Fusconaia mitchelli* (false spike) and all other mussel species, "Other Mussels" from Brushy Creek. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Brazos River (0 River Kilometers). CPUE = total number of either *F. mitchelli* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

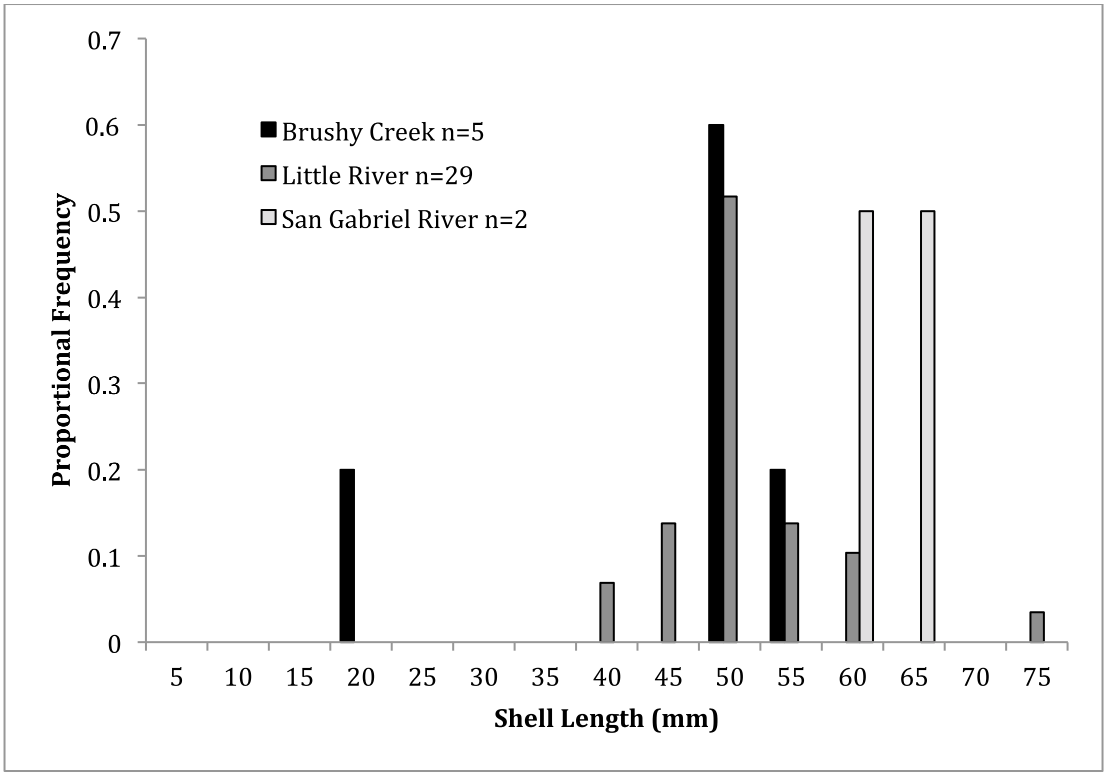

Figure 3. Proportional frequency of shell lengths for *Fusconaia mitchelli* (false spike) from Brushy Creek and the San Gabriel and Little Rivers. Shell lengths are binned into 5 mm groups.

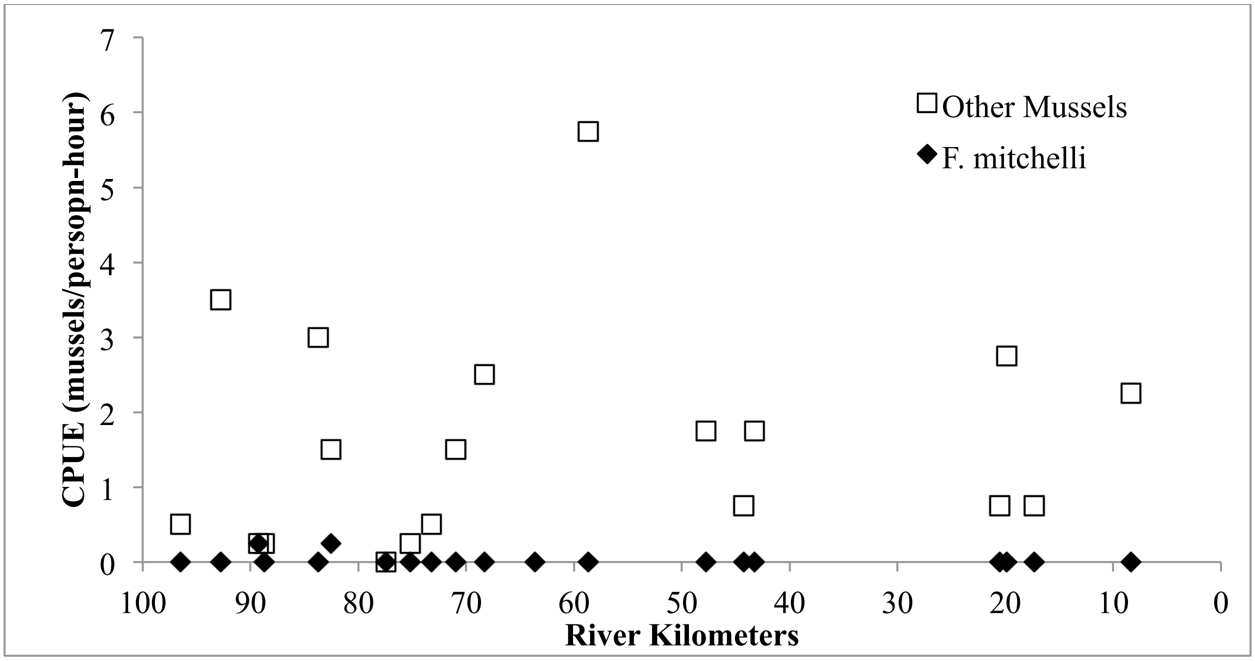

Figure 4. Relative abundance of *Fusconaia mitchelli* (false spike) and all other mussel species, "Other Mussels," on the San Gabriel River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Brazos River (0 River Kilometers). CPUE = total number of either *F. mitchelli* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

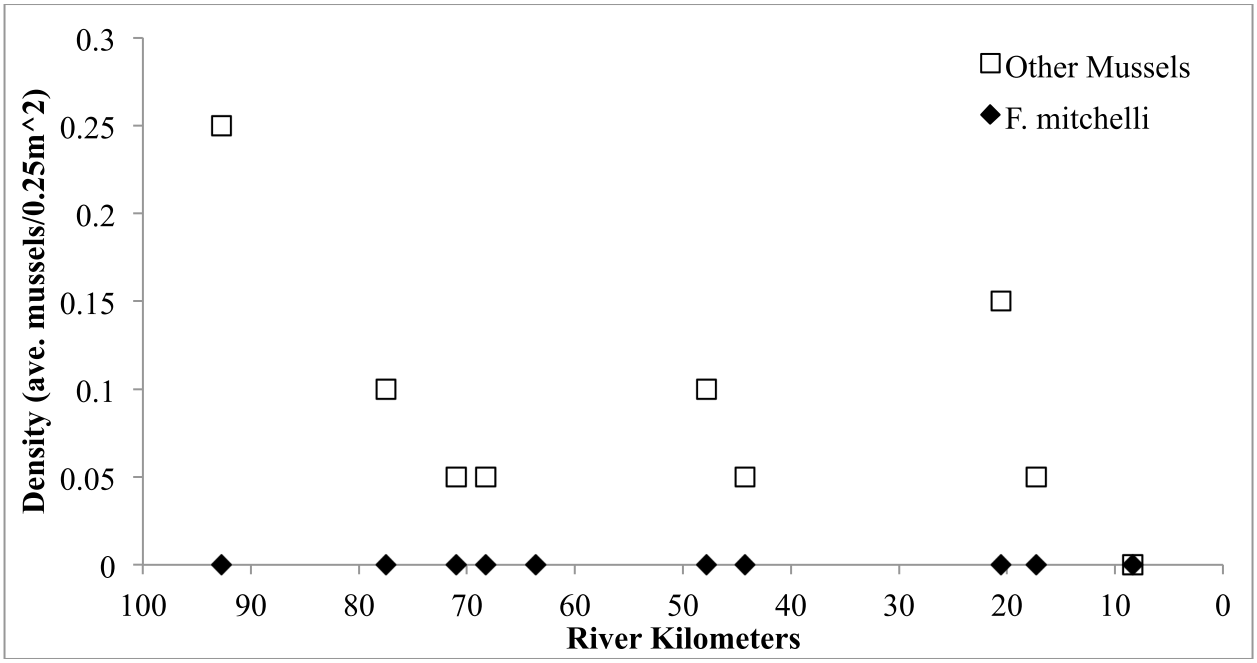

Figure 5. Density of *Fusconaia mitchelli* (false spike) and all other mussel species, "Other Mussels," on the San Gabriel River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Brazos River (0 River Kilometers). Density = mean number of *F. mitchelli* or other species found in 20 quadrats (0.25m^2 each) at each sampling location.

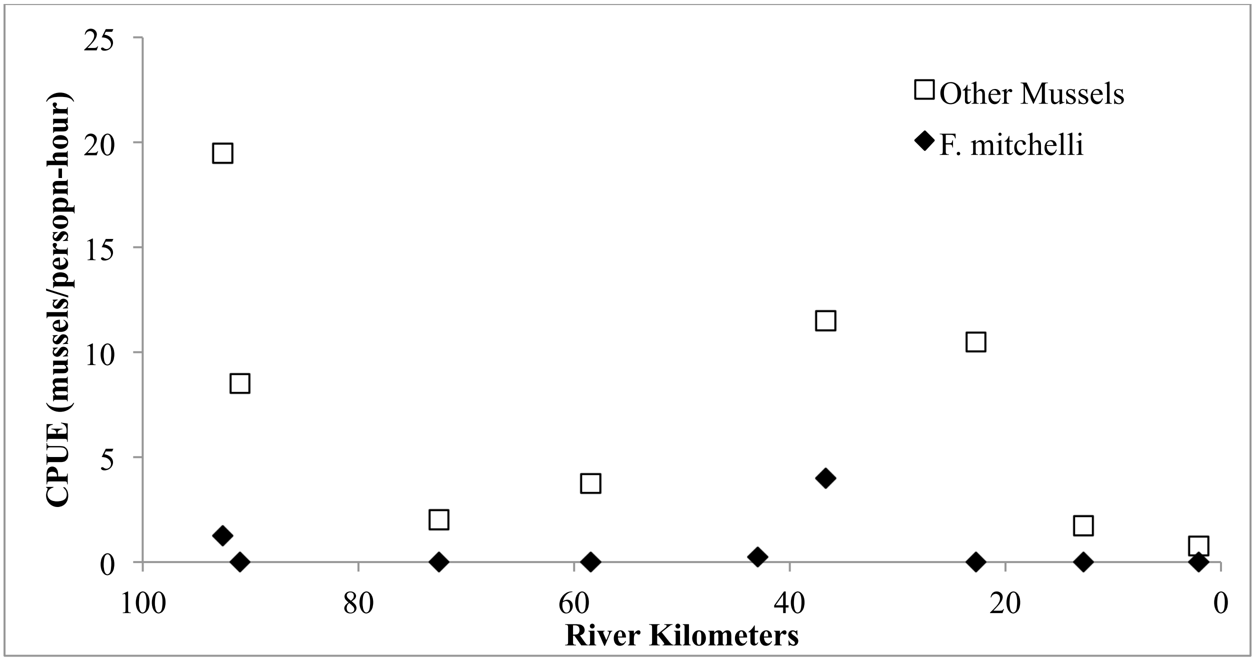

Figure 6. Relative abundance of *Fusconaia mitchelli* (false spike) and all other mussel species, “Other Mussels,” on the Little River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Brazos River (0 River Kilometers). CPUE = total number of either *F. mitchelli* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

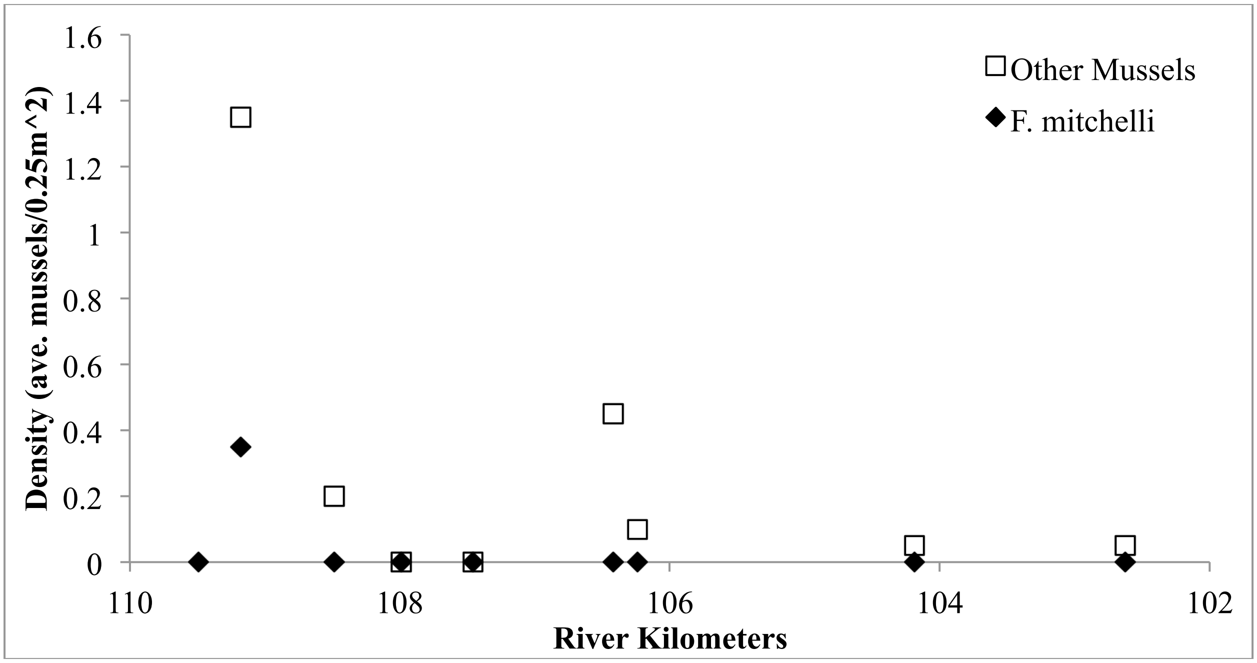

Figure 7. Density of *Fusconaia mitchelli* (false spike) and all other mussel species, "Other Mussels," on the Little River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Brazos River (0 River Kilometers). Density = mean number of *F. mitchelli* or other species found in 20 quadrats ($0.25m^2$ each) at each sampling location.

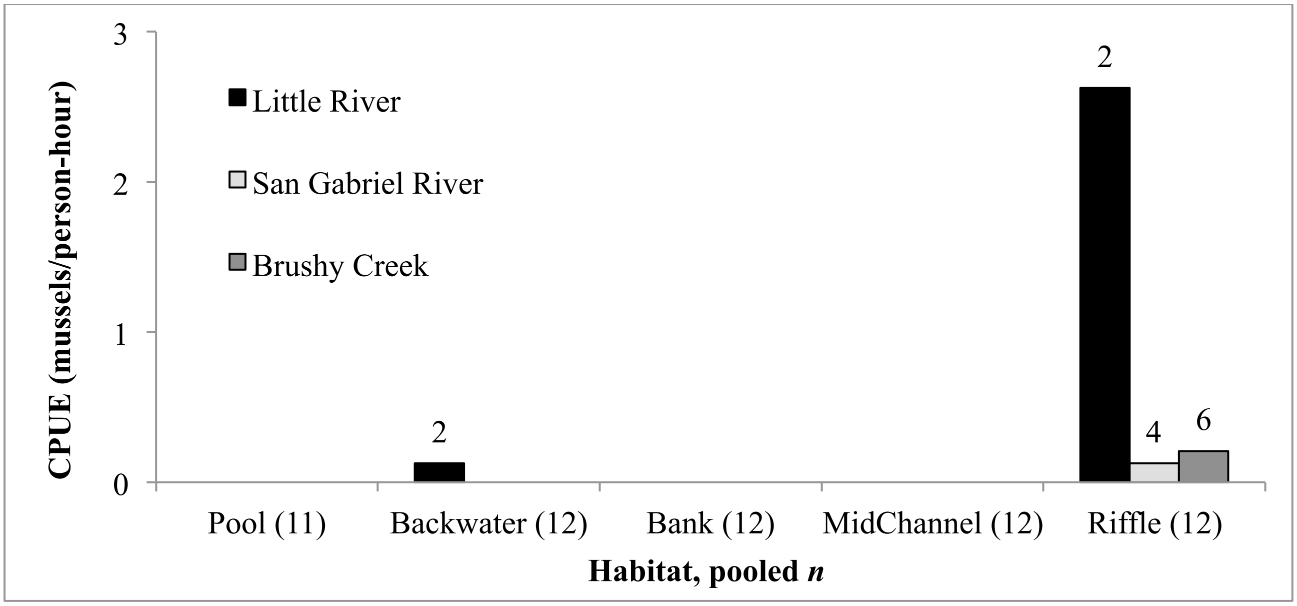

Figure 8. Abundance of *Fusconaia mitchelli* (false spike) by mesohabitat type in Brushy Creek and the San Gabriel and Little Rivers. The total number of sites sampled at each habitat are listed in parenthesis. Numbers above bars are the number of sites sampled of that habitat type in the corresponding river.

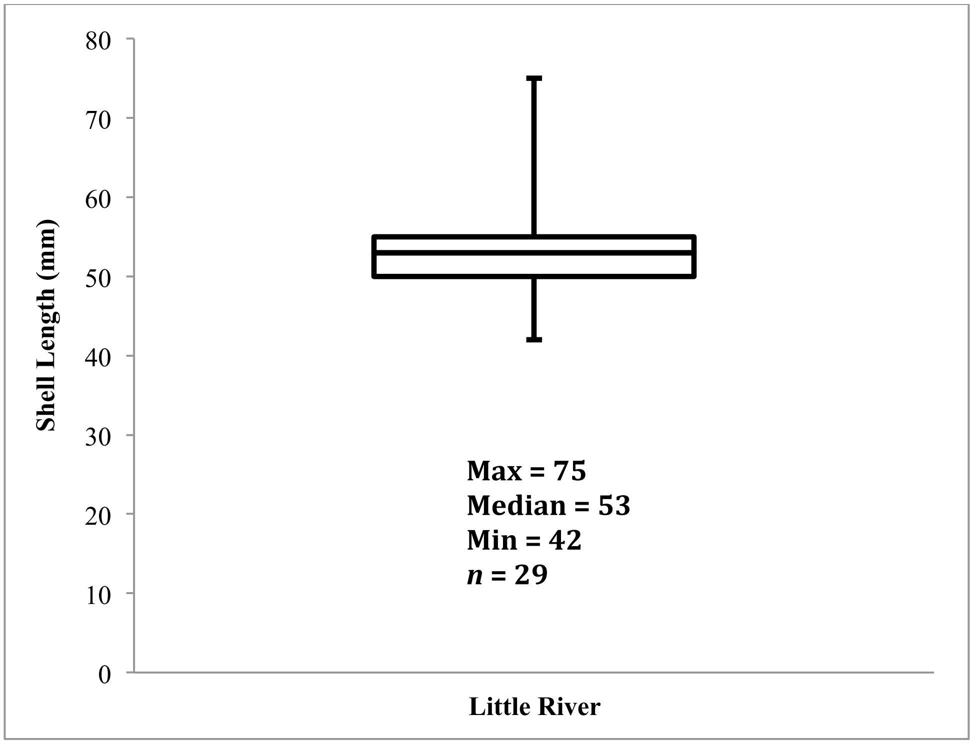

Figure 9. Box and whisker plot of shell length data for *Fusconaia mitchelli* (false spike) populations from the Little River. Brushy Creek (n = 5) and San Gabriel River (n = 2) *F. mitchelli* shell lengths are not included due to very small sample sizes.

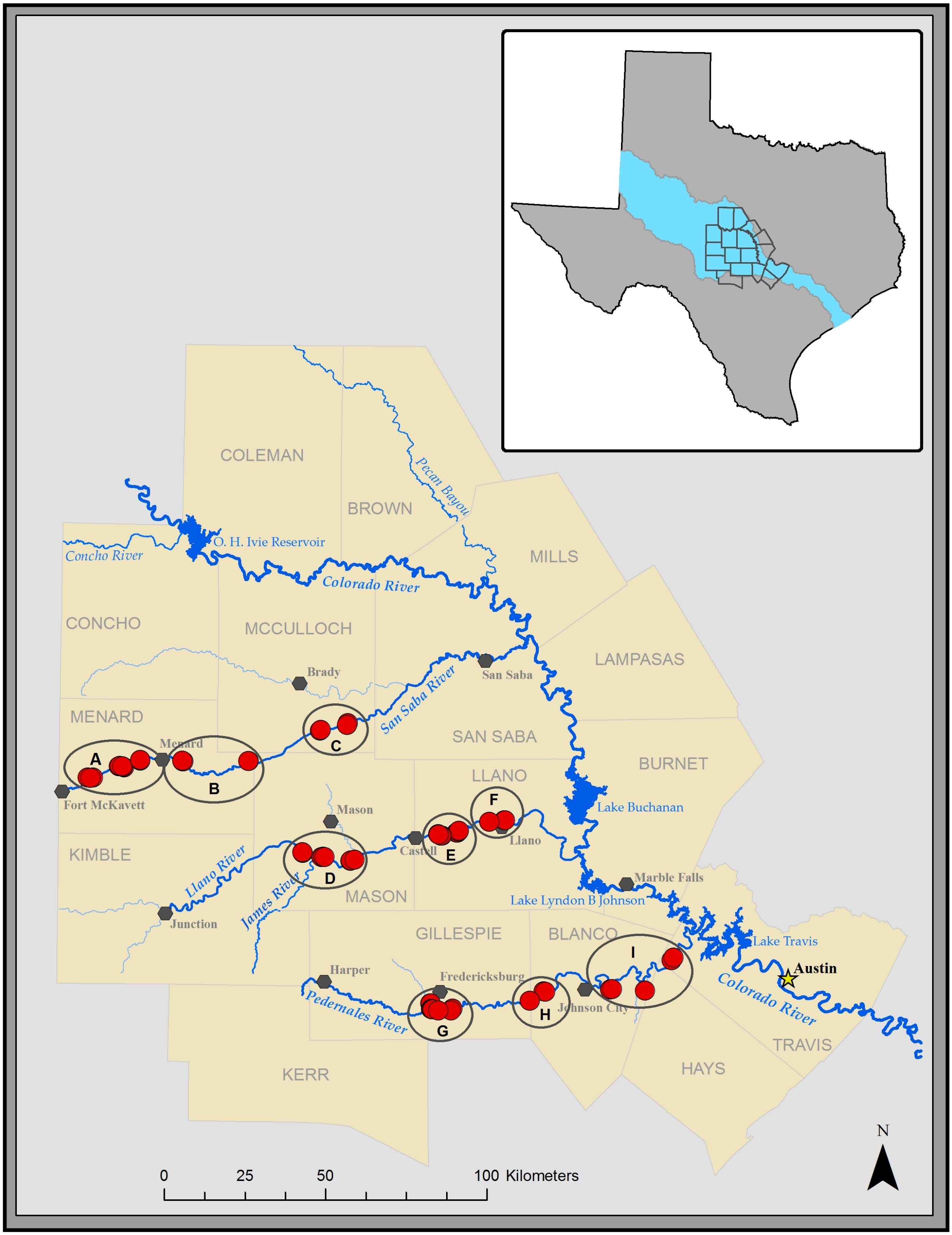

Figure 10. Map of Colorado drainage study area. Shaded circles denote sampling locations. Reaches are indicated by letter and correspond to Table 2.

Figure 11. Map of Guadalupe drainage study area. Shaded circles denote sampling locations. Reaches are indicated by Letter and correspond to Table 3.

Distribution and Habitat Use for *Lampsilis bracteata* (Texas fatmucket)

Section Summary

The goal of this study was to determine the status, distribution, and mesohabitat associations for *Lampsilis bracteata* (Texas fatmucket), a candidate for protection under the Endangered Species Act, in the San Saba, Llano, and Pedernales rivers of the Colorado River drainage. We used recent and historical data to inform a sampling program within the range of *L. bracteata*. In total, we surveyed 58 sites in the Colorado River drainage system and found 136 live individuals of *L. bracteata* from 20 (or 34 %) of the sites surveyed. *Lampsilis bracteata* was most abundant (*n* = 71 live individuals) but less prevalent (32% or 6/19 sites) in the San Saba River and least abundant (*n* = 18 live individuals) but more prevalent (37% or 7/19 sites) in the Pedernales River. In the Llano River, *L. bracteata* (*n* = 47 individuals) was found at 7 of the 20 sites (or 35%). *L. bracteata* was found only in bank and pool habitats with live individuals found in 45% of bank habitats (9/22 sites) and 33% of pool habitats (9/27 sites) across all drainages. No individuals were found in backwater, mid-channel, or riffle habitats. Population size frequency distributions, using shell length as a proxy for age, suggest that recruitment is occurring at a low level, with only the San Saba and Llano populations containing sub-adult individuals (1 live sub-adult per river). We also observed reproductively active females (gravid, i.e., gills containing a brood of either developing eggs or viable larvae) in each river surveyed which corroborates earlier observation of reproductive activity during this time period.

Introduction

Lampsilis bracteata, Texas fatmucket, is known historically from the upper and middle Guadalupe and Colorado River drainages of central Texas (Howells 2010). The type specimen was collected from the Llano River near present US Highway 87 crossing (Mason County) by Dr. T. H. Webb during the U.S. and Mexican Boundary Survey in 1850 and figured by Gould in 1855 (Taylor 1976). Records of questionable validity exist from the Brazos, Sabine, San Antonio and lower San Marcos rivers.

In the Guadalupe River, specimens of *L. bracteata* were collected near New Braunfels by A. L. Fitzpatrick (BU-MMC_MO33197-A-B) and in Kendall County by J. K. Strecker (BU-MMC_MO32493-A-B) (Strecker 1931). Singley (1893) reported specimens of *Lampsilis radiata* from the San Antonio River (Guadalupe River basin) and *Lampsilis powellii* from the Guadalupe River near New Braunfels. Frierson (1927) listed *L. powelli* from the Guadalupe River collected by Singley (1893) as a doubtful species, and placed the specimens to *L. bracteata*. Because *L. radiata* does not occur in Texas, these are also likely misidentified *L. bracteata*. Simpson (1914) listed *L. bracteata* in the Guadalupe River. Strecker (1931) also noted that *L. bracteata* was only known from the upper Guadalupe River in Comal, Kendall, and Kerr counties, while *L. hydiana* was known from the lower Guadalupe River in Victoria County. A specimen of *L. bracteata* was collected by J. Dobie in 1970 from the Guadalupe River at Kerrville (AUM_4157), and Horne and McIntosh (1979) reported *L. bracteata* in the Blanco River (Guadalupe River basin).

In the Colorado River drainage, Singley (1893) reported specimens of *L. radiata* from the San Saba River (Colorado River basin). These specimens are likely misidentified *L. bracteata* as verified specimens have been collected from Onion Creek by W.J. Williams and E.J. Cleveland (BU-MMC_MO31089-A-B), W.J. Williams and H.B. Parks (BU-MMC_MO34192-A-B), and J.K. Strecker (BU-MMC_MO33599-A-B). Simpson (1914) listed *L. bracteata* from the Llano River (a tributary of the Colorado River) and Colorado River. Strecker (1931) reported *L. bracteata* from the Colorado River system and noted that *L. bracteata* was common in the San Saba and Llano rivers but most abundant in the Concho River. Strecker also made a note of particular localities where *L. bracteata* had been collected: Colorado River in Runnels County by Frierson (BU-MMC_MO32866-A-B), San Saba River (a tributary of the Colorado River) in McCulloch and Menard counties by Fitzpatrick (BU-MMC_MO32751-A-B) and Smith, Llano River (a tributary of the Colorado River) in Mason County by A.L. Fitzpatrick (BU-MMC_MO32731-A-B), Cypress Creek (a tributary of the Pedernales River) by Goebel, and South Concho River (a tributary of the Colorado River) in Tom Green County by Williams (BU-MMC_MO33397-A-B) (Strecker 1931). Later years, specimens were collected in 1971 and 1976 by J. Dobie from multiple locations in the Llano River in Kimble and Llano counties (AUM_2947, AUM_2952, AUM_2976, AUM_4025, AUM_4080, and AUM_4044), while *L. bracteata* were collected from two locations in the San Saba River (Menard County) in 1963 by J. R. Preston (FWMSH_94V 2591) and 1971 by W.J. Voss & R. Pool (FWMSH_94V 2709). Cheatum et al. (1972) reported *L. bracteata* from Kimble County (Llano River drainage) and Menard County (San Saba River drainage). From these historical reports, *L. bracteata* ranged across the upper Colorado River and its tributaries.

Neck (1984) noted that large populations of this species occurred at numerous localities throughout central Texas; however, Athearn (1970) and Williams et al. (1993) identified *L. bracteata* as a species of special concern. NatureServe ranks *L. bracteata* as critically imperiled across its range. This species is currently listed as state threatened by the Texas Parks and Wildlife Department (TPWD 2010) and as a candidate for protection under the U.S. Endangered Species Act (USFWS 2011). Howells (2010) listed only 7 populations remaining in the Colorado and Guadalupe River basins since 1992: Elm Creek (a tributary of the upper Colorado River), Live Oak Creek (a tributary of the Pedernales River), Threadgill Creek (a tributary of the Llano River), San Saba River, Llano River, Spring Creek (a tributary of the Concho River, Colorado River basin), and Guadalupe River. More recent surveys have observed live individuals or very recently dead specimens of *L. bracteata* in the following drainages: Elm Creek, Live Oak Creek, Onion Creek (a tributary of the Colorado River), Rocky Creek (a tributary of the Pedernales River), Llano River, Pedernales River, San Saba River, and upper Guadalupe River (Burlakova and Karatayev 2010, Johnson and Groce 2011, Wilkins et al. 2011, Sowards et al. 2012, Randklev et al. 2013).

Lampsilis bracteata is considered a valid species (Burlakova and Karatayev 2010) and like other freshwater mussel species is an obligate ectoparasite on one or more host-fish species. Similar to other *Lampsilis* species, *L. bracteata* is a long-term brooder and has a modified mantle flap that is used to lure host fish and aid in the transmittal of glochidia. Howells et al. (2011) reported distinctive mantle flap forms for Llano River populations compared with populations in Elm Creek, Guadalupe River, Spring Creek, and San Saba River, though these differences likely represent ecophenotpic variation and not species divergence. Potential host fishes identified through artificial inoculations in the laboratory include: *Lepomis cyanellus* (green sunfish), *Lepomis macrochirus* (bluegill), *Micropterus salmoides* (largemouth bass), and *Micropterus treculii* (Guadalupe bass) (Howells 1997, Johnson et al. 2012). Braun et al. (2014) found that glochidia had parasitized a small percentage of these centrarchid fish species from the San Saba, Llano and Pedernales rivers, though the exact identities of these glochidia are unknown. Displaying females with full marsupia have been observed between July and October (Howells 2000, Johnson et al. 2012).

Lampsilis bracteata have been reported to occur in flowing waters in sand, mud, and gravel substrates among large cobble, boulders, bedrock ledges, horizontal cracks in bedrock slabs, and macrophyte beds (Howells 2010). Burlakova and Karatayev (2010) surveying the Llano River reported live *L. bracteata* inhabiting the roots of cypress tress and vegetation along steep banks. Despite these observations, habitat associations for adults remain untested and for juveniles, undescribed.

The objectives of this study were to assess the distribution, abundance, and habitat use for *L. bracteata* in the San Saba, Pedernales, and Llano rivers. The resulting survey information was then used to develop Conservation Status Assessment Maps for this species within the San Saba, Pedernales, and Llano River drainages.

Methods

Study Area

The Colorado River of Texas originates in northeastern Dawson County and is the largest river that is contained entirely within Texas, with an approximate length of 1,040 km and draining a total area of nearly 100,000 km^2 (Huser 2000). The San Saba, Llano, Pedernales rivers are three of the major tributaries of the Colorado River and all are spring-fed rivers, originating in the Edwards Plateau region (Higgins 2009). The San Saba River begins in Schleicher County where the North Valley Prong and Middle Valley Prong San Saba converge near Fort McKavett, and flows for 225 km passing through Menard, Mason, McCulloch, and San Saba counties until its confluence with the Colorado River (Belisle and Josselet 1977). The Llano River originates in Kimble County where the North Llano River and South Llano River converge in the town of Junction, TX. The Llano River flows for 161 km through Mason and Llano counties emptying into Lake Lyndon B. Johnson, an impoundment on the Colorado River. The Pedernales River originates in Kimble County and flows approximately 170 km through Gillespie, Blanco, Hays, and Travis counties, emptying into Lake Travis, an impoundment on the Colorado River (Perkin et al. 2010).

Sampling Methods

Survey sites in the San Saba, Llano, Pedernales rivers were selected using a random sampling design with the following strata: 1) upstream or downstream of a bridge crossing, 2) linear distance from the bridge; and 3) mesohabitat: (banks, backwater, mid-channel, riffles, and pools). The exact location and habitats were identified prior to field sampling using aerial imagery. Specifically, at each bridge crossing habitats within entire length of the river extending up to 2 river kilometers were identified and numbered. Then a random number generator was used to randomly select the linear distance from the bridge and the habitat type to be sampled at that location. For locations where specific habitats could not be identified using satellite imagery (e.g., riffles), we modified our sampling design by surveying the first habitat encountered for that target habitat type. In total, 20 sites in the Llano River and 19 sites in each the Pedernales and San Saba rivers were selected for sampling. Selected sites included sites that had both been sampled during previous efforts and sites that had not been sampled previously.

Qualitative surveys using the timed search method were performed in each selected mesohabitat type. The timed search method was chosen because it provides a more effective means of detecting rare species than quantitative sampling methodologies (Vaughn et al. 1997). At each site (i.e., mesohabitat type), we confined the search boundaries to the specific habitat type, ensuring that the search area did not exceed 150 m^2, though in some cases the search area included multiple mesohabitat types (e.g., pool-run or riffle-run habitats). Each site was surveyed tactilely and visually for a total of 4 person-hours (p-h). However, because we are interested in the amount of effort needed to detect *L. bracteata* (which will be important for designing long-term monitoring programs), we divided the total search time into 4, 1 p-h intervals. At the end of each search interval, surveyors combined all live specimens into a mesh bag, which was kept submerged in water until completion of the survey. During each interval, surveyors were spread out in the search area and every effort was made to search all available microhabitats. Following completion of the survey, all live mussels from each time period were identified to species, counted, measured, checked for gravidity, and then returned back to the river into the appropriate habitat.

Data analysis

Scatter plots of relative abundance (CPUE: number of individuals/total person-hours) vs. river kilometer (RKM) were used to examine the effect of stream position on total mussel and *L. bracteata* abundance in each river. Boxplots and length-frequency histograms were developed for *L. bracteata* to assess demographic patterns and population structuring within populations. Generally, multimodal size class distribution may indicate recruitment, whereas truncated distributions (absence of a particular age class, large, or small individuals) may indicate a lack of recent recruitment or a localized extinction event. Bar graphs were also used to visually represent presence of *L. bracteata* by mesohabitat type (i.e., riffle, mid-channel, pool, bank, and backwater).

Results/Discussion

A total of 232 person-hours were spent surveying 58 sites located in the San Saba, Llano, and Pedernales rivers of the Colorado River drainage (Figure 1). A total of 136 live individuals of *L. bracteata* were found at 6 of 19 (32%) sites in the San Saba River (n = 71 individuals), 7 of 20 (35%) sites in the Llano River (n = 47 individuals), and 7 of 19 (37%) sites in the Pedernales River (n = 18 individuals). Relative abundance ranged from 0 to 8 mussels/hr across all drainages, with the highest average occurring in the San Saba River (0.93 ± 2.14 mussels/hr; mean ± SD) followed by the Llano River (0.59 ± 0.99 mussels/hr; mean ± SD) then the Pedernales River (0.24 ± 0.43 mussels/hr; mean ± SD). These results indicate that the San Saba has the highest abundance of *L. bracteata*; the single site with the highest abundance of mussels (n = 32 individuals) also occurred in the San Saba River. Across all three rivers, an average of 2.34 ± 5.63 mussels were found per site (mean ± SD).

Previous surveys in the San Saba River reported a total of 5 live individuals from surveys in 1997 and 2005 in Menard County (Howells 2006, Burlakova and Karatayev 2010). Recent surveys from 2011 to 2013 by Burlakova, FWS, and TPWD biologists found 37 live individuals across 2 sites in McCulloch and Menard counties. Our survey efforts in Menard and McCulloch Counties detected 71 live individuals within 6 of the 19 sites surveyed with densities generally higher in sites located further upstream from the confluence with the Colorado River (Figure 2). Though our survey efforts did not include sites within 100 km of the confluence with the Colorado River, a survey by Tsakiris et al. (unpublished data) sampled 18 sites on the San Saba River between the confluences of Brady Creek and the Colorado River; no *L. bracteata* were found within that study area. Our results combined with those from recent surveys indicate that *L. bracteata* may be more abundant in the San Saba River than prior efforts have suggested.

In the Llano River, previous surveys reported a total of 11 individuals from 4 sites from Kimble, Mason, and Llano counties over a 3 year period (Burlakova and Karatayev 2010, Randklev et al. 2013). Recent independent surveys by IRNR found a total of 73 live individuals at 3 sites in Mason County. In the present study, we observed 47 individuals from 7 of 20 sites surveyed.

Densities were generally higher in sites further upstream from the confluence with the Colorado River (Figure 3). Additionally, recent surveys from 2015 by TPWD biologists in tributaries of the Llano River, found 9 live *L. bracteata* individuals in the James River, 11 live individuals in the North Llano River, and 9 live individuals in the South Llano River. Our results combined with those from recent surveys by IRNR and TPWD indicate that *L. bracteata* may be more abundant in the Llano River and its tributaries than previous efforts have suggested.

In the Pedernales River, live individuals were known from the mainstem Pedernales River (Gillespie and Hays counties) and 2 of its tributaries: Live Oak Creek (Gillespie County), and Rocky Creek (Blanco County) (Burlakova and Karatayev 2010, Wilkins et al. 2011, Sowards et al. 2012, Randklev et al. 2013). Low numbers were reported from all 2 tributaries in those studies. Previous surveys reported a total of 6 live individuals from Live Oak Creek over a 3 year period (Howells et al. 2003, Howells 2004, 2006, Burlakova and Karatayev 2010). In the present study, we observed 18 individuals across 7 of 19 sites surveyed, with higher densities in sites furthest upstream from the confluence with the Colorado River (Figure 4). We found 4 individuals across 2 sites in Live Oak Creek in the present study, which support the low occurrence of *L. bracteata* in tributaries of the Pedernales River. However, our study also found 7 individuals at a site on the mainstem of the Pedernales River upstream of the confluence with Live Oak Creek. This represents the largest number of live individuals at a single site found to date on the Pedernales River.

In the Llano River, *L. bracteata* was the dominant species and was occasionally found with *Fusconaia mitchelli* (false spike), *Quadrula houstonensis* (smooth pimpleback), and *Q. petrina* (Texas pimpleback). *Lampsilis bracteata* was also the dominant species in the Pedernales River and was occasionally found with *Utterbackia imbecillis* (paper pondshell). In the San Saba River, however, it was the second most observed species after *U. imbecillis* and was also occasionally found with *Potamilus purpuratus* (bleufer), *Quadrula apiculata* (southern mapleleaf), *Q. houstonensis*, *Q. petrina*, and *Q. verrucosa* (pistolgrip). In all 3 rivers, *L. bracteata* was found primarily in bank and pool habitats, and no individuals were found in backwater, mid-channel, or riffle habitats (Figure 5). Live individuals were found at 45% of bank habitats (9/22 sites) and 33% of pool habitats (9/27 sites) across all drainages. In the Llano River, the only habitats surveyed were bank, pool, and pool-run mesohabitats, and *L. bracteata* was found in all 3 of those habitat types. In the case of 2 sites on Live Oak Creek, the entire width of the stream was sampled which included multiple mesohabitats; however, the live individuals were found in bank habitats at each site.

Size frequency distributions for the Pedernales and San Saba rivers were similar (median = 57.5 and 56 mm, respectively). Median shell length for the population in the Llano River was approximately 45 mm (Figure 6). The minimum and maximum shell lengths differed slightly between drainages: the San Saba River (23 mm and 74 mm), Llano River (28 mm and 59 mm), and Pedernales River (38 mm and 70 mm). However, such differences are likely due to the small sample sizes. Small sample size may also account for the low numbers of sub-adults found (individuals <30 mm); only the San Saba and Llano River populations had any sub-adult individuals (n = 1 live sub-adult individual per river). The shape of shell length distributions for the San Saba River population approximated an "inverted teardrop" (Miller and Payne 1993) except for the 60 mm size class, which may indicate a localized extinction event for that size class, or may be an artifact of small sample size (Figure 7). Otherwise, the size frequency distribution seems to be indicative of consistent annual recruitment. However, both the Llano and Pedernales rivers had gaps in their size frequency distributions likely due in some part to the small sample sizes. The Pedernales River had no small individuals less than 30 mm and a gap at the 45 mm size class, while the size distribution of the Llano River was shifted toward slightly smaller individuals than those seen in the Pedernales and San Saba rivers. To determine if these differences are significant between populations and point towards low recruitment or impacts on growth in local populations, further studies are needed that increase the current sample size.

During the course of this study, June through early August, we observed reproductively active females (gravid—i.e., gills containing a brood of either developing eggs or viable larvae), which coincides with previous observations in the Llano River (Johnson et al. 2012). Additionally, since *L. bracteata* are sexually dimorphic, it was possible to identify mussel gender by their shell morphologies. The sex ratio for *L. bracteata* was male biased to varying degrees in all three rivers (2.46:1 in the Llano River, 3.25:1 in the Pedernales River, and 1.16:1 in the San Saba River). Haag (2012) noted that male biased sex ratios are more common in lampsiline species, possibly because lure display may incur a higher female mortality. However, bias from our small sample size made it difficult to infer sex ratios over a whole population.

In summary, our results indicate *Lampsilis bracteata* continues to occur within the Llano, Pedernales and San Saba rivers. Our results along with those within the past 5 years indicate that *L. bracteata* is abundant in portions of the San Saba and the Llano rivers (Burlakova and Karatayev 2010, Johnson and Groce 2011, Wilkins et al. 2011, Sowards et al. 2012, Randklev et al. 2013). For the Pedernales River, *L. bracteata* occurred in low abundance compared to the Llano and San Saba rivers, but was more prevalent (37% or 7/19 sites). Because much of our sampling focused on sites near bridge crossings, due to limited points of public access, large sections of the 3 river drainages are still unsampled. Despite this limitation, we tried to ensure adequate spatial coverage by distributing our sites evenly throughout each river.

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Table 1. Locality and collection information for mussel survey sites in the Colorado River drainage. Habitat key: P = pool, B = bank, BW = backwater, PR = pool-run, R = riffle, RR = riffle-run, MC = mid-channel, All = site was entire width of stream and encompassed all habitat types present.

Site	Habitat	Locality	County	Date of collection	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
1	B	San Saba	Menard	8/5/2015	0	0	-	4	150
2	P	San Saba	Menard	8/5/2015	0	0	-	4	150
3	P	San Saba	Menard	8/5/2015	1	0.25	N	4	150
4	B	San Saba	Menard	8/3/2015	32	8	Y	4	150
5	P	San Saba	Menard	8/4/2015	23	5.75	N	4	150
6	P	San Saba	Menard	8/4/2015	5	1.25	N	4	150
7	BW	San Saba	Menard	8/4/2015	0	0	-	4	150
8	B	San Saba	Menard	8/4/2015	9	2.25	N	4	150
9	B	San Saba	Menard	8/5/2015	0	0	-	4	150
10	P	San Saba	Menard	8/5/2015	0	0	-	4	150
11	R	San Saba	Menard	8/6/2015	0	0	-	4	150
12	B	San Saba	Menard	8/6/2015	0	0	-	4	150
13	BW	San Saba	Menard	8/6/2015	0	0	-	4	150
14	P	San Saba	Menard	8/6/2015	0	0	-	4	150
15	B	San Saba	Menard	8/6/2015	1	0.25	N	4	150
16	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
17	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
18	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
19	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
20	P	Llano	Mason	7/30/2015	13	3.25	N	4	150
21	PR	Llano	Mason	7/30/2015	10	2.5	Y	4	150
22	PR	Llano	Mason	7/30/2015	0	0	-	4	150
23	PR	Llano	Mason	7/30/2015	0	0	-	4	150
24	B	Llano	Mason	7/30/2015	7	1.75	N	4	150
25	PR	Llano	Mason	7/29/2015	0	0	-	4	150
26	B	Llano	Mason	7/29/2015	0	0	-	4	150
27	P	Llano	Mason	7/31/2015	0	0	-	4	150
28	B	Llano	Llano	7/29/2015	0	0	-	4	150
29	P	Llano	Llano	7/29/2015	0	0	-	4	150
30	B	Llano	Llano	7/29/2015	3	0.75	N	4	150
31	P	Llano	Llano	7/29/2015	9	2.25	N	4	150
32	P	Llano	Llano	7/28/2015	0	0	-	4	150
33	B	Llano	Llano	7/28/2015	1	0.25	N	4	150
34	B	Llano	Llano	7/28/2015	4	1	N	4	150
35	PR	Llano	Llano	7/28/2015	0	0	-	4	150
36	P	Llano	Llano	7/27/2015	0	0	-	4	150
37	B	Llano	Llano	7/28/2015	0	0	-	4	150
38	PR	Llano	Llano	7/27/2015	0	0	-	4	150
39	B	Llano	Llano	7/27/2015	0	0	-	4	150
40	P	Flat Creek	Blanco	6/25/2015	0	0	-	4	150
41	P	Live Oak Creek	Gillespie	6/24/2015	3	0.75	N	4	150
42	P	Live Oak Creek	Gillespie	6/24/2015	1	0.25	N	4	150
43	All	Live Oak Creek	Gillespie	6/23/2015	1	0.25	N	4	150

Table 1. Continued.

Site	Habitat	Locality	County	Date of collection	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
44	All	Live Oak Creek	Gillespie	6/23/2015	1	0.25	N	4	150
45	B	Pedernales	Gillespie	6/24/2015	2	0.5	N	4	150
46	P	Pedernales	Gillespie	6/24/2015	7	1.75	N	4	150
47	B	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
48	B	Pedernales	Gillespie	6/24/2015	3	0.75	N	4	150
49	BW	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
50	MC	Pedernales	Gillespie	6/23/2015	0	0	-	4	150
51	B	Pedernales	Gillespie	6/23/2015	0	0	-	4	150
52	RR	Pedernales	Blanco	6/23/2015	0	0	-	4	150
53	B	Pedernales	Blanco	6/23/2015	0	0	-	4	150
54	BW	Pedernales	Blanco	6/23/2015	0	0	-	4	150
55	P	Pedernales	Blanco	6/22/2015	0	0	-	4	150
56	RR	Pedernales	Blanco	6/22/2015	0	0	-	4	150
57	BW	Pedernales	Travis	6/25/2015	0	0	-	4	150
58	B	Pedernales	Travis	6/25/2015	0	0	-	4	150

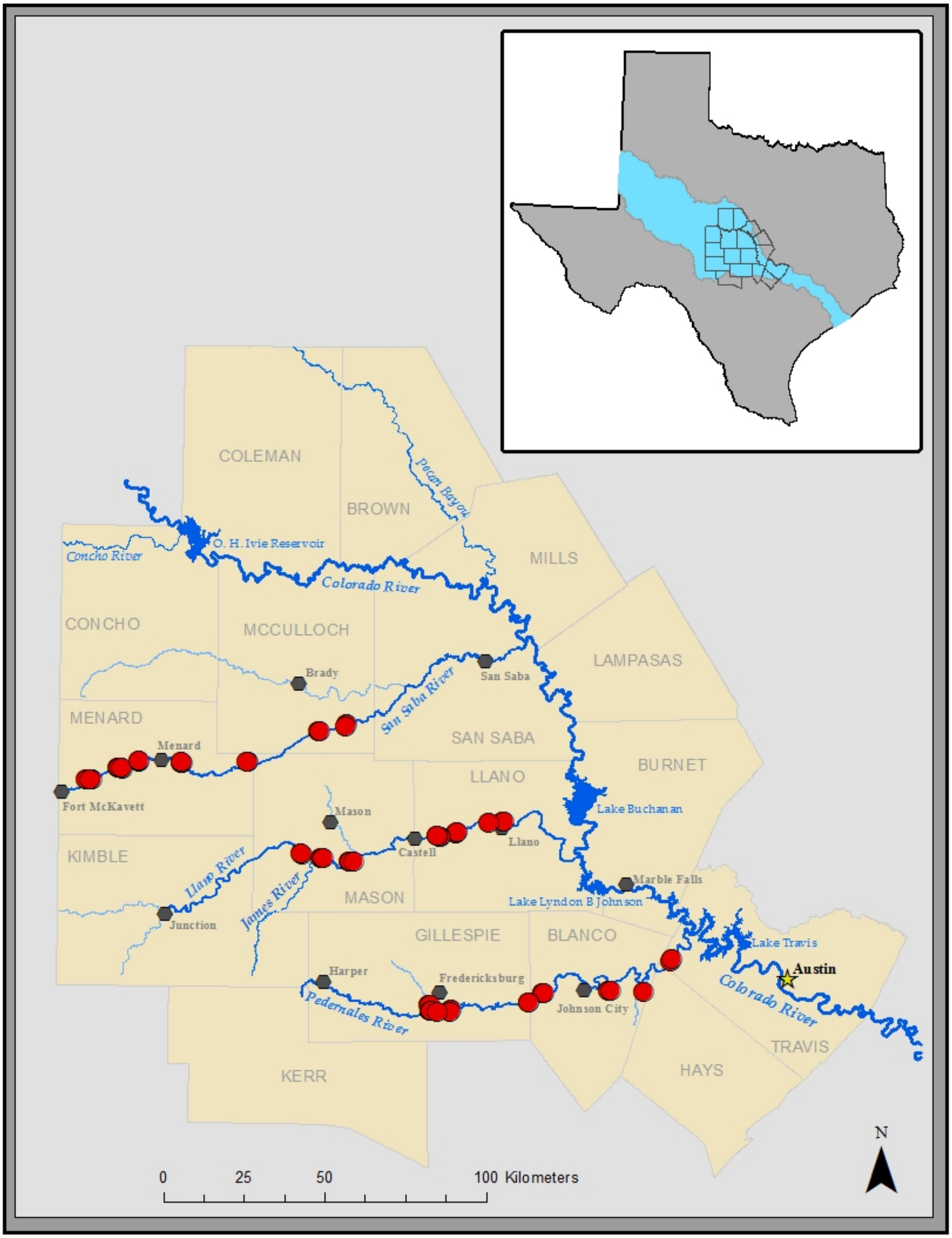

Figure 1. Map of study area. Shaded circles denote sampling locations.

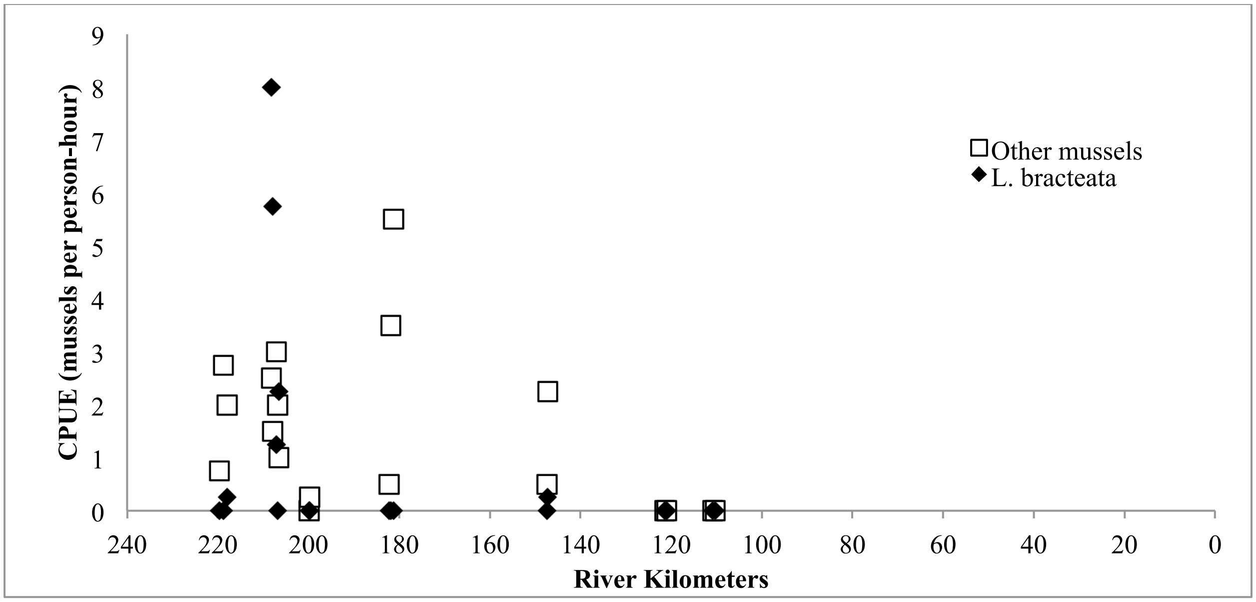

Figure 2. Relative abundance of *Lampsilis bracteata* (Texas fatmucket) and all other mussel species, “Other Mussels,” on the San Saba River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Colorado River (0 River Kilometers). CPUE = total number of either *L. bracteata* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

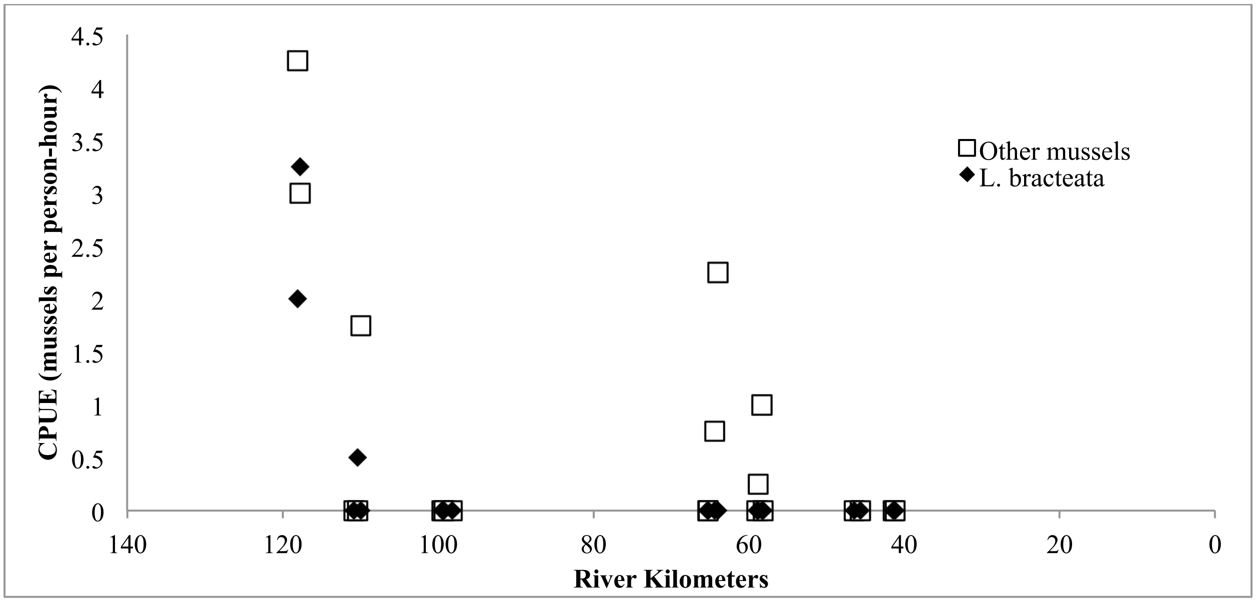

Figure 3. Relative abundance of *Lampsilis bracteata* (Texas fatmucket) and all other mussel species, "Other Mussels," on the Llano River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Colorado River (0 River Kilometers). CPUE = total number of either *L. bracteata* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

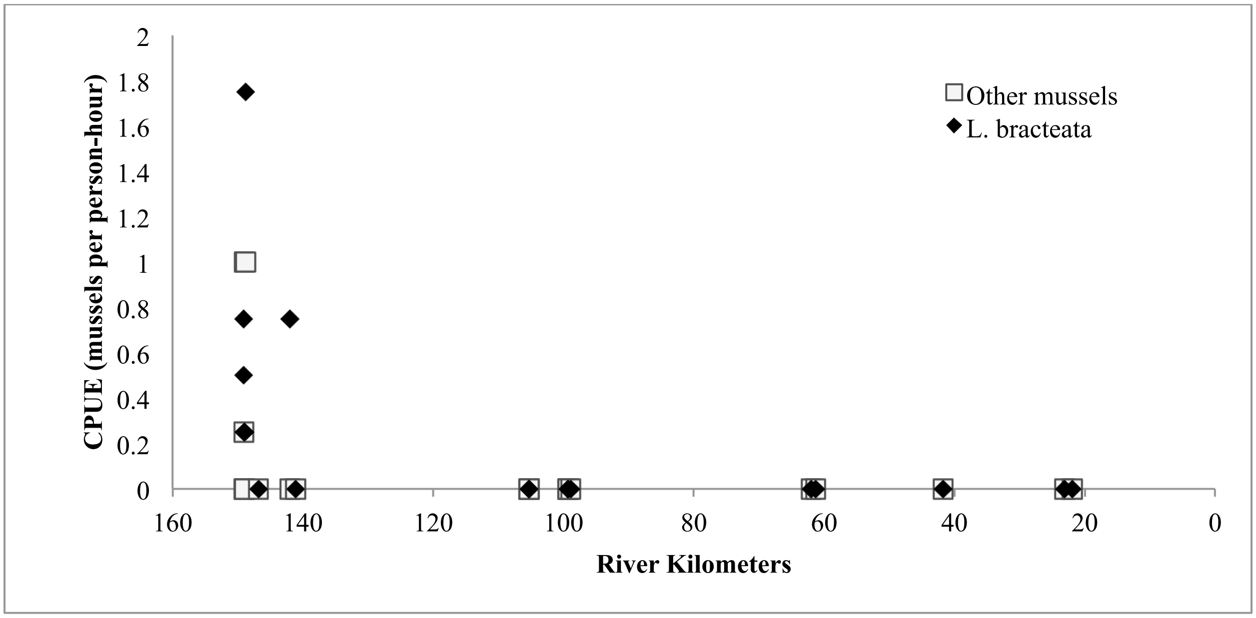

Figure 4. Relative abundance of *Lampsilis bracteata* (Texas fatmucket) and all other mussel species, "Other Mussels," on the Pedernales River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Colorado River (0 River Kilometers). CPUE = total number of either *L. bracteata* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

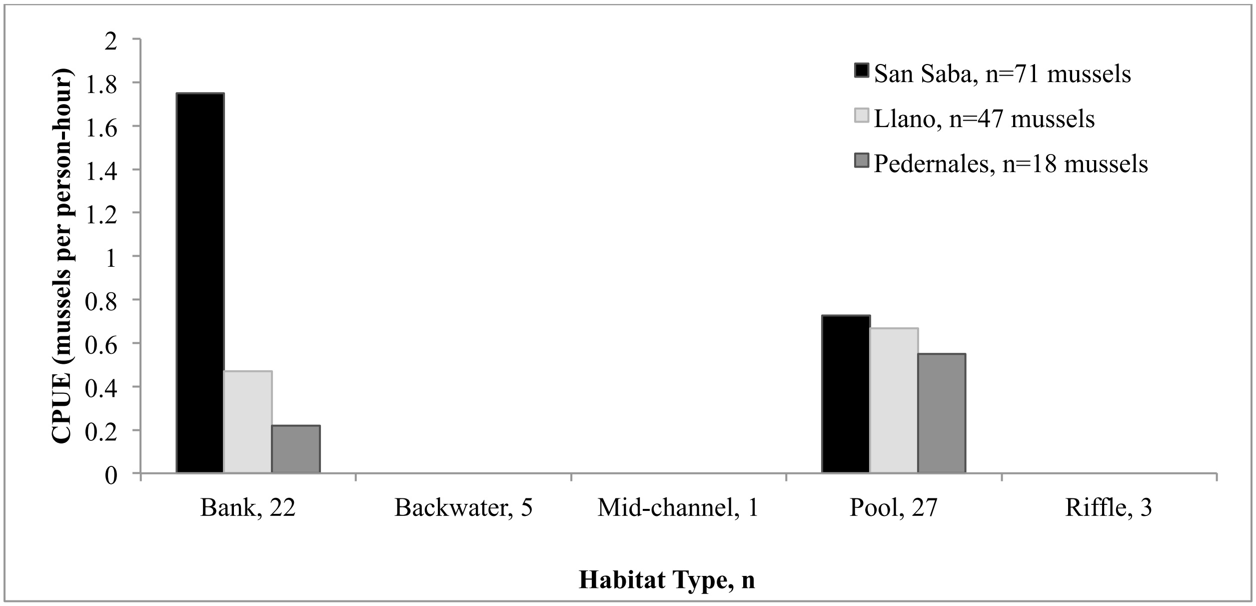

Figure 5. Distribution of *Lampsilis bracteata* (Texas fatmucket) by mesohabitat type in the San Saba, Llano, and Pedernales Rivers. The number of sites sampled in each habitat are listed next to each habitat type.

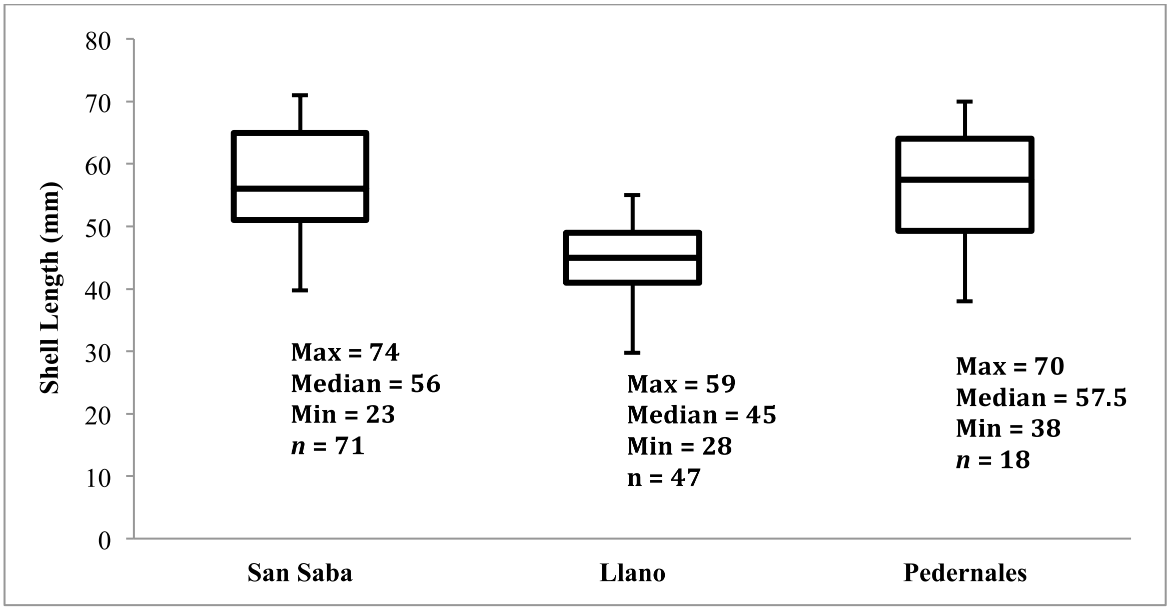

Figure 6. Box and whisker plot of shell length data for *Lampsilis bracteata* (Texas fatmucket) populations from the San Saba, Llano, and Pedernales Rivers.

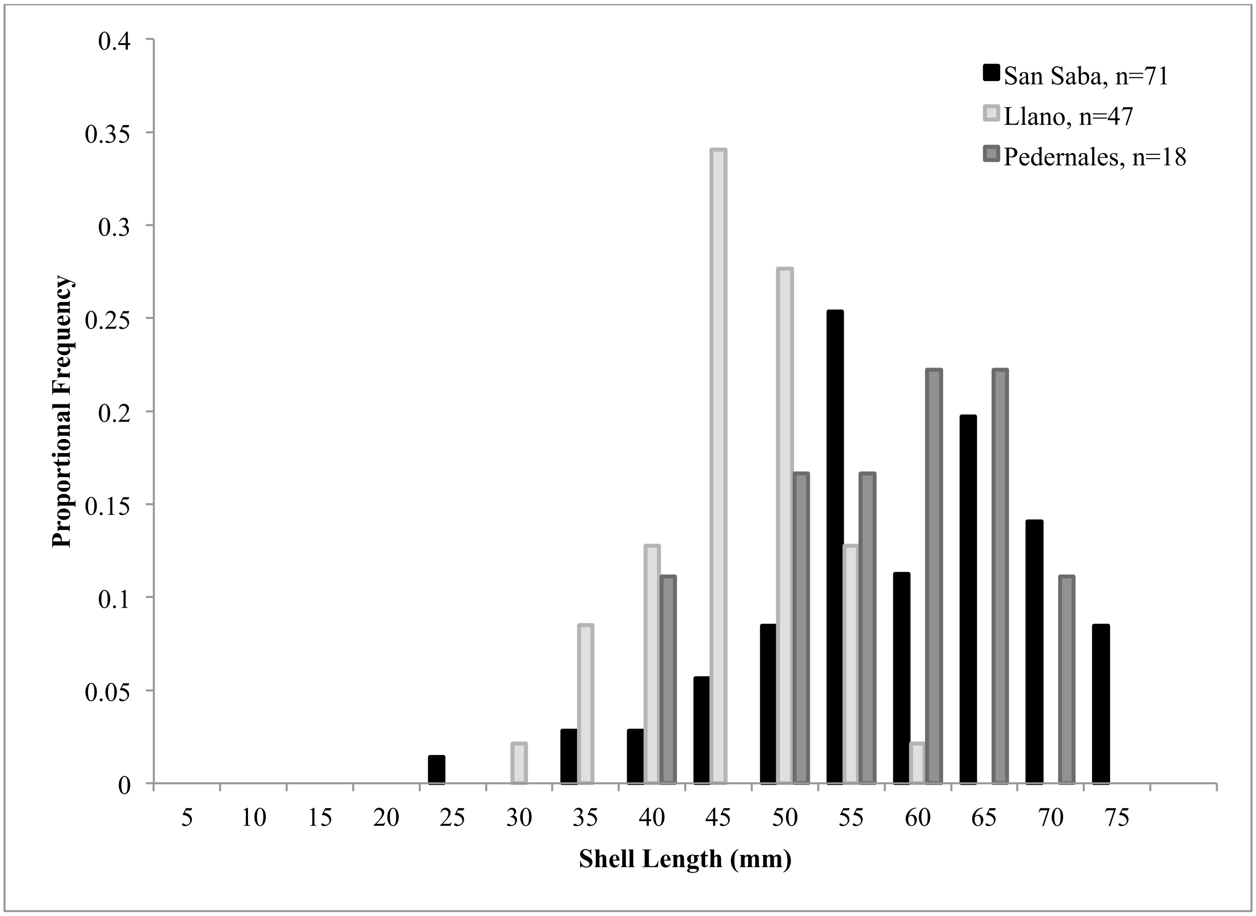

Figure 7. Proportional frequency of shell lengths for *Lampsilis bracteata* (Texas fatmucket) from the San Saba, Llano, and Pedernales Rivers. Shell lengths are binned into 5 mm groups.

Distribution and Habitat Use for *Quadrula aurea* (golden orb)

Section Summary

The goal of this study was to determine the status, distribution, and mesohabitat associations of *Quadrula aurea* (golden orb), a candidate for protection under the U.S. Endangered Species Act, in the Guadalupe River. We used recent and historical data to inform a sampling program within its presumptive range in the selected basin. In total, we surveyed 13 sites within the Guadalupe River, and found 377 live individuals of *Q. aurea* from 10 of the 13 (or 76.9%) sites surveyed. The majority of live individuals (*n* = 284) found occurred in pool habitat; however, not all habitat types were sampled equally due to persistent high flows throughout central Texas which prevented sampling of riffle habitats, an optimal mesohabitat for this species. Size frequency distributions, using shell length as a proxy for age, suggest that recruitment is occurring in the Guadalupe River populations.

Introduction

Quadrula aurea (Lea 1859), golden orb, is known historically from the San Antonio-Guadalupe and Nueces River basins of central Texas (Howells 2002, 2010). The holotype specimen was collected by W. Newcomb and described formally as *Unio aureus* by Lea (1859). The species was subsequently assigned to *Quadrula* by Simpson (1900). Howells (2002, 2010a) and Turgeon et al. (1998) assert *Q. aurea* is a taxonomically valid species.

In the San Antonio-Guadalupe River basin, historic records of *Q. aurea* have come from the mainstem of the Guadalupe and San Antonio rivers and several of their tributaries. *Quadrula aurea* has been reported in the mainstem of the Guadalupe River from the following counties: Comal, Gonzales, Kendall, Kerr, and Victoria. Others have reported *Q. aurea* from two tributaries of the Guadalupe River: Geronimo Creek in Guadalupe County and the San Marcos River in Caldwell and Gonzales counties. In the San Antonio River, *Q. aurea* has been reported from Bexar, Goliad, Karnes, Victoria/Refugio, and Wilson counties. Tributaries of the San Antonio River have also been reported to harbor the species: Cibolo Creek in Guadalupe, Karnes, and Wilson counties; Medina River in Bexar County; and Salado Creek in Bexar County.

Strecker (1931) originally remarked that *Q. aurea* was abundant in the Guadalupe and San Antonio rivers based on prevalence of shell material. In recent years, investigators have suggested that *Q. aurea* has become increasingly rare throughout its range (Howells 2002, 2010). Currently, Texas Parks and Wildlife Department lists *Q. aurea* as state-threatened (TPWD 2010) and U.S. Fish and Wildlife Service has listed this species as a candidate for protection under the U.S. Endangered Species Act (USFWS 2011). Presently, the American Fisheries Society lists *Q. aurea* as endangered (Williams et al. 1993) and NatureServe ranks it as imperiled. Since the 1990s, surveys in central Texas have led to the discovery of live individuals or very recently dead specimens of *Q. aurea* in the following rivers within the San Antonio-Guadalupe River drainage: Cibolo Creek (Guadalupe, Karnes, and Wilson counties); Guadalupe River (Comal, Gonzales, Kerr, and Victoria counties); San Antonio River (Bexar, Goliad, Karnes, Refugio, Victoria, and Wilson counties); and San Marcos River (Caldwell and Gonzales counties) (Howells 2006, 2010, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2003, Burlakova and Karatayev 2010, Sowards et al. 2012, Randklev et al. 2013, Braun et al. 2014, Tsakiris and Randklev 2016). Similar survey efforts have led to observations of *Q. aurea* in the following waterbodies of the Nueces River drainage: Frio River (Live Oak and McMullan counties); Nueces River (Live Oak, and San Patrico counties); and Lake Corpus Christi (Live Oak and San Patrico counties) (Burlakova and Karatayev digital data, Howells 1996, 1997, 2006, Randklev et al. 2016, unpublished data).

Currently, little is known about the life history or reproductive requirements of *Q. aurea* (Howells 2010). Like other freshwater mussel species, it is an obligate ectoparasite on one or more host-fish species and its congeners are short-term brooders that use mantle lures known as mantle magazines to attract fish species from the catfish family, Ictaluridae. Based on recent observations from field surveys throughout *Q. aurea's* range, sub-adults and adults appear to occur most often in riffle and run mesohabitats in sand, gravel, and cobble substrates, including gravel-filled cracks in bedrock slabs (Howells 2010, Tsakiris and Randklev 2016). These mesohabitat types appear to serve as flow refuges (*sensu* Strayer 1999), where near bed shear stress remains low during high flow events.

The objectives of this study were to assess the distribution, abundance, and habitat use for *Q. aurea* in selected reaches within the Guadalupe River basin. The resulting survey information was then used to develop Conservation Status Assessment Maps for this species**.**

Methods

Study Area

The Guadalupe River is spring fed and originates on the Edwards Plateau in Kerr County, Texas and drains an area of 15,539 km^2. The river runs 402 km in length to the Guadalupe and San Antonio bays (Huser 2000). The major tributaries are the Blanco-San Marcos and the San Antonio rivers. The Guadalupe River has 10 mainstem impoundments with Canyon Lake in Comal County as the largest followed by Lake McQueeney in Guadalupe County (Huser 2000, Roach et al. 2014). Many small dams are located on the San Marcos and Guadalupe rivers with the most downstream dam occurring at the confluence near the city of Gonzales.

Sampling Methods

Survey sites within the Guadalupe River were selected using a random sampling design. Specifically, we delineated the entire length of the Guadalupe between Cuero and Victoria, TX, into 10 km reaches and randomly chose a subset of those reaches to survey. Within each reach, sites were selected randomly by mesohabitat. For locations where habitats could not be identified using satellite imagery (e.g., riffles), we modified our sampling design by surveying the first habitat encountered for that target habitat type. In total, only three sites in DeWitt County and 10 sites in Victoria County were selected for sampling as a result of higher than normal flow conditions during 2015.

Qualitative surveys using the timed search method were performed at each site (i.e., mesohabitat type). The timed search method was chosen because it provides a more effective means of detecting rare species than quantitative sampling methodologies (Vaughn et al. 1997). At each site we confined the search boundaries within the randomly selected mesohabtiat and standardized the search area to 150 m^2. Each site was surveyed tactilely and visually for a total of 4 person-hours (p-h). Because our focus was to determine the amount of effort needed to detect *Q. aurea* (which will be important for designing long-term monitoring programs), we divided the total search time into 4, 1 p-h intervals. At the end of each search interval, surveyors combined all live specimens into a mesh bag, which was kept submerged in water until completion of the survey. During each interval, surveyors were spread out in the search area and every effort was made to search all available microhabitats. Following completion of the survey, all live mussels from each time period were identified to species, counted, measured, checked for gravidity, and then returned back to the river into the appropriate habitat.

Quantitative surveys using the quadrat sampling method were performed in each selected mesohabitat in the Guadalupe River. The quadrat method provides a more effective means of detecting juveniles and sub-adults of a species, and thus, can provide a more accurate estimate of demographic parameters (Vaughn et al. 1997). At each site (i.e., mesohabitat type), we confined the search boundaries to the specific habitat type, ensuring that the search area did not exceed 150 m^2. Each site was then first surveyed quantitatively and then afterwards surveyed qualitatively using the timed-search method as explained above. The 150 m^2 search area was subdivided into a square meter grid and 20 points were selected within the grid using a random number generator. At each randomly selected point, quadrats were sampled by excavating sediment up to 15 cm in depth using a modified Surber sampler with a 0.25 m^2 search area. Sediment was sieved through 3.175 mm mesh screen, and all live specimens from each quadrat were placed into a mesh bag, which was kept submerged in water until completion of the survey. Following completion of the survey, all live mussels from each quadrat were identified to species, counted, measured, checked for gravidity, and then returned back to the river into the appropriate habitat.

Data Analysis

Scatter plots of abundance (CPUE: number of individuals/total person-hours) vs. river kilometer (RKM) were used to examine the effect of stream position on total mussel and *Q. aurea* abundance in each river. Boxplots and length-frequency histograms were developed for *Q. aurea* to assess demographic patterns and population structure. Generally, multimodal size class distribution may indicate recruitment, whereas truncated distributions (absence of a particular age class; skewed towards large or small individuals) may indicate inconsistent recruitments over time. Bar graphs were also used to visually represent presence of *Q. aurea* by mesohabitat type (i.e., riffle, mid-channel, pool, bank, and backwater).

Results/Discussion

In total, 52 p-h were spent surveying mussels at 13 sites in the Guadalupe River (Figure 1). Overall, we found 377 live individuals of *Q. aurea*, which were found at 10 of 13 (76.9%) sites during both qualitative (n = 307 individuals) and quantitative sampling (n = 70 individuals). Catch-per-unit-effort (CPUE) ranged from 0 to 41.5 mussels/p-h and averaged 5.9 ± 3.2 mussels/p-h (± SE) for *Q. aurea* (Table 1), while CPUE averaged 54.1 ± 25.1 mussels/p-h for all mussels (Figure 2). Relative abundance of *Q. aurea* was 10.9% of all mussels collected within the Guadalupe River. Density of *Q. aurea* in the Guadalupe River ranged from 0 to 12.0 mussels/m^2 and averaged 1.1 ± 0.9 mussels/m^2 (Table 1). In comparison, density measured for all mussels averaged 5.4 ± 3.5 mussels/m^2 (Figure 3). The highest abundance (18.4 mussels/p-h) and density (4.2 mussels/m^2) of *Q. aurea* were observed in pool habitat (Figures 4 and 5). Median shell length for this population was 53 mm and minimum and maximum shell lengths were 7 mm and 90 mm, respectively (Figure 6). Shell length distributions for *Q. aurea* in the Guadalupe River population were left skewed with mussels predominately belonging to larger size classes, although the presence of smaller size-classes indicates recruitment in recent years (Figure 7).

Our findings indicate that *Q. aurea* is present within the Guadalupe River at moderate abundances, particularly in pool habitat. However, a previous study conducted in the Guadalupe River between Gonzales and Cuero, TX (Reach A in Figure 1) in 2014–2015 found a higher average CPUE and density of *Q. aurea* (13.7 ± 4.2 mussels/p-h and 4.0 ± 1.2 mussels/m^2, respectively) at a larger number of sites (*n* = 52 sites) (Tsakiris and Randklev 2016). An Indicator Species Analysis (ISA) of the 2014 data (IV = 0.558, p-value = 0.005, and frequency = 52) indicated that this species occurred primarily in riffle habitat (Randklev, unpublished data). Our current study sampled only one riffle due to elevated water levels and unsafe sampling conditions. Thus, the abundance of *Q. aurea* occurring in the Guadalupe between Cuero and Victoria, TX, is likely higher than what was observed during our survey. Our results, along with previous observations from the Guadalupe River, indicate that *Q. aurea* continues to persist within the Guadalupe River at moderate abundances, primarily in riffle and pool habitats.

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Table 1. Locality and collection information for mussel survey sites in the Guadalupe River drainage. CPUE = total number of *Q. aurea* encountered at each site during qualitative sampling divided by the number of person hours (4) searched at each site. Density = mean number of *Q. aurea* per 0.25 m^2 quadrat. Habitat key: P = pool, BH = Bank, BW = backwater, R = riffle, FPB = front of point bar, BPB = behind point bar. Sites are ordered upstream to downstream.

Site/ Reach	Habitat	Locality	County	Date of collection	Number of live	CPUE	Density /m²	Sub adult	Effort		Area (m²)
									Hours	Quadrats	
1A	P	Guadalupe	DeWitt	8/27/2015	53	12.5	0.6	Y	4	20	150
2A	P	Guadalupe	DeWitt	8/27/2015	5	1.25	0	N	4	20	150
3A	BW	Guadalupe	DeWitt	8/27/2015	15	3.75	0	Y	4	20	150
4B	BH	Guadalupe	Victoria	8/19/2015	13	3	0.2	Y	4	20	150
5B	R	Guadalupe	Victoria	8/25/2015	0	0	0	-	4	20	150
6B	BW	Guadalupe	Victoria	8/19/2015	6	1.25	0.2	N	4	20	150
7B	FPB	Guadalupe	Victoria	8/19/2015	0	0	0	-	4	20	150
8B	P	Guadalupe	Victoria	8/20/2015	226	41.5	12	Y	4	20	150
9B	BH	Guadalupe	Victoria	8/18/2015	3	0.75	0	N	4	20	150
10B	FPB	Guadalupe	Victoria	8/18/2015	0	0	0	-	4	20	150
11B	BPB	Guadalupe	Victoria	8/25/2015	1	0.25	0	N	4	20	150
12B	BPB	Guadalupe	Victoria	8/25/2015	5	1	0.2	N	4	20	150
13B	BW	Guadalupe	Victoria	3/9/2015	50	11.5	0.8	N	4	20	150

Figure 1. Map of Guadalupe River study area. Shaded circles denote sampling locations. Reaches are indicated by letter and correspond to Table 1.

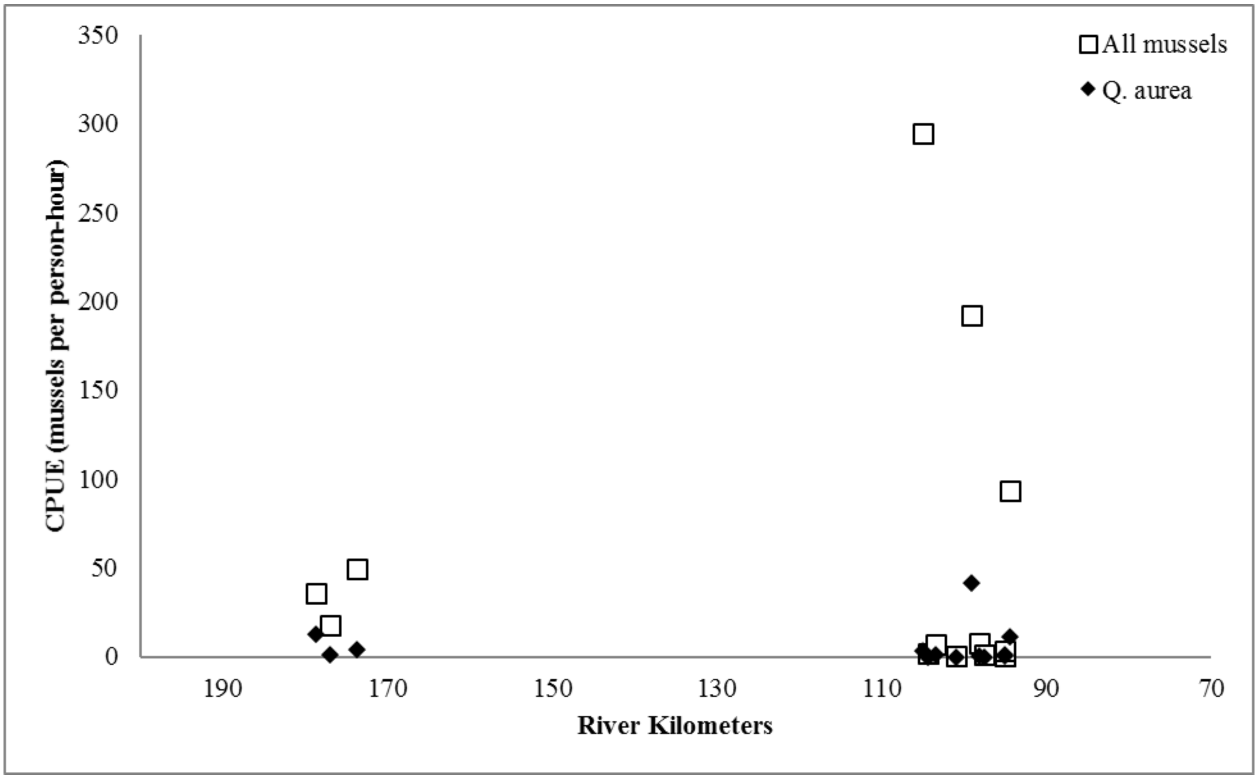

Figure 2. Catch-per-unit effort (CPUE) of *Quadrula aurea* (golden orb) and all mussel species (All mussels) in the Guadalupe River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the San Antonio Bay (0 River Kilometers). CPUE = total number of either *Q. aurea* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

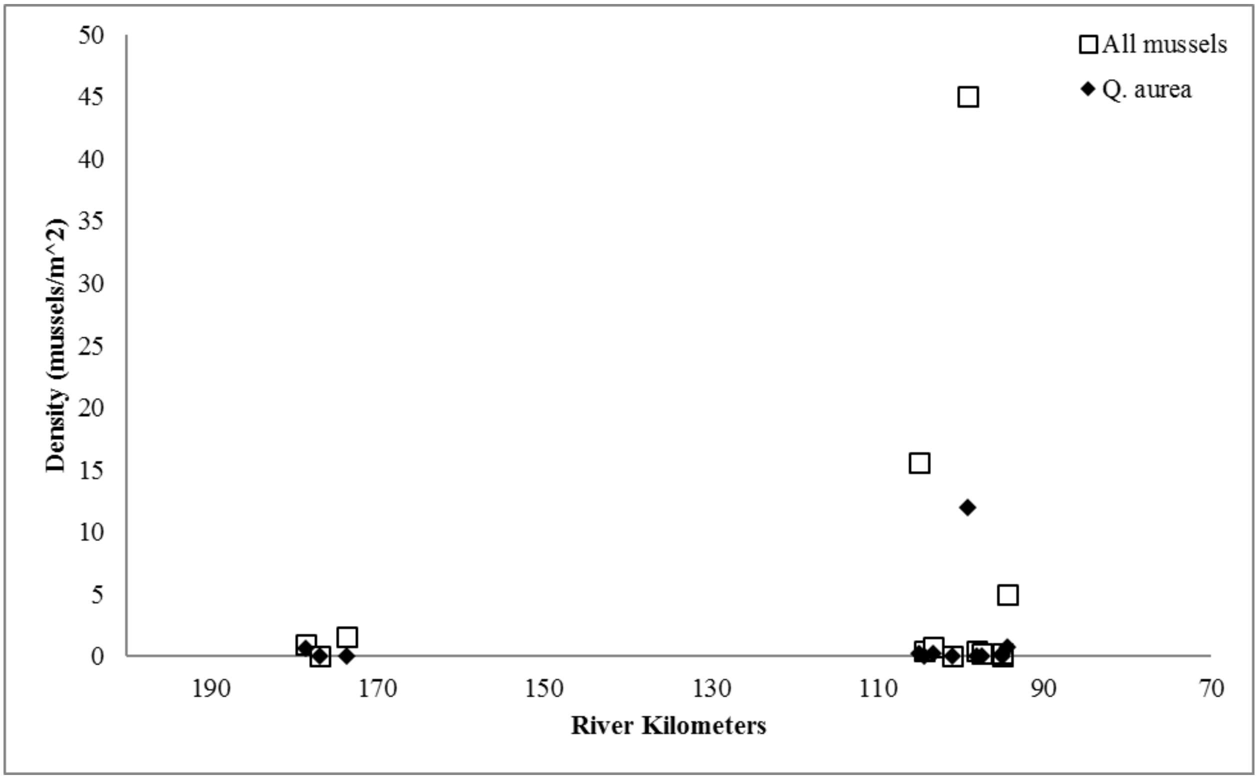

Figure 3. Densities of *Quadrula aurea* (golden orb) and all mussel species (All mussels) in the Guadalupe River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the San Antonio Bay (0 River Kilometers). Density = total number of either *Q. aurea* or all mussels encountered at each site divided by the total area of quadrats searched at each site.

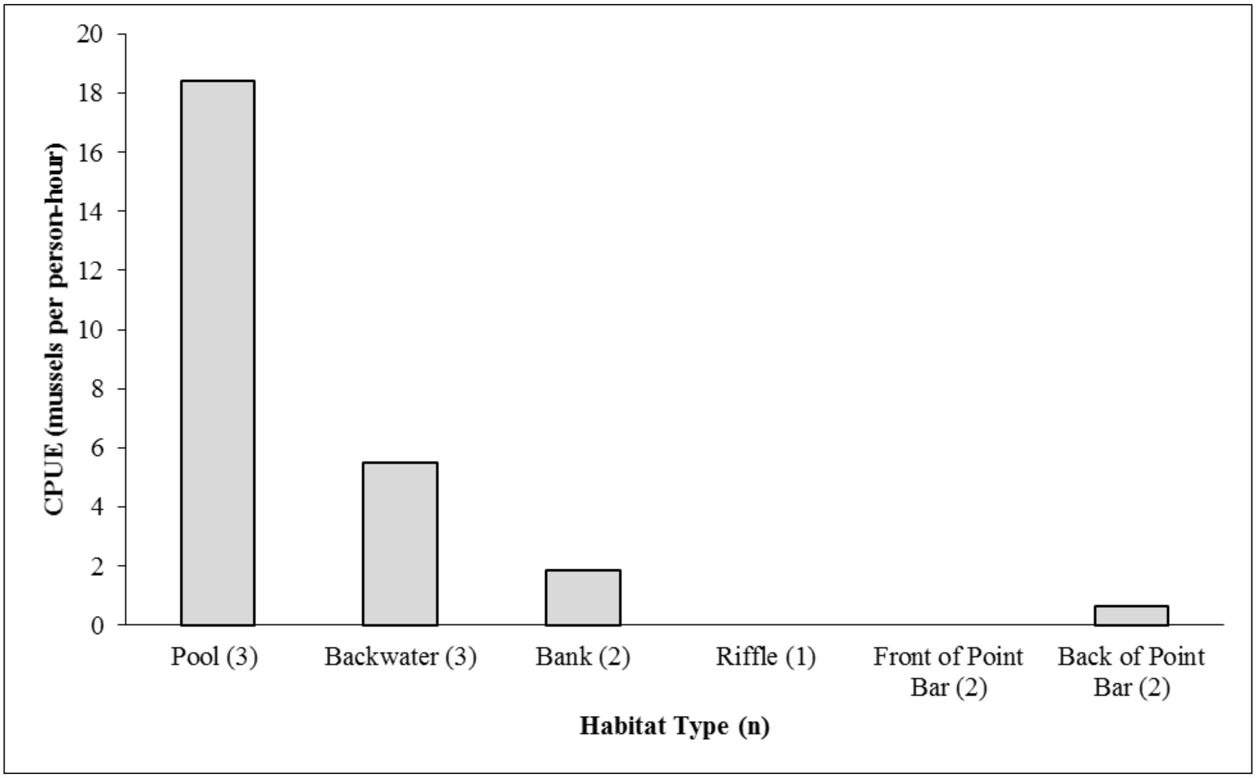

Figure 4. Catch-per-unit-effort (CPUE) of *Quadrula aurea* (golden orb) by mesohabitat type in the Guadalupe River. The number of sites sampled for each habitat are listed in parenthesis.

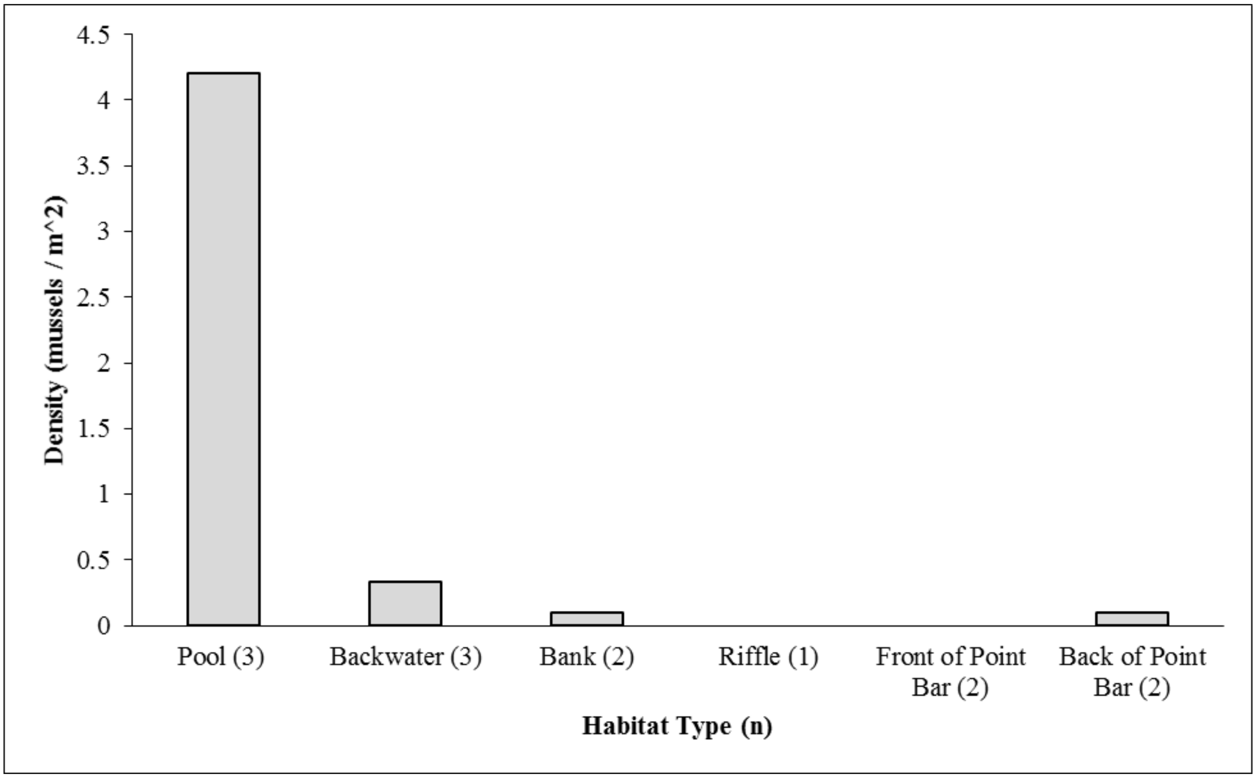

Figure 5. Density of *Quadrula aurea* (golden orb) by mesohabitat type in the Guadalupe River. The number of sites sampled at each habitat are listed in parenthesis.

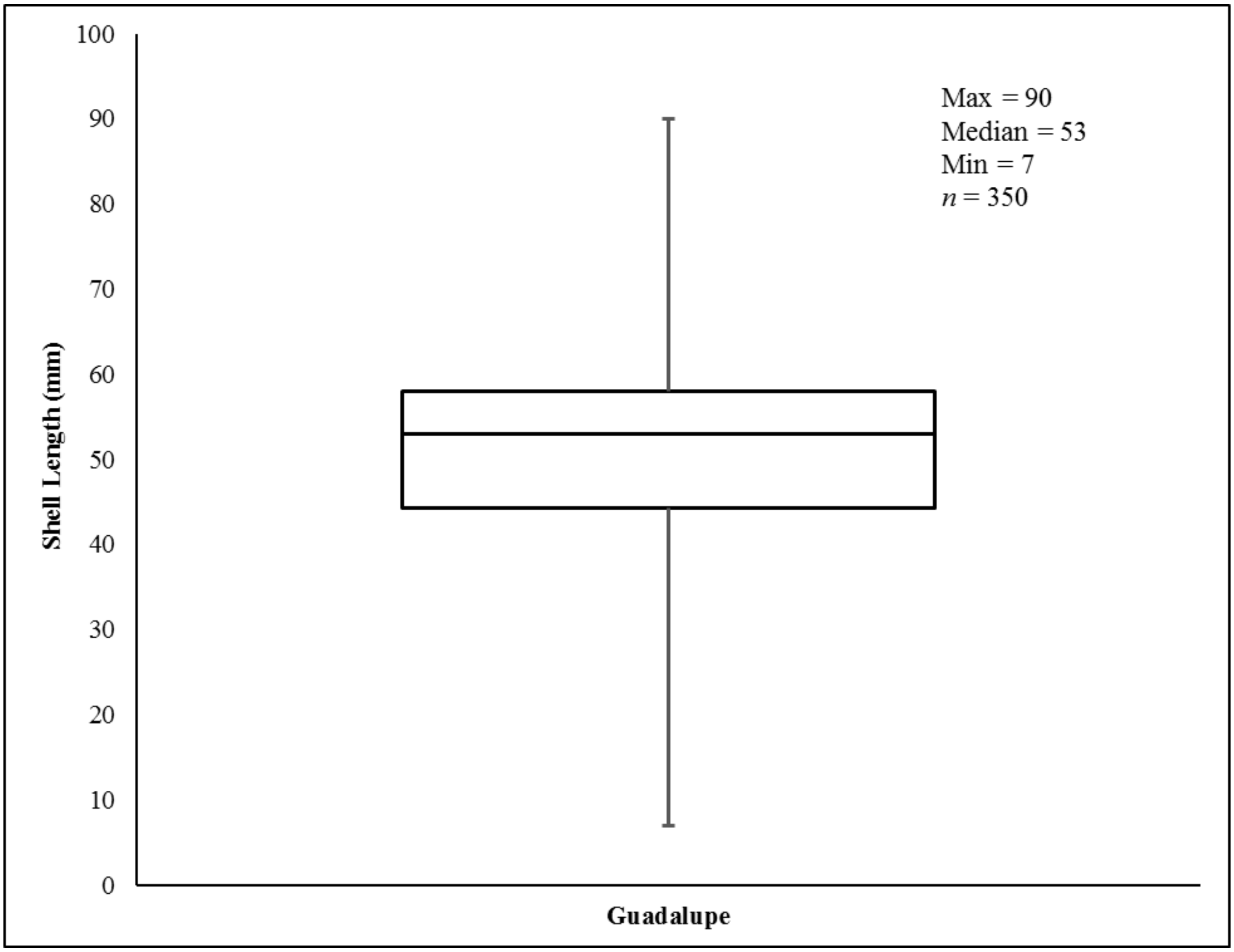

Figure 6. Shell length data of *Quadrula aurea* (golden orb) populations in the Guadalupe River.

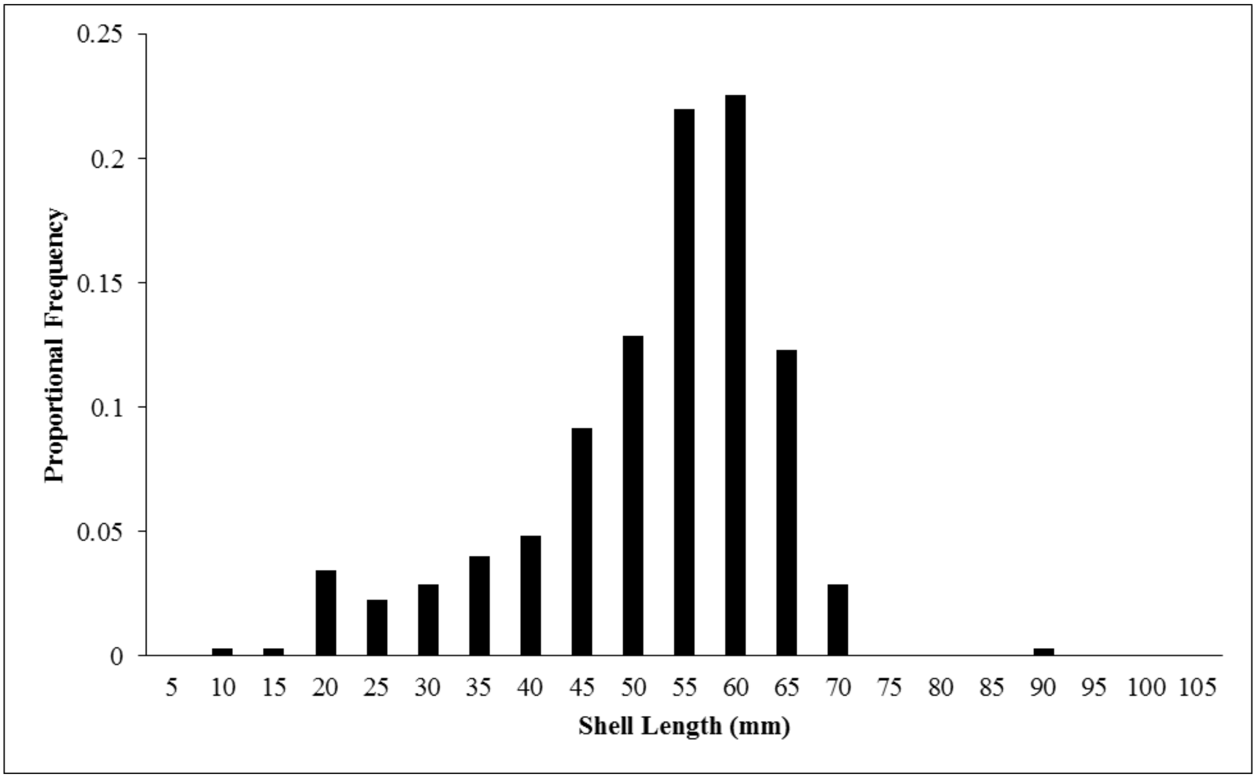

Figure 7. Proportional frequency of shell length of *Quadrula aurea* (golden orb) in the Guadalupe River. Shell lengths are binned into 5 mm groups.

Distribution and Habitat Use for *Quadrula houstonensis* (smooth pimpleback)

Section Summary

The goal of this study was to determine the status, distribution, and mesohabitat associations of *Quadrula houstonensis* (smooth pimpleback), a candidate for protection under the U.S. Endangered Species Act, in Brushy Creek, San Gabriel River, and Little River of the Brazos River drainage and in the San Saba, Llano, and Pedernales rivers of the Colorado River drainage. We used recent and historical data to inform a sampling program in select basins within its presumptive range. In total, we surveyed 117 sites across the Brazos and Colorado River basins, and found 295 live individuals of *Q. houstonensis* from 31 of the 117 (or 24.5%) sites surveyed. In the Brazos River drainage, we surveyed 59 sites across Brushy Creek, San Gabriel River, and Little River and found 282 live individuals. *Quadrula houstonensis* was most abundant and prevalent in the Little River, though it was present in parts of the San Gabriel River and Brushy Creek. The majority of *Q. houstonensis* found in Brushy Creek and the Little River occurred in riffle habitat, while in the San Gabriel River live individuals were mainly observed in midchannel habitats. In the Colorado River drainage, we surveyed 58 sites across the Llano, San Saba, and Pedernales rivers and found a combined total of 13 live *Q. houstonensis* from the San Saba and Llano rivers. In both rivers, live individuals were collected primarily from pool and backwater habitats. No live individuals or shell material of *Q. houstonensis* were observed from the Pedernales River. Size frequency distributions, using shell length as a proxy for age, suggest that some level of recruitment is occurring in the San Saba, Llano, San Gabriel, and Little River populations.

Introduction

Quadrula houstonensis, smooth pimpleback, is known historically from the Brazos and Colorado River drainages of Central Texas (Howells 2010). The type specimen was collected from the Colorado River near Rutersville, TX by C. G. Forshey and described as *Unio houstonensis* by Lea (1859) (USNM_85768). The species was subsequently placed into the genus *Margaron* and then *Quadrula* by Simpson (1900) (Howells 2010). Howells (2002a, 2010) and Turgeon et al. (1998) asserted *Q. houstonensis* is a taxonomically valid species.

In the Brazos River basin, historic records of *Q. houstonensis* have come from both the mainstem of the Brazos River and several large tributaries. *Quadrula houstonensis* has been reported in the Brazos River from the following counties: Austin/Waller, Brazos, Burleson, Falls, Fort Bend, Grimes/Washington, McLennan, Milam, and Robertson. Others have reported *Q. houstonensis* from several tributaries of the Brazos River: Bosque River in McLennan County; Lampasas River in Bell County; Leon River in Bell, Comanche, Coryell, and Hamilton counties; Little Brazos River in Brazos, Milam, and Robertson counties; Navasota River in Brazos, Grimes, Leon/Robertson, Limestone, Madison, and Washington counties; North Bosque River in McLennan County; Sandy Creek in Robertson County; San Gabriel River in Milam County; and Yegua Creek in Burleson/Washington County. A recently dead specimen was found in Belton Reservoir in Bell County (Howells 2001), and a long dead specimen was observed in Lake Whitney in Bosque County (Howells 2000). Live specimens have been collected in Lake Waco in McLennan County (USAO 3743), Lake Brazos in McClennan County (N. Ford pers comm.), and Lake Graham in Young County (Howells 2004).

In the Colorado River basin, historic records of *Q. houstonensis* have come from both the mainstem of the Colorado River and several large tributaries. *Quadrula houstonensis* has been reported in the Colorado River from Bastrop, Burnet, Concho, Fayette, San Saba, Travis, and Wharton counties. It has also been reported in several tributaries of the Colorado River: Llano River in Kimble, Llano, and Mason counties; Pedernales River in Blanco County; Pecan Bayou in Brown and Mills counties; San Saba River in Menard and San Saba counties; and South Concho River in Tom Green County. Specimens have been observed from several reservoirs in Burnet County: Inks Lake (Howells 1994, 1999), Lake LBJ (Howells 2002b), and Lake Marble Falls (Howells 1996b).

Strecker (1931) originally remarked that *Q. houstonensis* was abundant in the Brazos and Colorado River drainages, based on the prevalence of shell material. However, Howells (2010) noted that based on survey data at the time that this species appeared to have declined throughout its range. Currently Texas Parks and Wildlife Department lists *Q. houstonensis* as state-threated (TPWD 2010) and U.S. Fish and Wildlife Service has listed this species as a candidate for protection under the U.S. Endangered Species Act (USFWS 2011). Presently, the American Fisheries Society lists *Q. houstonensis* as endangered (Williams et al. 1993) and NatureServe ranks it as imperiled. Since the 1990s, live and recently dead individuals have been observed in the Brazos River drainage from the Brazos River (Austin, Brazos, Burleson, Falls, Fort Bend, Grimes, McLennan, Robertson, and Waller, Washington counties), Lampasas River (Bell county), Leon River (Bell, Comanche, Coryell, and Hamilton counties), Little Brazos River (Brazos, Milam, and Robertson counties), Navasota River (Brazos, Grimes, Harris, Leon, and

Milam counties), Sandy Creek (Robertson county), and Yegua Creek (Brazos, Burleson, and Washington counties) (Howells 1994, 1995, 1996a, 1996b, 1997, 2001, 2002, 2009, 2010; Karatayev and Burlakova 2008, 2010; Randklev et al. 2010, 2013a, 2013c, 2014, Tsakiris and Randklev 2016, Zara pers. comm.). In the Colorado River drainage, live and recently dead individuals have been observed recently in the Colorado River (Coleman, Colorado, San Saba, Travis, and Wharton counties), Llano River (Llano county), Pecan Bayou (Brown and Mills counties), and San Saba River (San Saba county) (Howells 1994, 1996a, 1996b, 1997, 2000, 2001, 2002, 2009, 2010; Karatayev and Burlakova 2010; Randklev et al. 2013c, Sowards et al. 2013).

Currently, little is known about the life history or reproductive requirements of *Q. houstonensis* (Howells 2010). Like other freshwater mussel species, it is an obligate ectoparasite on one or more host-fish species, and its congeners appear to be short-term brooders that use mantle lures known as mantle magazines to attract fish species from the catfish family, Ictaluridae (Haag 2012, Sietman et al. 2012). Based on recent observations from field surveys throughout *Q. houstonensis'* range, sub-adults and adults appear to occur most often in riffle and run mesohabitats in sand, gravel, and cobble substrates, including gravel-filled cracks in bedrock slabs (Howells 2010, Tsakiris and Randklev 2014). These mesohabitat types appear to serve as flow refuges (*sensu* Strayer 1999), where near bed shear stress remains low during high flow events.

The objectives of this study were to assess the distribution, abundance, and habitat use for *Q. houstonensis* in the Brazos and Colorado drainages. The resulting survey information was then used to develop Conservation Status Assessment Maps for this species within the Brazos and Colorado drainages.

Methods

Study Area

The Brazos River originates near the Texas-New Mexico border, runs southeast for approximately 1,900 km into the Gulf of Mexico, and drains a total of 118,000 km^2 (Kammerer 1990). The Little River, San Gabriel River, and Brushy Creek are all part of the same sub-drainage within the Brazos River system, which drains the Edwards Plateau and Blackland Prairie regions of Central Texas (Rose and Echelle 1981). The Little River system is characterized by clay and fine sediments, woody debris, and generally slow currents (Labay 2010), and land use types within the lower Little River drainage consist of urban and agricultural (Labay 2010). The Little River itself is formed by the confluence of the Leon and Lampasas rivers and flows for 258 km, draining an area of approximately 12,485 km^2 before emptying into the Brazos River (Rose and Echelle 1981). The San Gabriel River begins in Georgetown where the North and South Forks converge and flows east for roughly 80 km until reaching its confluence with the Little River (Belisle and Josselet 1977). The San Gabriel is impounded by Granger Lake, which is a 16.2 km^2 reservoir used primarily for flood control (Mcalister et al. 2013). Brushy Creek originates in Williamson County and flows east for 111 km before emptying into the San Gabriel River near Rockdale (Belisle and Josselet 1977).

The Colorado River originates in northeastern Dawson County, TX, runs approximately 1,040 km to the Gulf of Mexico, and drains an area of 100,000 km^2 (Huser 2000). The San Saba, Llano, and Pedernales rivers are three major tributaries of the upper Colorado River that originate in the Edwards Plateau region (Higgins 2009). The San Saba River begins in Schleicher County and flows 225 km until its confluence with the Colorado River (Belisle and Josselet 1977). The Llano River originates in Kimble County and flows 161 km until emptying into Lake Lyndon B. Johnson, an impoundment on the Colorado River. The Pedernales River also originates in Kimble County and flows approximately 170 km until emptying into Lake Travis, another major impoundment on the Colorado River (Perkin et al. 2010).

Sampling Methods

Survey sites within the Brazos and Colorado River drainages were selected using a stratified random sampling design with the following strata: (1) upstream or downstream of an access point (e.g., bridge crossing); (2) linear distance (river-kilometers) from an access point; and (3) mesohabitat (banks, backwater, mid-channel, riffles, and pools). We first used aerial imagery to delineate mesohabitat between bridge crossings that could be accessed by canoe or motor boat, and then assigned each mesohabitat a unique number. A random number generator was then used to randomly select mesohabitat type and distance from a bridge. For locations where specific habitats could not be identified using satellite imagery (e.g., riffles), we modified our sampling design by surveying the first habitat encountered for that target habitat type. In total, 20 sites in the Llano River, 19 sites in the Pedernales River, and 19 sites in the San Saba River were selected for sampling in the Colorado River basin. In the Brazos River basin, we randomly selected 30 sites in Brushy Creek, 20 sites in the San Gabriel River, and 9 sites in the Little River for sampling. These sites include both sites that have and have not been sampled historically.

Qualitative surveys using the timed search method were performed at each site (i.e., mesohabitat type). The timed search method was chosen because it provides a more effective means of detecting rare species than quantitative sampling methodologies (Vaughn et al. 1997). At each site, we confined the search boundaries within the randomly selected mesohabtiat and standardized the search area to 150 m^2, though in some cases the search area included multiple mesohabitat types (e.g., pool-run or riffle-run habitats). Each site was surveyed tactilely and visually for a total of 4 person-hours (p-h). Because our focus was to determine the amount of effort needed to detect *Q. houstonensis* (which will be important for designing long-term monitoring programs), we divided the total search time into 4, 1 p-h intervals. At the end of each search interval, surveyors combined all live specimens into a mesh bag, which was kept submerged in water until completion of the survey. During each interval, surveyors were spread out in the search area and every effort was made to search all available microhabitats. Following completion of the survey, all live mussels from each time period were identified to species, counted, measured, checked for gravidity, and then returned back to the river into the appropriate habitat.

Quantitative surveys using the quadrat sampling method were performed in each mesohabitat in the Little River, and a portion of the sites in the San Gabriel River. The quadrat method provides a more effective means of detecting juveniles, and thus, can estimate demographic parameters in areas with high abundance more accurately (Vaughn et al. 1997). For quantitative sampling, we subdivided the 150 m^2 search area into a square meter grid and 20 points were selected within the grid using a random number generator. At each randomly selected point, quadrats were sampled by excavating sediment up to 15 cm in depth using a modified Surber sampler with a 0.25 m^2 search area. Sediment was sieved through 3.175 mm mesh screen, and all live specimens from each quadrat were placed into a mesh bag, which was kept submerged in water until completion of the survey. Following completion of the survey, all live mussels from each quadrat were identified to species, counted, measured, checked for gravidity, and then returned to the river into the appropriate habitat. For sites where both sampling methods were used, quantitative sampling occurred prior to the timed-search method as explained above.

Data Analysis

We estimated catch-per-unit-effort (CPUE: number of individuals/total p-h) and density (number of individuals/m^2) of mussels for each site from qualitative and quantitative data, respectively. Total abundance (number of individuals) and relative abundance (number of individuals of a species/total number of individuals of all species) were calculated for each river. Scatter plots of CPUE vs. river kilometer were used to examine the relationship between stream position and both abundance of *Q. houstonensis* and all mussel species. Shell lengths of *Q. houstonensis* were plotted using boxplots and length-frequency histograms to examine demographic patterns and population structure. Generally, multimodal size class distribution may indicate recruitment, whereas truncated distributions (absence of a particular age class, large, or small individuals) may indicate a lack of recent recruitment or a localized extinction event (Miller and Payne 1993, Haag and Warren 2007). Bar graphs were also used to visually represent presence of *Q. houstonensis* by mesohabitat type (i.e., riffle, mid-channel, pool, bank, and backwater).

Results/Discussion

Brazos River Basin

In total, 236 p-h were spent surveying mussels across 59 sites in Brushy Creek, San Gabriel River, and Little River of the Brazos River drainage (Figure 1). Overall, we found 282 live individuals of *Q. houstonensis*, which were found at 7 of 30 (11.4%) sites in Brushy Creek (n = 32 individuals), 11 of 20 (55.0%) sites in the San Gabriel River (n = 28 individuals), and 9 of 9 (100%) sites in the Little River (n = 221 individual). Catch-per-unit-effort (CPUE) of *Q. houstonensis* ranged from 0 to 16.75 mussels/p-h across rivers and averaged 0.27 ± 0.18 mussels/p-h (± SE) in Brushy Creek, 0.30 ± 0.11 mussels/p-h in the San Gabriel River, and 5.17 ± 1.83 mussels/p-h in the Little River (Table 1). In comparison, CPUE measured for all mussels averaged 1.48 ± 0.72 mussels/p-h in Brushy Creek (Figure 2), 1.81 ± 0.37 mussels/p-h in the San Gabriel (Figure 3), and 7.67 ± 2.29 mussels/p-h in the Little River (Figure 4). Thus, relative abundance of *Q. houstonensis* was highest in the Little River (67.4%), followed by Brushy Creek (16.1%) and the San Gabriel River (3.6%). Similarly, density of *Q. houstonensis* was highest in the Little River (0.78 ± 0.52 mussels/m^2), followed by the San Gabriel River (0.10 ± 0.04 mussels/m^2; Table 1). Mussel density measured for all mussels averaged 0.36 ± 0.05 and 1.16 ± 0.73 mussels/m^2 in the San Gabriel and Little Rivers, respectively (Figures 5 and 6). However, it is important to note that quantitative sampling only occurred at a portion of the sites in the San Gabriel River and did not occur in Brushy Creek. Finally, data analyzed by habitat type indicated average CPUE and density of *Q. houstonensis* in the Little River (14.00 mussels/p-h and 3.75 mussels/m², respectively) and CPUE in Brushy Creek (1.25 mussels/p-h) was highest in riffles (Figures 7, 8, and 9). In contrast, CPUE (0.81 mussels/p-h) and density (0.38 mussels/m²) of *Q. houstonensis* in the San Gabriel River was highest in mid-channel habitats (Figures 10 and 11). Median shell length for populations of *Q. houstonensis* from Brushy Creek (37 mm), San Gabriel River (39 mm), and Little River (43 mm) were similar. However, minimum and maximum lengths varied across rivers with Brushy Creek ranging from 29 to 51 mm, San Gabriel River ranging from 15 to 62 mm, and the Little River ranging from 12 to 68 mm (Figure 12). Length-frequency distributions using shell length as a proxy for age indicate that recruitment is occurring in the Little and San Gabriel rivers (Figure 13).

Prior to this survey, abundance of *Q. houstonensis* in the Little River had only been reported from one site near the confluence with the Brazos River (0.40 mussels/p-h). Our findings confirmed that *Q. houstonensis* persists in the Brazos River drainage and appears to be relatively abundant. In addition to our results, recent surveys have found relatively high abundances of *Q. houstonensis* within other nearby tributaries of the Brazos River. In Yegua Creek, Tsakiris and Randklev (2016) documented an average CPUE of 8.97 mussels/p-h for *Q. houstonensis* across 53 sites. Other investigators have noted a high number of *Q. houstonensis* in Yegua Creek, though a limited number of sites were surveyed during these efforts (Howells 1999, 2001, Karatayev and Burlakova 2008, Randklev et al. 2010). In the Navasota River, Randklev et al. (2010) found high density of *Q. houstonensis* (9.70 mussels/m^2) across 3 sites, and Karatayev and Burlakova (2008) found high abundance of *Q. houstonensis* (1.78 mussels/p-h) across 4 sites within the same river. Randklev et al. (2013a) surveyed the Leon River, a tributary of the Little River, and found a relatively high average abundance of *Q. houstonensis* (3.94 mussels/p-h) upstream of Belton Lake; below this reservoir and upstream of Lake Proctor the authors reported this species as absent or rare. Within the mainstem of the Brazos River, Karatayev and Burlakova (2008) reported high abundances for *Q. houstonensis* from 18 sites between Palo Pinto and Fort Bend counties (2.2 mussels/p-h); however, the middle reaches of the Brazos River near College Station, TX were reported as being a hotspot for this species. In the lower Brazos River, between Sealy, TX, to the Fort Bend/Brazoria County line, Randklev et al. (2014) reported low abundances of *Q. houstonensis* (0.60 ± 0.27 mussels/p-h) across a larger number of sites (n = 92 sites). In the upper Brazos River, there are only two recent accounts of live individuals or shell material of *Q. houstonensis* (Howells 1996a, Karatayev and Burlakova 2008). Taken together these data indicate that *Q. houstonensis* continues to persist throughout most of the Brazos River basin and is most abundant in the middle reaches of the Brazos River and its associated tributaries, including Yegua Creek and the Little and Navasota Rivers (Karatayev and Burlakova 2008, Randklev et al. 2010, Tsakiris and Randklev 2016).

Colorado River Basin

In total, 232 p-h were spent surveying mussels at 58 sites in the San Saba, Llano, and Pedernales rivers of the Colorado River basin of Texas (Figure 14). Overall, we found 13 live individuals of *Q. houstonensis*, which were found at 2 of 19 (10.5%) sites in the San Saba River (n = 8 individual) and 2 of 20 (10.0%) sites in the Llano River (n = 5 individuals). No live individuals or shell material of *Q. houstonensis* were found in the Pedernales River. *Quadrula houstonensis* is only known from the Pedernales River from a single weathered shell collected in 1985 (USAO

4254), which has not been verified, and so there is little to no evidence that this species ever occurred in this river. Catch-per-unit-effort (CPUE) of *Q. houstonensis* ranged from 0 to 1.75 mussels/p-h across sites and averaged 0.11 ± 0.09 mussels/hr (± SE) in the San Saba River and 0.06 ± 0.04 mussels/p-h in the Llano River (Table 2). In comparison, CPUE measured for all mussels averaged 2.41 ± 0.66 and 0.95 ± 0.43 mussels/p-h in the San Saba and Llano Rivers, respectively (Figures 15 and 16). In both rivers, relative abundance of *Q. houstonensis* was low (4.4 % and 6.6 % of all mussels collected in the San Saba and Llano Rivers, respectively). Finally, data analyzed by habitat type indicated average CPUE of *Q. houstonensis* was highest in backwaters in the San Saba River (0.875 mussels/p-h; Figure 17) and pools in the Llano River (0.10 mussels/p-h; Figure 18). Median shell lengths for populations of *Q. houstonensis* from the San Saba River and Llano River was 33 and 53 mm, and minimum and maximum shell were 21 and 34 mm and 27 and 58 mm, respectively (Figures 19). Length-frequency distributions of the San Saba and Llano Rivers were bimodal and intermediate size classes were absent. However, small size classes (<30 mm) were present in both rivers, suggesting some level of recruitment (Figure 20).

Our findings indicate that *Q. houstonensis* continues to persist within the San Saba and Llano rivers at low abundances. In addition to our results, recent surveys conducted within the Colorado River drainage have also yielded accounts of live individuals. In the lower Colorado River (in Colorado and Wharton counties), Burlakova and Karatayev (2010) found live individuals at densities ranging from 0.31 ± 1.1 to 1.33 ± 2.3 mussels/m^2. Live mussels were also present at a site on the middle Colorado River (San Saba County) (Braun et al. 2014). In the lower San Saba River, Tsakiris et al. (2014) collected mussels across two sites and documented an average CPUE of 2.3 mussels/p-h for *Q. houstonensis* and 8.9 mussels/p-h for all mussels, which indicates higher relative abundance in this part of the river. Our results combined with those from recent surveys indicate that *Q. houstonensis* occurs at low abundance in the middle and lower Colorado River as well as the San Saba and Llano Rivers.

In summary, our results demonstrate that *Q. houstonensis* continues to persist within Brushy Creek, San Gabriel River, and Little River of the Brazos River drainage and the Llano and San Saba rivers of the Colorado River drainage. Our results, along with observations within the past 15 years (Karatayev and Burlakova 2008, Burlakova and Karatayev 2010, Howells 2010, Randklev et al. 2013a, 2013b, 2014, Braun et al. 2014, Tsakiris and Randklev 2014, 2016), indicate that *Q. houstonensis* occurs in moderate abundance in the Little River and other tributaries of the Brazos River and low abundance in the Llano and San Saba rivers. In the present surveys, no live individuals or shell material of *Q. houstonensis* were observed from the Pedernales River. The only record of *Q. houstonensis* from the river is a weathered shell collected in 1985, which raises the question as to whether this species historically occurred in the Pedernales River. In our study, *Q. houstonensis* was collected in a variety of habitat types depending on the river system, which indicates this species may be a habitat generalist. Within both drainages, size frequency distributions using shell length as a proxy for age show recruitment is occurring in the Little, San Gabriel, San Saba, and Llano rivers, though it is unknown whether it is sufficient to ensure long-term persistence in these rivers.

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Table 1. Locality and collection information for mussel survey sites in the Brazos River drainage. CPUE = total number of *Q. houstonensis* encountered at each site during qualitative sampling divided by the number of person hours (4) searched at each site. Density=mean number of *Q. houstonensis* per 0.25 m^2 quadrat. Habitat key: B = Bank, BW = Backwater, MC = Mid-Channel, P = Pool, R = Riffle. Sites are ordered upstream to downstream.

Site/ Reach	Habitat	Locality	County	Date of collection	Number of Live	CPUE	Density	Sub Adult	Effort		Area (m2)
									Hours	Quadrats	
1A	B	Brushy Creek	Williamson	7/13/2015	0	0	n/a	-	4	0	150
2A	MC	Brushy Creek	Williamson	7/13/2015	2	0.5	n/a	N	4	0	150
3A	P	Brushy Creek	Williamson	7/14/2015	1	0.25	n/a	N	4	0	150
4A	MC	Brushy Creek	Williamson	7/14/2015	0	0	n/a	-	4	0	150
5A	P	Brushy Creek	Williamson	7/14/2015	0	0	n/a	-	4	0	150
6A	B	Brushy Creek	Williamson	7/14/2015	0	0	n/a	-	4	0	150
7A	MC	Brushy Creek	Williamson	7/16/2015	0	0	n/a	-	4	0	150
8A	BW	Brushy Creek	Williamson	7/16/2015	0	0	n/a	-	4	0	150
9A	R	Brushy Creek	Williamson	7/16/2015	0	0	n/a	-	4	0	150
10A	BW	Brushy Creek	Williamson	7/16/2015	0	0	n/a	-	4	0	150
11B	R	Brushy Creek	Williamson	7/15/2015	0	0	n/a	-	4	0	150
12B	B	Brushy Creek	Williamson	7/15/2015	0	0	n/a	-	4	0	150
13B	R	Brushy Creek	Williamson	7/15/2015	0	0	n/a	-	4	0	150
14B	MC	Brushy Creek	Williamson	7/15/2015	0	0	n/a	-	4	0	150
15B	P	Brushy Creek	Williamson	7/15/2015	0	0	n/a	-	4	0	150
16B	B	Brushy Creek	Milam	7/15/2015	0	0	n/a	-	4	0	150
17B	B	Brushy Creek	Milam	7/15/2015	0	0	n/a	-	4	0	150
18B	MC	Brushy Creek	Milam	7/16/2015	1	0.25	n/a	Y	4	0	150
19B	P	Brushy Creek	Milam	7/16/2015	0	0	n/a	-	4	0	150
20B	B	Brushy Creek	Milam	7/16/2015	0	0	n/a	-	4	0	150
21C	R	Brushy Creek	Milam	7/7/2015	22	5.5	n/a	Y	4	0	150
22C	P	Brushy Creek	Milam	7/7/2015	1	0.25	n/a	N	4	0	150
23C	R	Brushy Creek	Milam	7/10/2015	3	0.75	n/a	Y	4	0	150
24C	B	Brushy Creek	Milam	7/7/2015	0	0	n/a	-	4	0	150
25C	BW	Brushy Creek	Milam	7/6/2015	2	0.5	n/a	Y	4	0	100
26C	P	Brushy Creek	Milam	7/10/2015	0	0	n/a	-	4	0	150
27C	MC	Brushy Creek	Milam	7/7/2015	0	0	n/a	-	4	0	150
28C	BW	Brushy Creek	Milam	7/7/2015	0	0	n/a	-	4	0	150
29C	MC	Brushy Creek	Milam	7/10/2015	0	0	n/a	-	4	0	150
30C	B	Brushy Creek	Milam	7/6/2015	0	0	n/a	-	4	0	100
31F	BW	Little	Milam	4/28/2015	3	0.75	0	N	4	20	105
32F	R	Little	Milam	4/28/2015	69	11.25	4.8	Y	4	20	100
33F	BW	Little	Milam	4/29/2015	25	5.75	0.4	Y	4	20	140
34F	MC	Little	Milam	4/29/2015	7	1.75	0	Y	4	20	100
35F	P	Little	Milam	4/29/2015	1	0.25	0	N	4	20	100
36F	R	Little	Milam	4/30/2015	73	16.75	1.2	Y	4	20	104
37F	B	Little	Milam	4/30/2015	21	5	0.2	Y	4	20	100
38F	MC	Little	Milam	4/30/2015	9	2	0.2	N	4	20	100
39F	B	Little	Milam	4/30/2015	13	3	0.2	N	4	20	100
40D	MC	San Gabriel	Williamson	7/8/2015	5	1.25	n/a	N	4	0	150
41D	B	San Gabriel	Williamson	7/8/2015	1	0.25	n/a	N	4	0	150
42D	BW	San Gabriel	Williamson	7/8/2015	1	0.25	n/a	N	4	0	150

Table 1. Continued.

Site/ Reach	Habitat	Locality	County	Date of collection	Number of Live	CPUE	Density	Sub Adult	Effort		Area (m2)
									Hours	Quadrats	
43D	P	San Gabriel	Williamson	7/9/2015	0	0	n/a	-	4	0	150
44D	MC	San Gabriel	Williamson	7/8/2015	2	0.5	n/a	N	4	0	150
45D	BW	San Gabriel	Williamson	7/9/2015	0	0	n/a	-	4	0	150
46D	R	San Gabriel	Williamson	7/9/2015	1	0.25	n/a	N	4	0	150
47D	B	San Gabriel	Williamson	7/8/2015	0	0	n/a	-	4	0	150
48D	R	San Gabriel	Williamson	7/9/2015	0	0	n/a	-	4	0	150
49D	P	San Gabriel	Williamson	7/9/2015	0	0	n/a	-	4	0	150
50E	BW	San Gabriel	Milam	4/27/2015	0	0	0	-	4	20	100
51E	P	San Gabriel	Milam	4/20/2015	0	0	0	-	4	20	90
52E	MC	San Gabriel	Milam	4/20/2015	8	1.25	0.6	Y	4	20	100
53E	B	San Gabriel	Milam	4/21/2015	1	0	0.2	N	4	20	90
54E	R	San Gabriel	Milam	4/21/2015	0	0	0	-	4	20	100
55E	R	San Gabriel	Milam	4/22/2015	0	0	0	-	4	20	100
56E	MC	San Gabriel	Milam	4/23/2015	1	0.25	0	N	4	20	90
57E	BW	San Gabriel	Milam	4/22/2015	7	1.75	0	N	4	20	90
58E	P	San Gabriel	Milam	4/27/2015	1	0	0.2	N	4	20	100
59E	B	San Gabriel	Milam	4/23/2015	1	0.25	0	N	4	20	100

Table 2. Locality and collection information for mussel survey sites in the Colorado River drainage. CPUE = total number of *Q. houstonensis* encountered at each site during qualitative sampling divided by the number of person hours (4) searched at each site. Density = mean number of *Q. houstonensis* per 0.25 m^2 quadrat. Habitat key: P = pool, BH = Bank, BW = backwater, R = riffle, FPB = front of point bar, BPB = behind point bar. Sites are ordered upstream to downstream.

Site/ Reach	Habitat	Locality	County	Date of collection	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m2)
1A	B	San Saba	Menard	8/5/2015	0	0	-	4	150
2A	P	San Saba	Menard	8/5/2015	0	0	-	4	150
3A	P	San Saba	Menard	8/5/2015	0	0	-	4	150
4A	B	San Saba	Menard	8/3/2015	0	0	-	4	150
5A	P	San Saba	Menard	8/4/2015	0	0	-	4	150
6A	P	San Saba	Menard	8/4/2015	0	0	-	4	150
7A	BW	San Saba	Menard	8/4/2015	0	0	-	4	150
8A	B	San Saba	Menard	8/4/2015	0	0	-	4	150
9A	B	San Saba	Menard	8/5/2015	0	0	-	4	150
10A	P	San Saba	Menard	8/5/2015	0	0	-	4	150
11B	R	San Saba	Menard	8/6/2015	1	1	Y	4	150
12B	B	San Saba	Menard	8/6/2015	0	0	-	4	150
13B	BW	San Saba	Menard	8/6/2015	7	0	Y	4	150
14B	P	San Saba	Menard	8/6/2015	0	0	-	4	150
15B	B	San Saba	Menard	8/6/2015	0	0	-	4	150
16C	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
17C	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
18C	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
19C	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
20D	P	Llano	Mason	7/30/2015	3	2	Y	4	150
21D	PR	Llano	Mason	7/30/2015	2	5	Y	4	150
22D	PR	Llano	Mason	7/30/2015	0	0	-	4	150
23D	PR	Llano	Mason	7/30/2015	0	1	-	4	150
24D	B	Llano	Mason	7/30/2015	0	0	-	4	150
25D	PR	Llano	Mason	7/29/2015	0	0	-	4	150
26D	B	Llano	Mason	7/29/2015	0	0	-	4	150
27D	P	Llano	Mason	7/31/2015	0	0	-	4	150
28E	B	Llano	Llano	7/29/2015	0	0	-	4	150
29E	P	Llano	Llano	7/29/2015	0	0	-	4	150
30E	B	Llano	Llano	7/29/2015	0	0	-	4	150
31E	P	Llano	Llano	7/29/2015	0	0	-	4	150
32E	P	Llano	Llano	7/28/2015	0	0	-	4	150
33E	B	Llano	Llano	7/28/2015	0	0	-	4	150
34E	B	Llano	Llano	7/28/2015	0	0	-	4	150
35E	PR	Llano	Llano	7/28/2015	0	0	-	4	150
36F	P	Llano	Llano	7/27/2015	0	0	-	4	150
37F	B	Llano	Llano	7/28/2015	0	0	-	4	150
38F	PR	Llano	Llano	7/27/2015	0	0	-	4	150
39F	B	Llano	Llano	7/27/2015	0	0	-	4	150
40G	P	Live Oak Creek	Gillespie	6/24/2015	0	0	-	4	150
41G	P	Live Oak Creek	Gillespie	6/24/2015	0	0	-	4	150
42G	All	Live Oak Creek	Gillespie	6/23/2015	0	0	-	4	150
43G	All	Live Oak Creek	Gillespie	6/23/2015	0	0	-	4	150

Table 2. Continued.

Site Reach	Habitat	Locality	County	Date of collection	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m2)
44G	B	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
45G	P	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
46G	B	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
47G	B	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
48G	BW	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
49H	MC	Pedernales	Gillespie	6/23/2015	0	0	-	4	150
50H	B	Pedernales	Gillespie	6/23/2015	0	0	-	4	150
51H	RR	Pedernales	Blanco	6/23/2015	0	0	-	4	150
52H	B	Pedernales	Blanco	6/23/2015	0	0	-	4	150
53H	BW	Pedernales	Blanco	6/23/2015	0	0	-	4	150
54I	P	Pedernales	Blanco	6/22/2015	0	0	-	4	150
55I	RR	Pedernales	Blanco	6/22/2015	0	0	-	4	150
56I	P	Flat Creek	Blanco	6/25/2015	0	0	-	4	150
57I	BW	Pedernales	Travis	6/25/2015	0	0	-	4	150
58I	B	Pedernales	Travis	6/25/2015	0	0	-	4	150

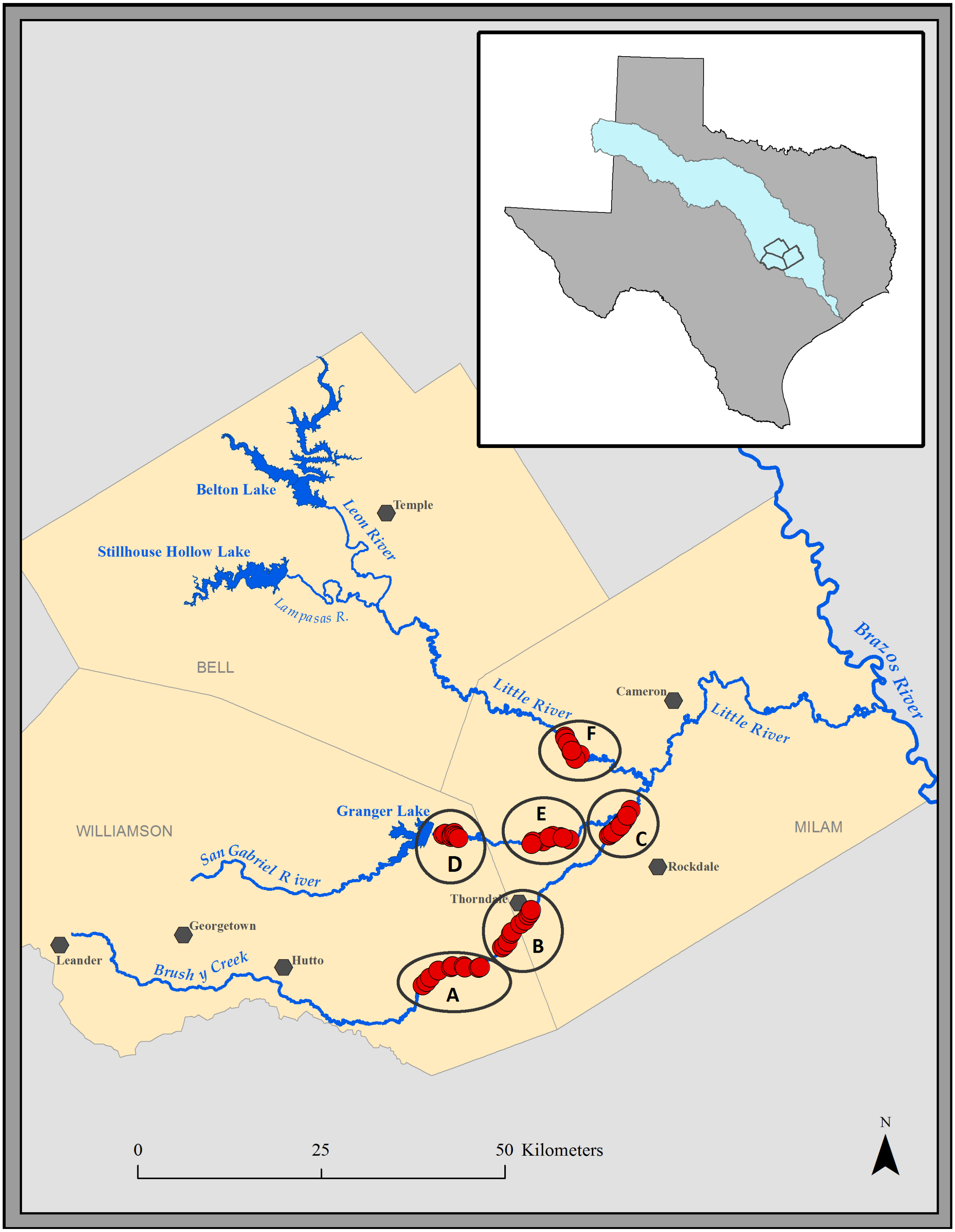

Figure 1. Map of Brazos drainage study area. Shaded circles denote sampling locations. Reaches are indicated by letter and correspond to Table 1.

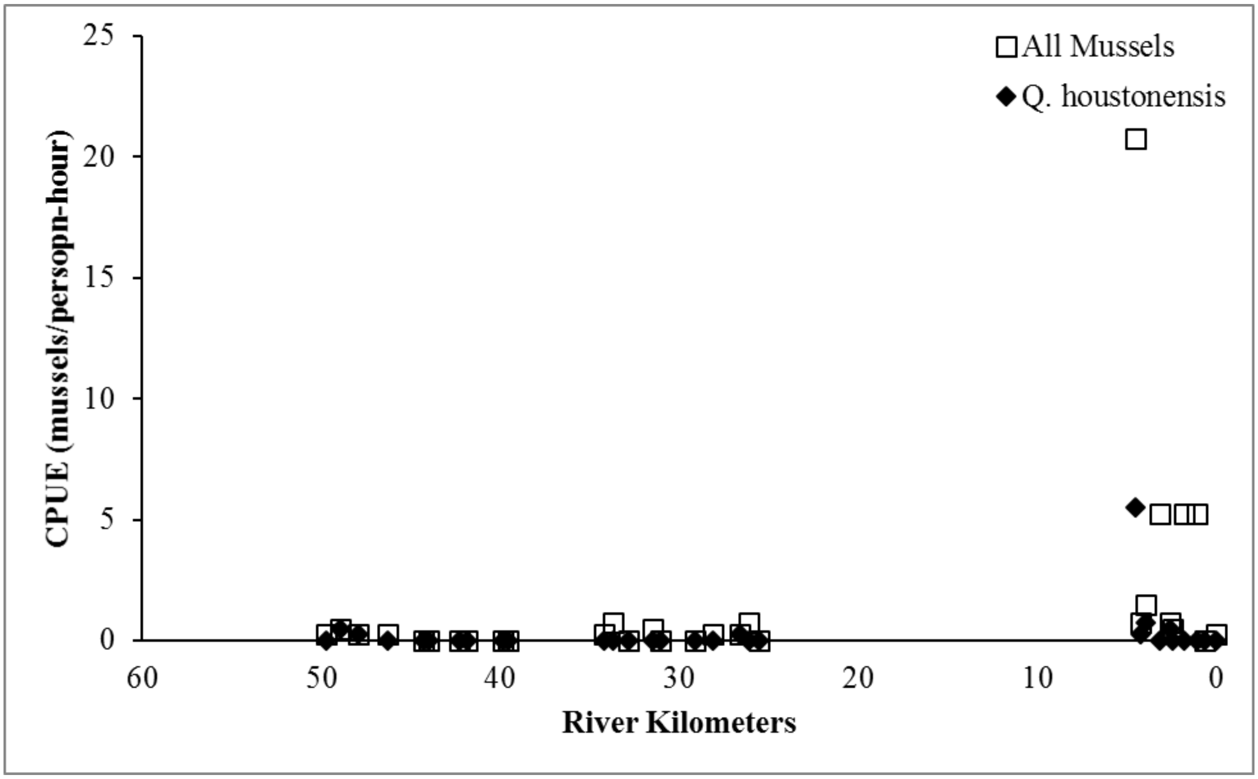

Figure 2. Catch-per-unit effort (CPUE) of *Quadrula houstonensis* (smooth pimpleback) and all mussel species (All mussels) in Brushy Creek. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the San Gabriel River (0 River Kilometers). CPUE = total number of either *Q. houstonensis* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

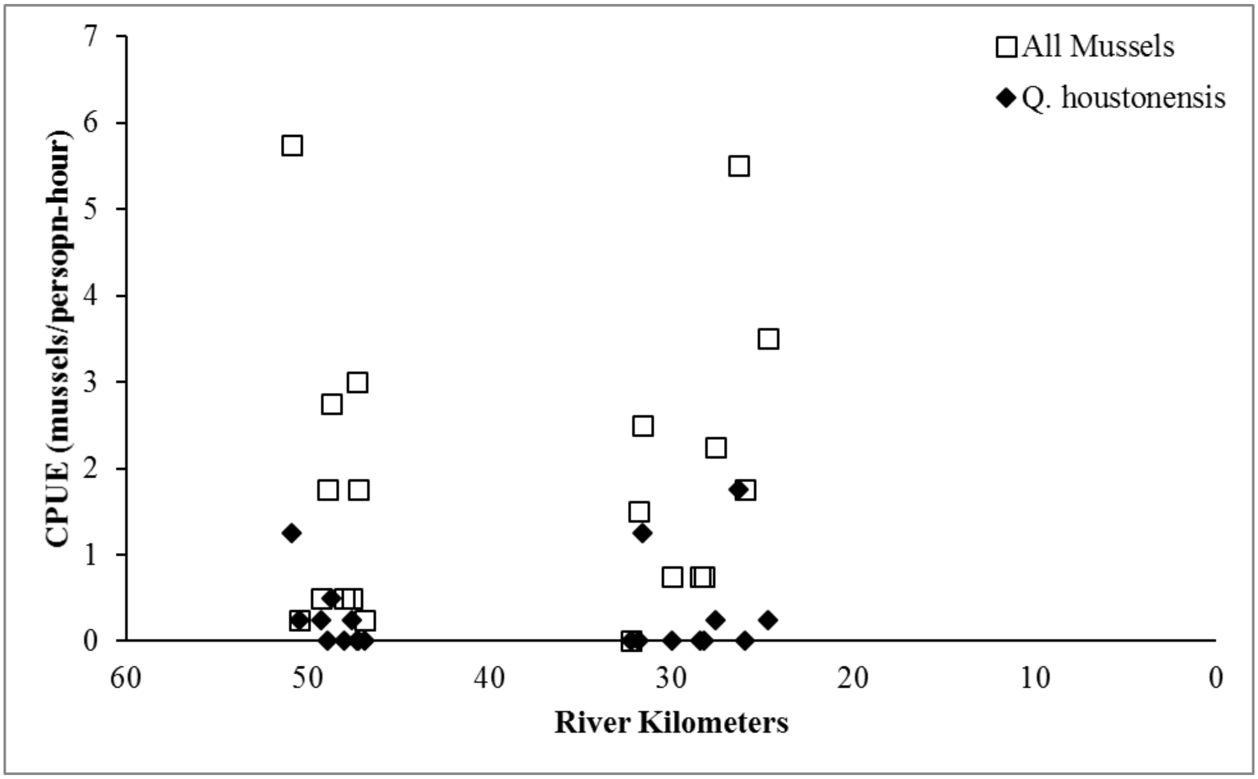

Figure 3. Catch-per-unit effort (CPUE) of *Quadrula houstonensis* (smooth pimpleback) and all mussel species (All mussels) in the San Gabriel River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Little River (0 River Kilometers). CPUE = total number of either *Q. houstonensis* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

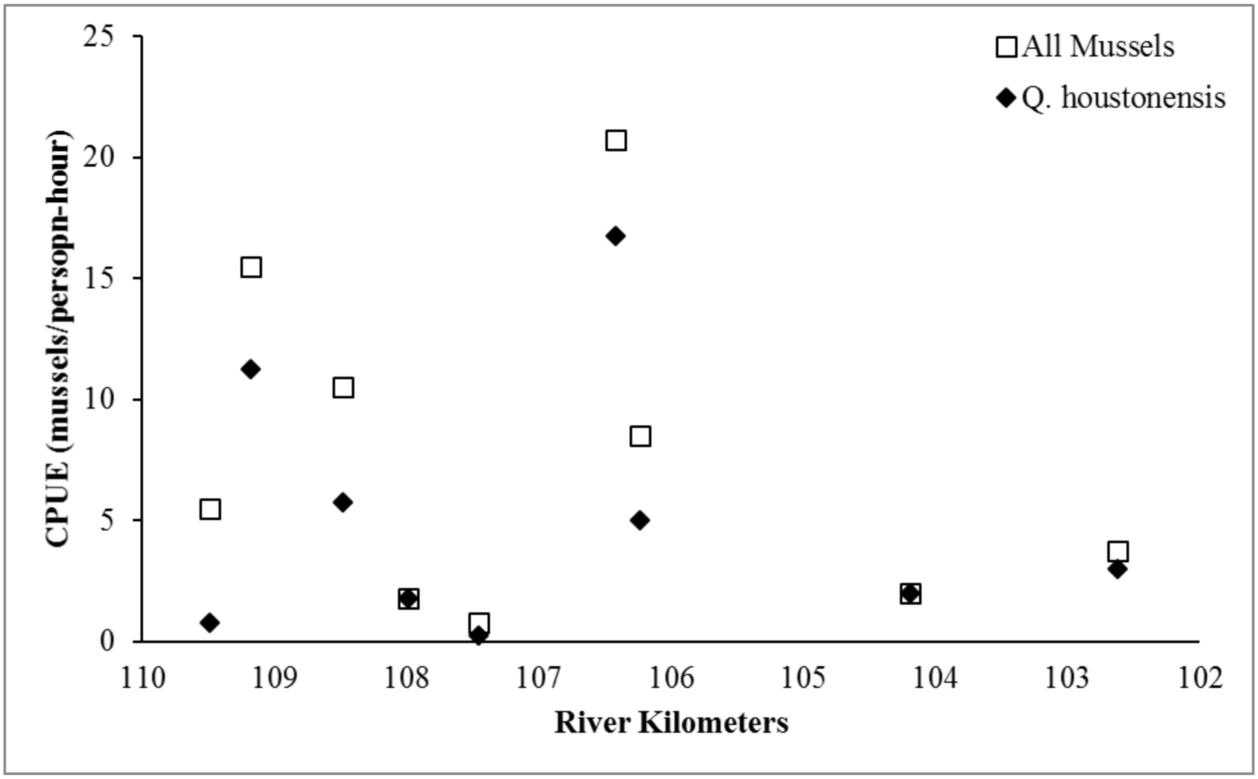

Figure 4. Catch-per-unit effort (CPUE) of *Quadrula houstonensis* (smooth pimpleback) and all mussel species (All mussels) in the Little River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Brazos River (0 River Kilometers). CPUE = total number of either *Q. houstonensis* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

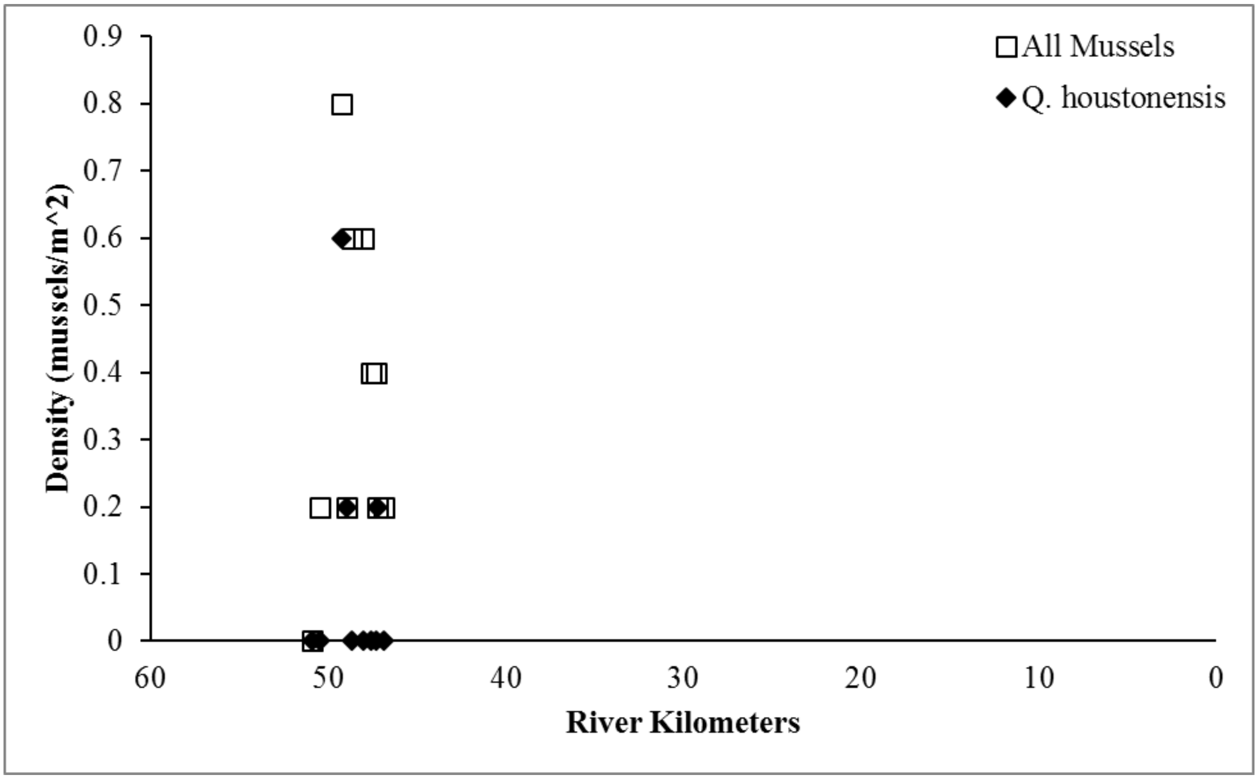

Figure 5. Densities of *Quadrula houstonensis* (smooth pimpleback) and all mussel species (All mussels) in the San Gabriel River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the Little River (0 River Kilometers). Density = total number of either *Q. houstonensis* or all mussels encountered at each site divided by the total area of quadrats searched at each site.

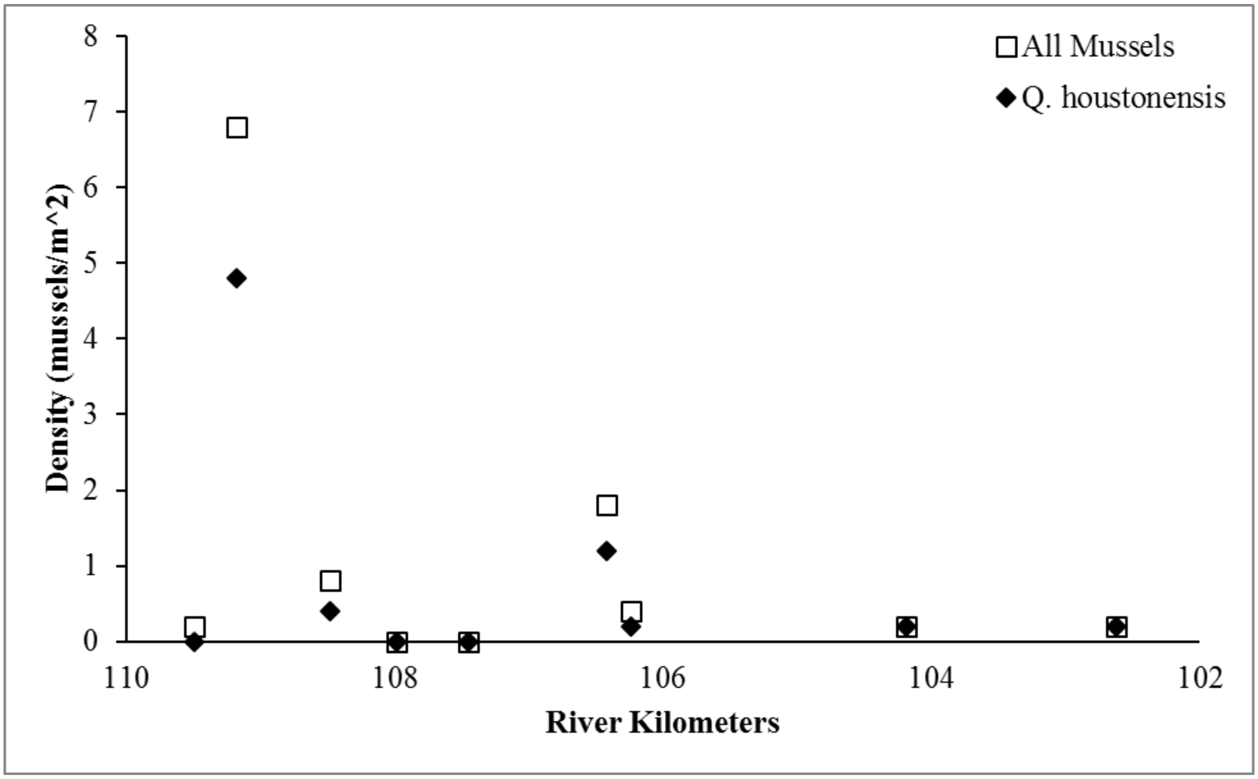

Figure 6. Densities of *Quadrula houstonensis* (smooth pimpleback) and all mussel species (All mussels) in the Little River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Brazos River (0 River Kilometers). Density = total number of either *Q. houstonensis* or all mussels encountered at each site divided by the total area of quadrats searched at each site.

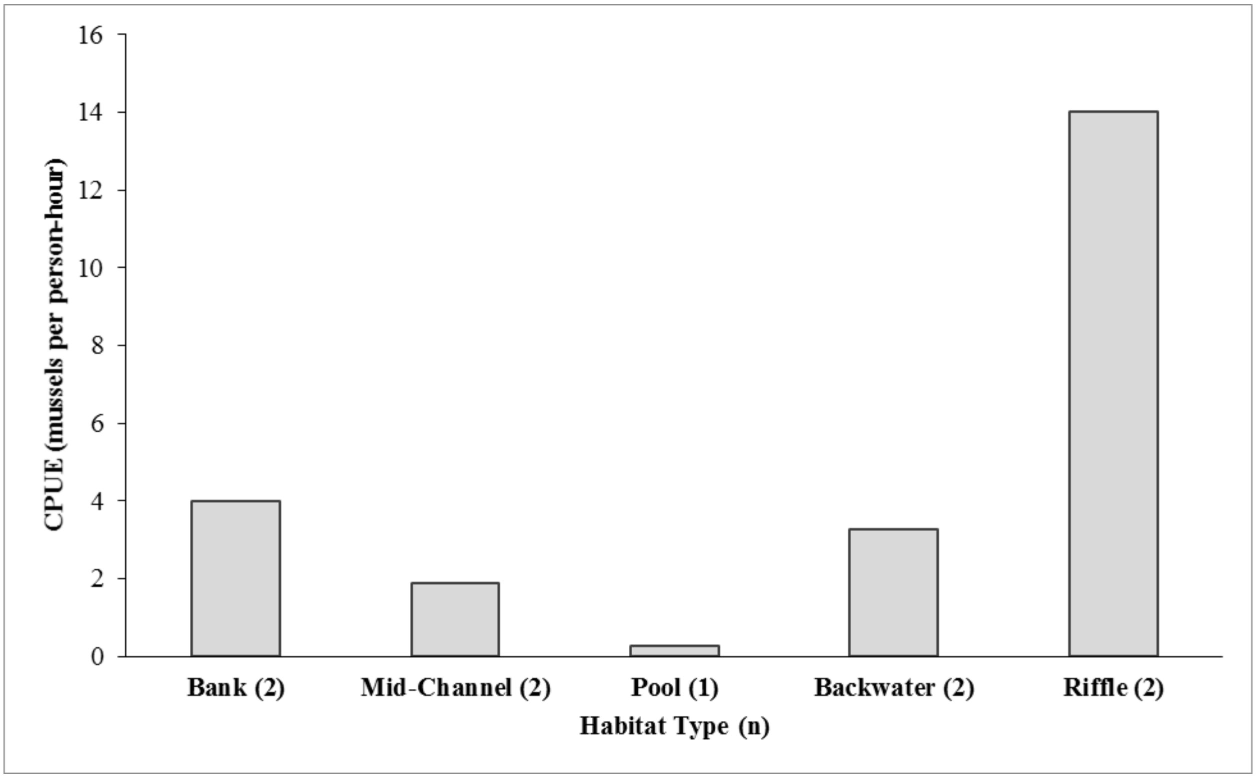

Figure 7. Catch-per-unit-effort (CPUE) of *Quadrula houstonensis* (smooth pimpleback) by mesohabitat type in the Little River. The total number of sites sampled for each habitat are listed in parenthesis.

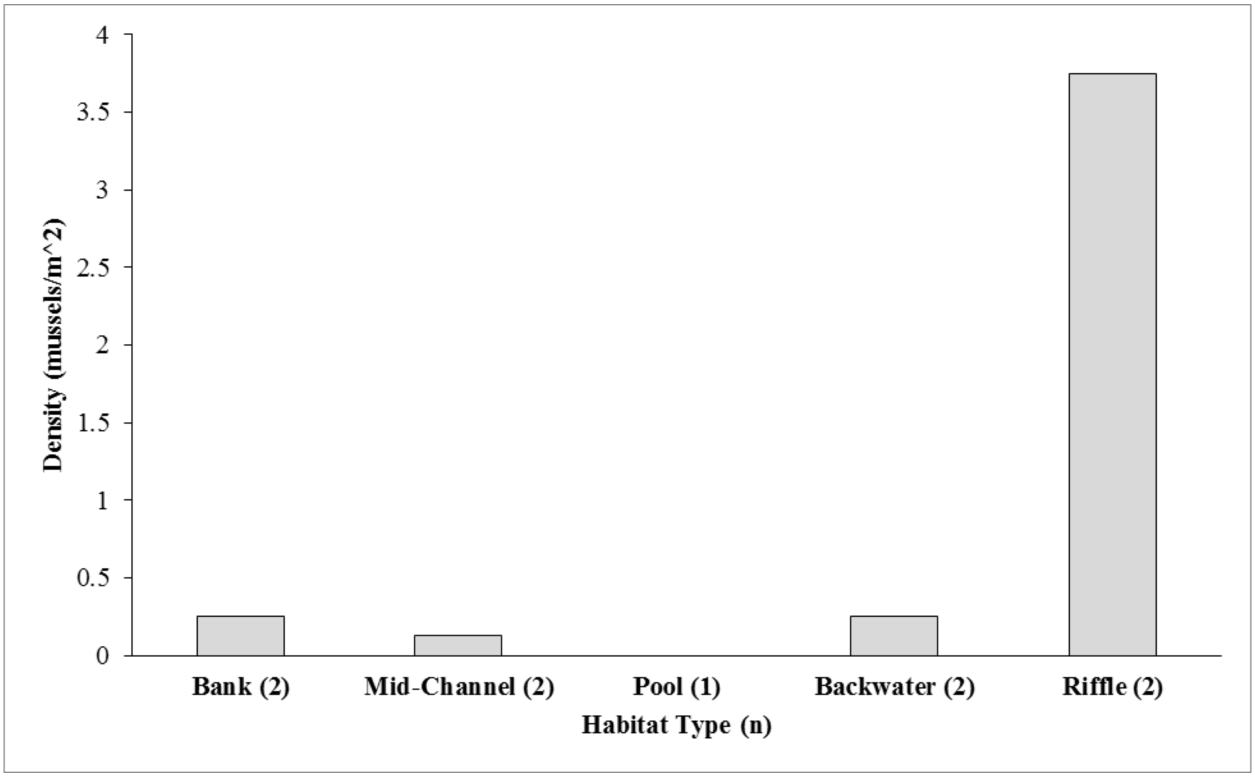

Figure 8. Density of *Quadrula houstonensis* (smooth pimpleback) by mesohabitat type in the Little River. The total number of sites sampled for each habitat are listed in parenthesis.

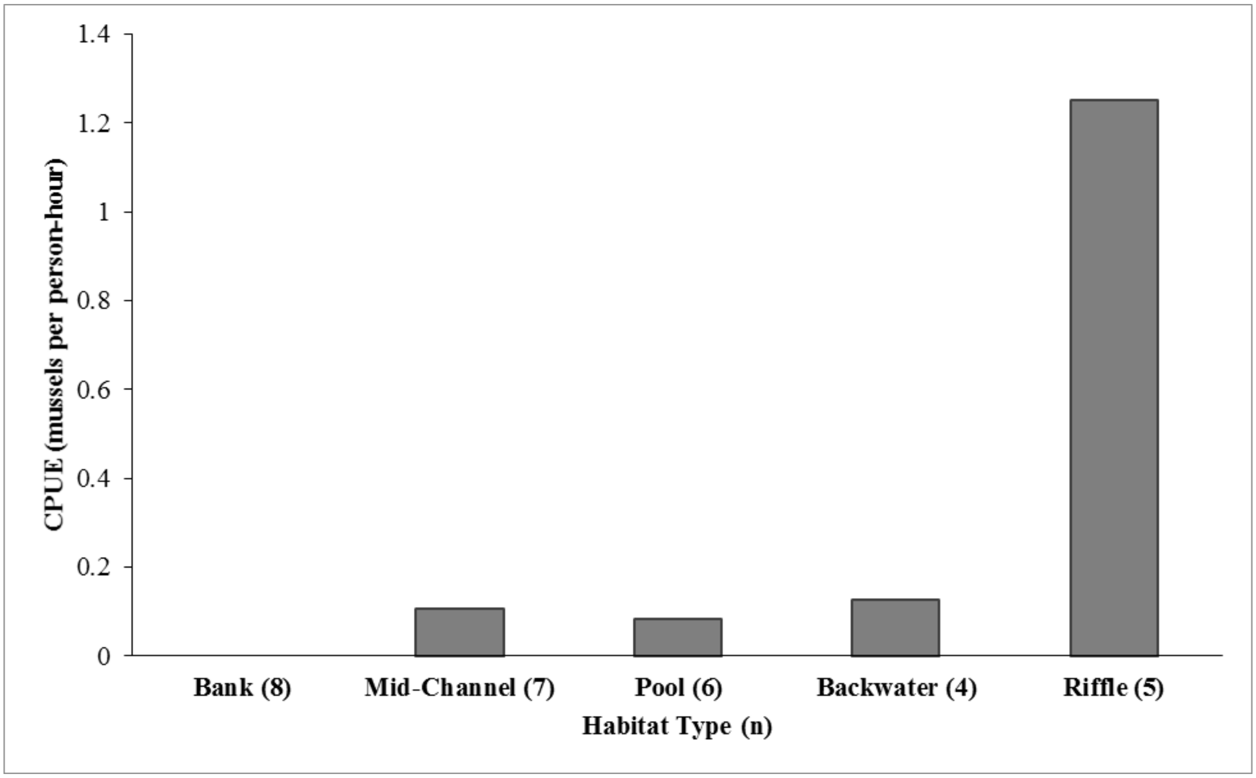

Figure 9. Catch-per-unit-effort (CPUE) of *Quadrula houstonensis* (smooth pimpleback) by mesohabitat type in Brushy Creek. The total number of sites sampled for each habitat are listed in parenthesis.

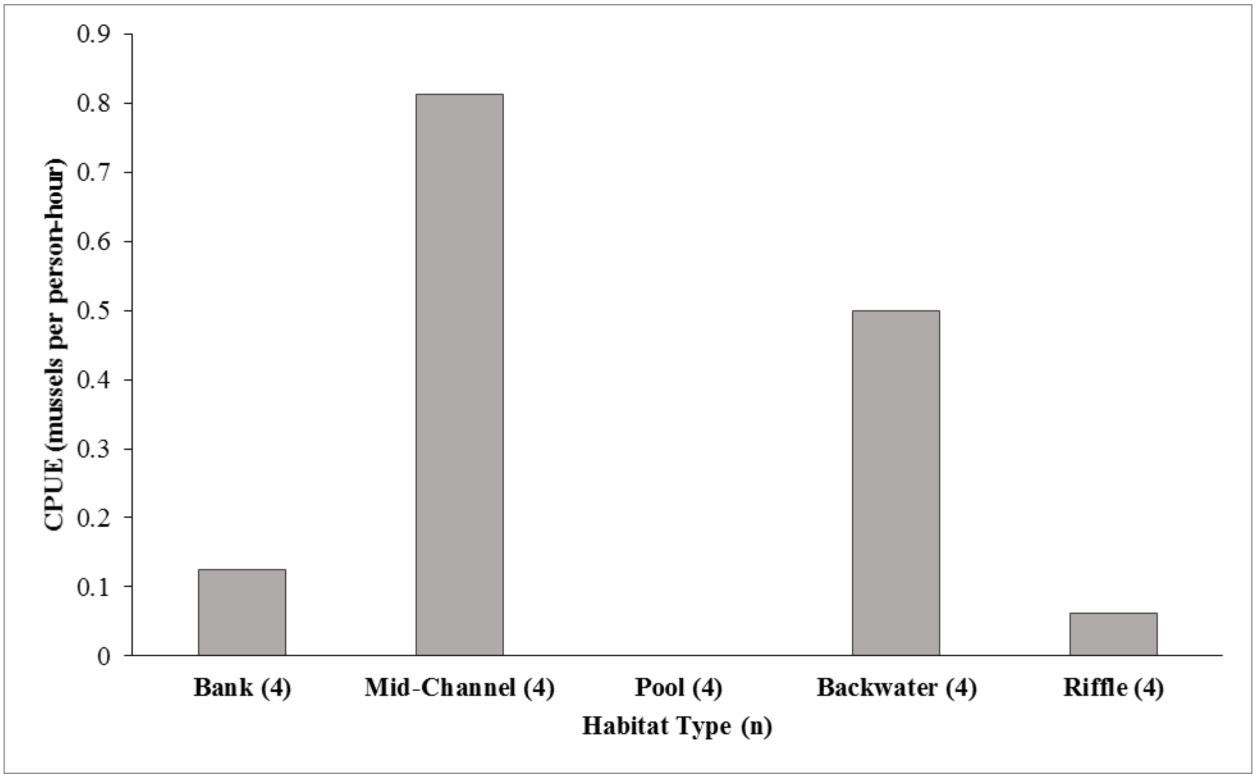

Figure 10. Catch-per-unit-effort (CPUE) of *Quadrula houstonensis* (smooth pimpleback) by mesohabitat type in the San Gabriel River. The total number of sites sampled for each habitat are listed in parenthesis.

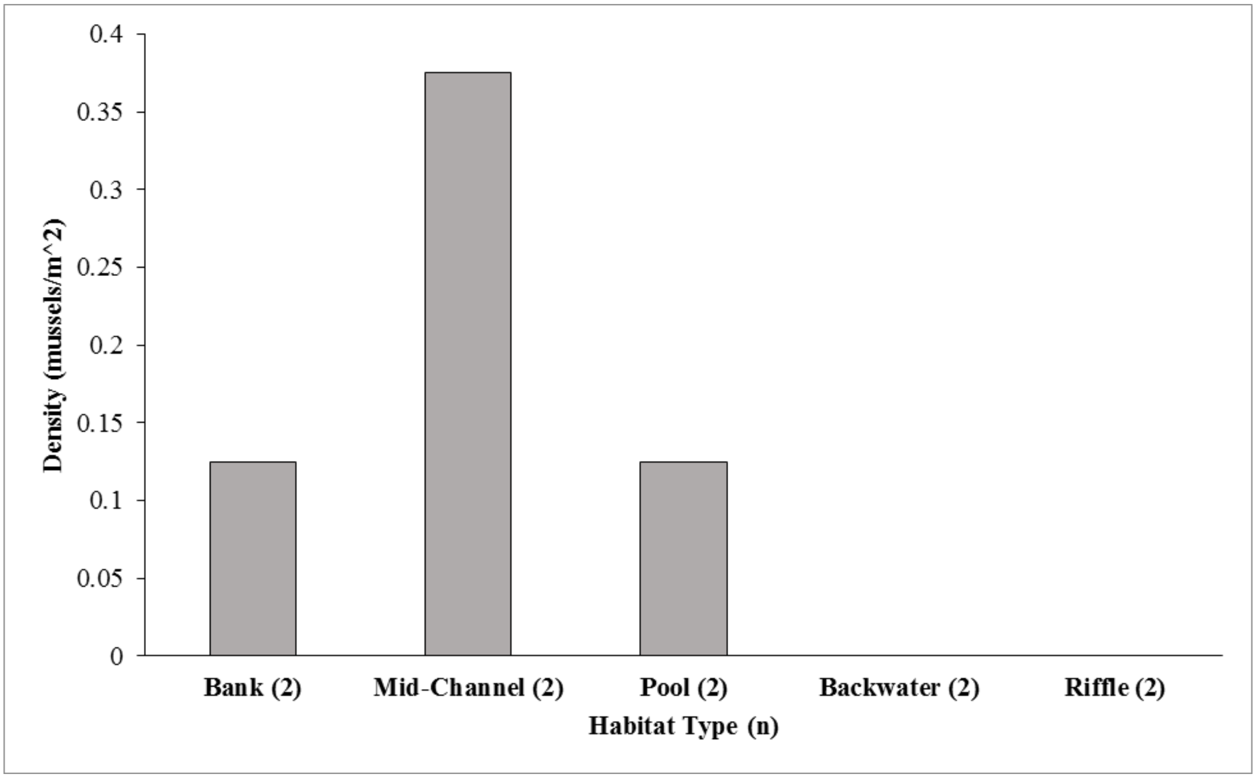

Figure 11. Density of *Quadrula houstonensis* (smooth pimpleback) by mesohabitat type in the San Gabriel River. The total number of sites sampled for each habitat are listed in parenthesis.

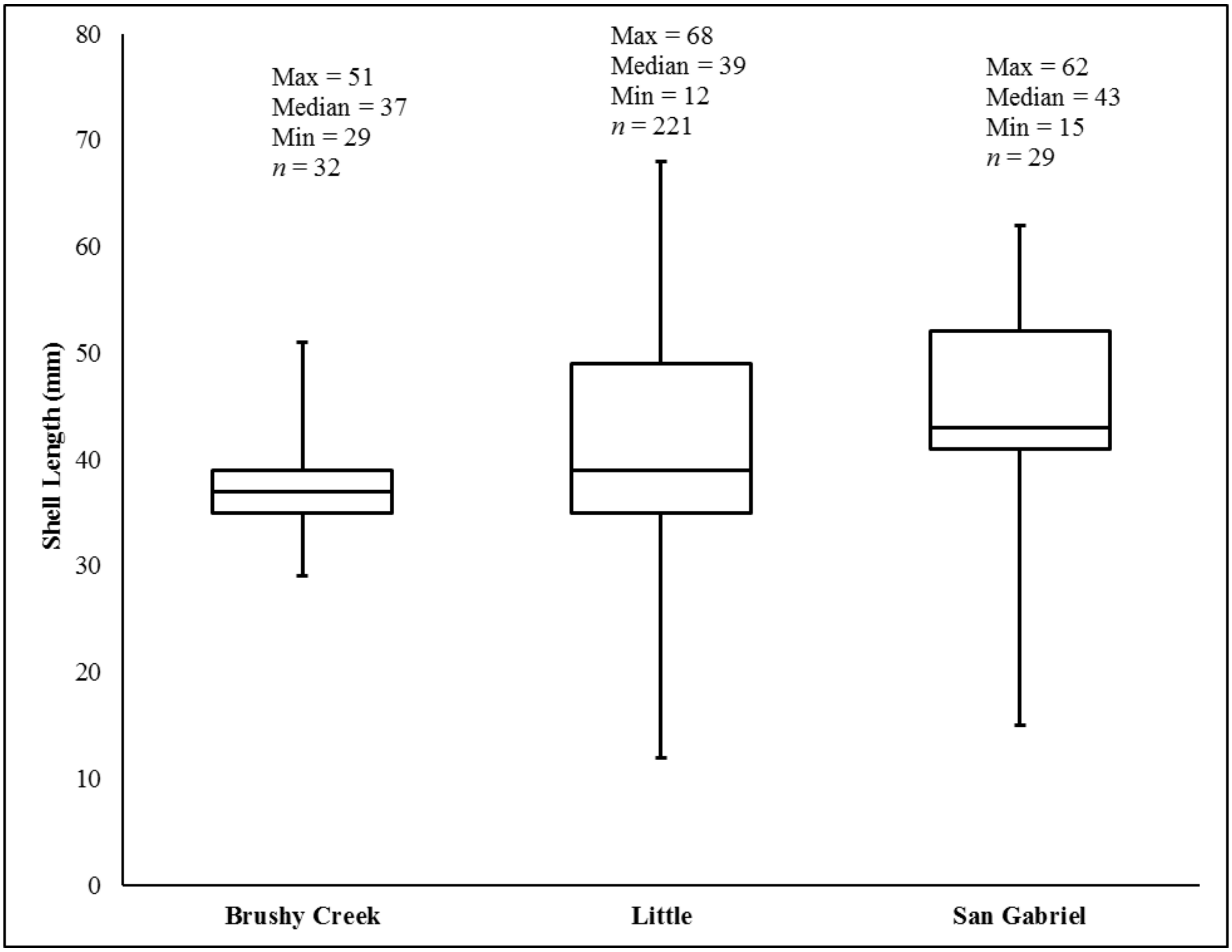

Figure 12. Shell length data of *Quadrula houstonensis* (smooth pimpleback) populations in Brushy Creek, the Little River, and the San Gabriel River of the Brazos River drainage.

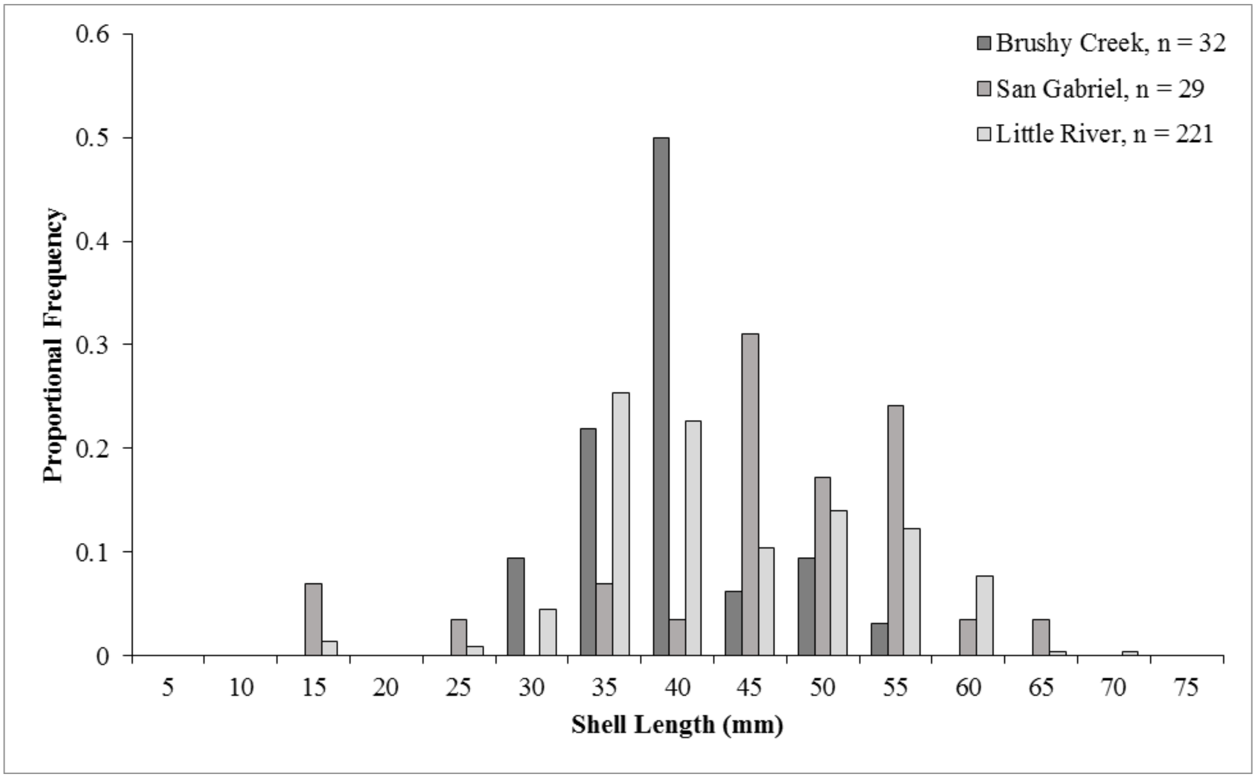

Figure 13. Proportional frequency of shell length of *Quadrula houstonensis* (smooth pimpleback) in Brushy Creek, Little River, San Gabriel River. Shell lengths are binned into 5 mm groups.

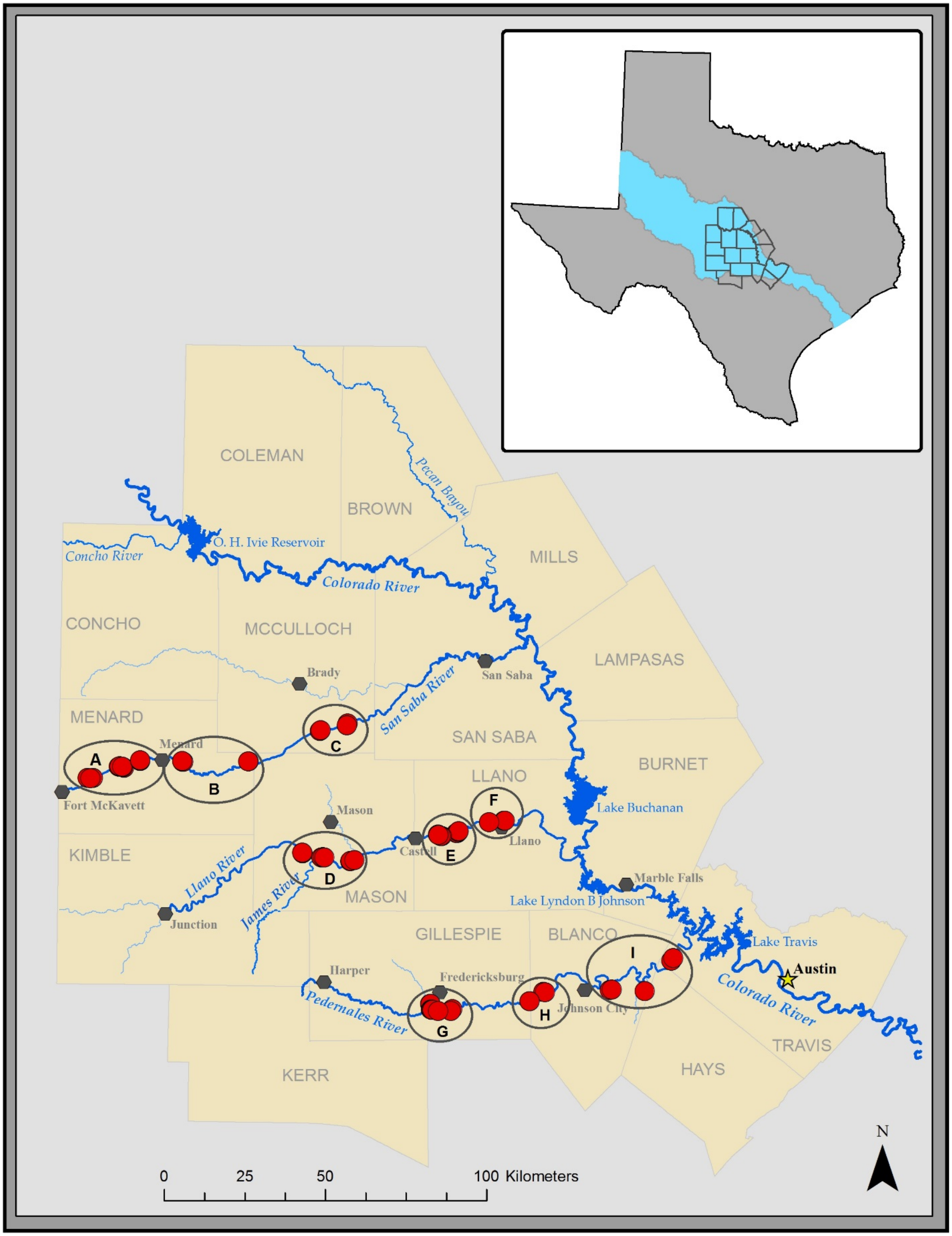

Figure 14. Map of Colorado drainage study area. Shaded circles denote sampling locations. Reaches are indicated by letter and correspond to Table 2.

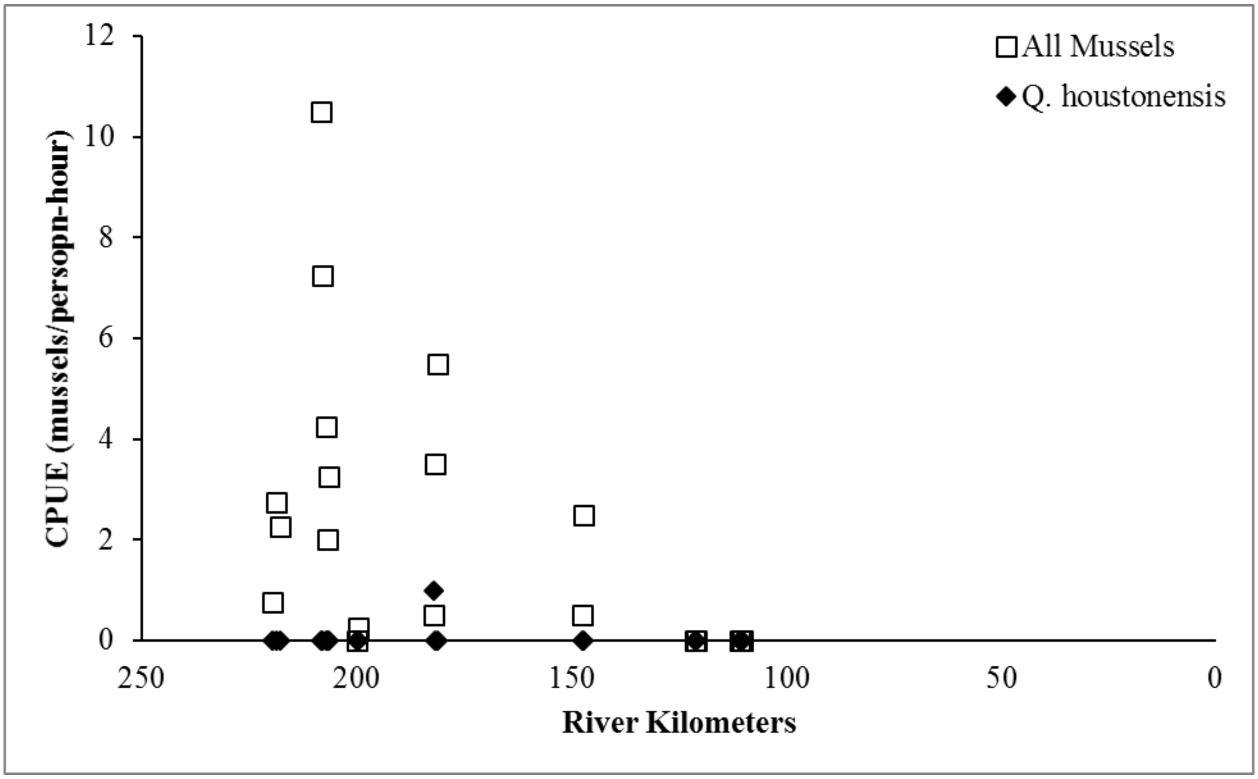

Figure 15. Catch-per-unit effort (CPUE) of *Quadrula houstonensis* (smooth pimpleback) and all mussel species (All mussels) in the San Saba River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Colorado River (0 River Kilometers). CPUE = total number of either *Q. houstonensis* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

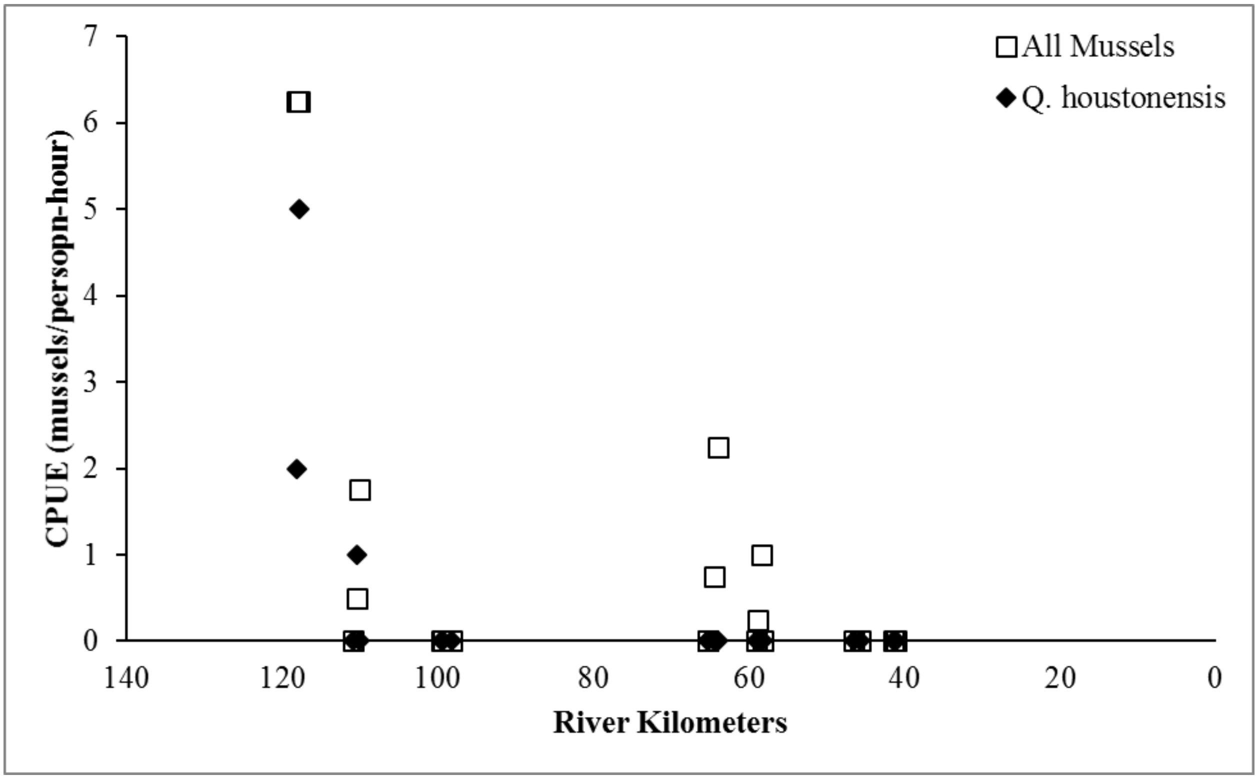

Figure 16. Catch-per-unit effort (CPUE) of *Quadrula houstonensis* (smooth pimpleback) and all mussel species (All mussels) in the Llano River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Colorado River (0 River Kilometers). CPUE = total number of either *Q. houstonensis* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

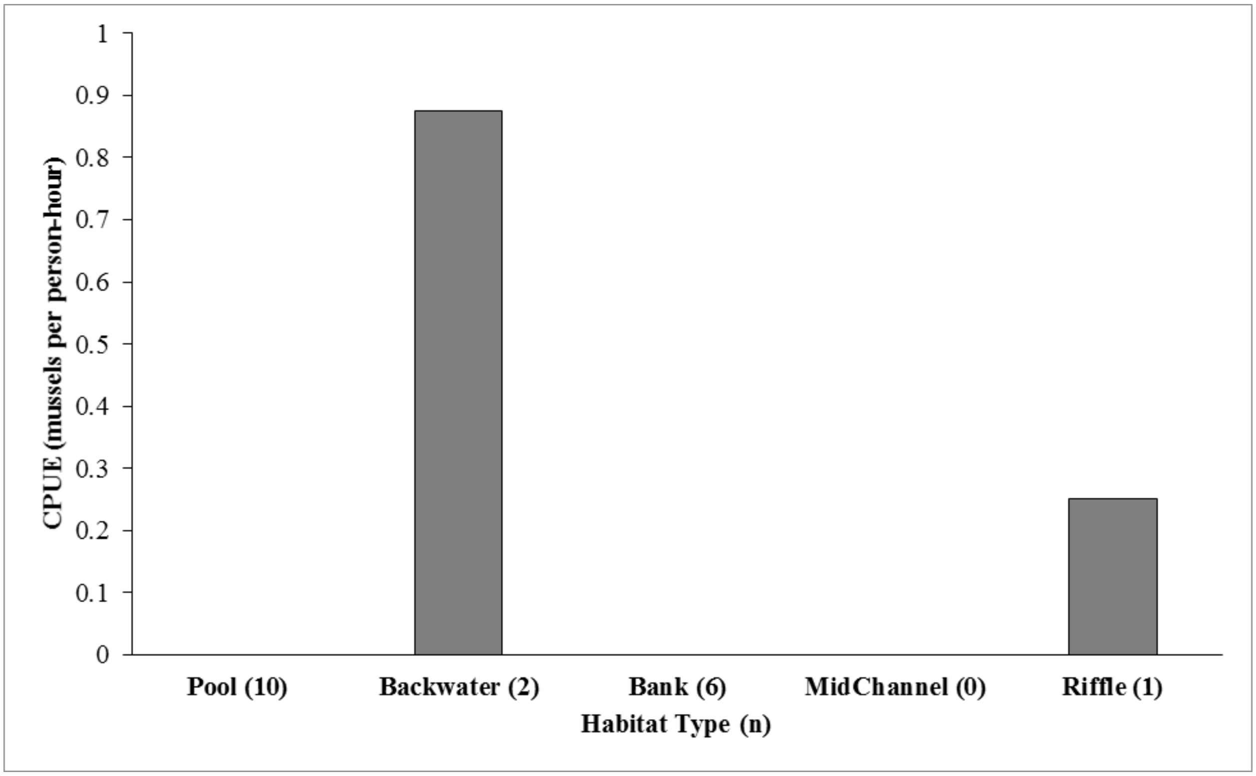

Figure 17. Catch-per-unit-effort (CPUE) of *Quadrula houstonensis* (smooth pimpleback) by mesohabitat type in the San Saba River. The total number of sites sampled for each habitat are listed in parenthesis.

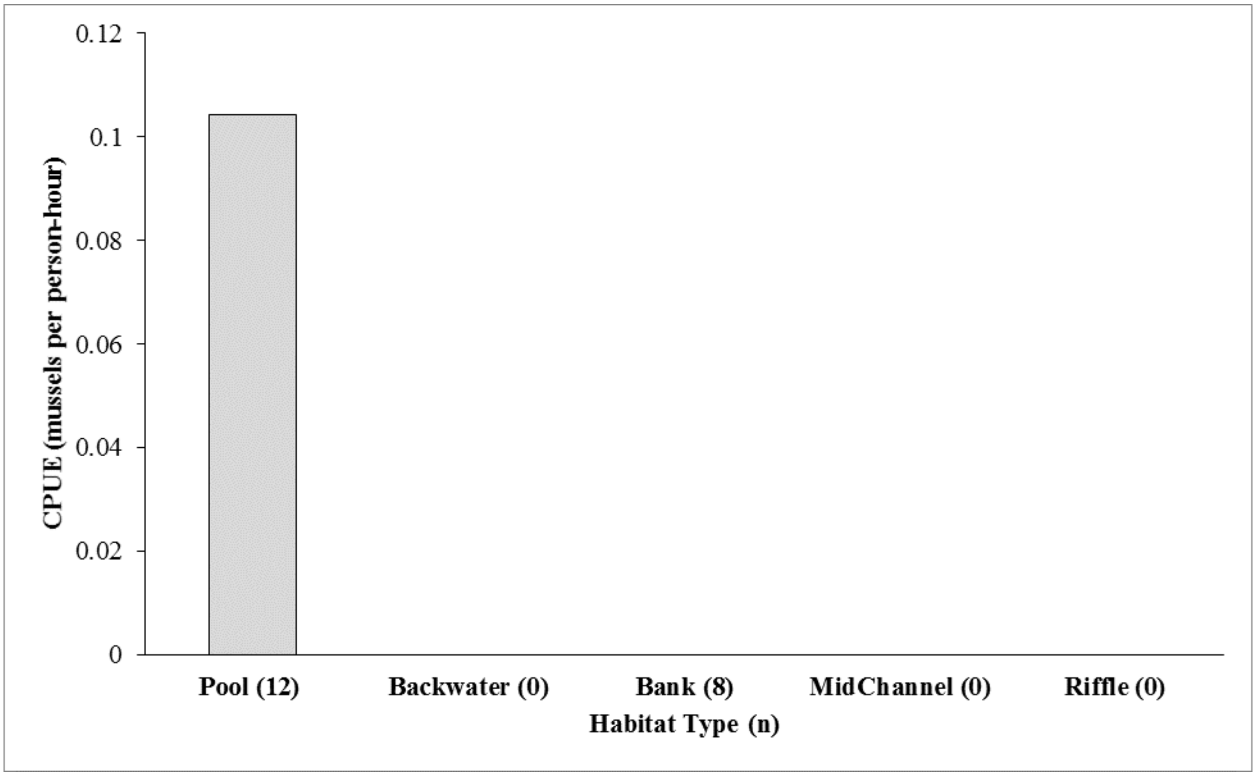

Figure 18. Catch-per-unit-effort (CPUE) of *Quadrula houstonensis* (smooth pimpleback) by mesohabitat type in the Llano River. The total number of sites sampled for each habitat are listed in parenthesis.

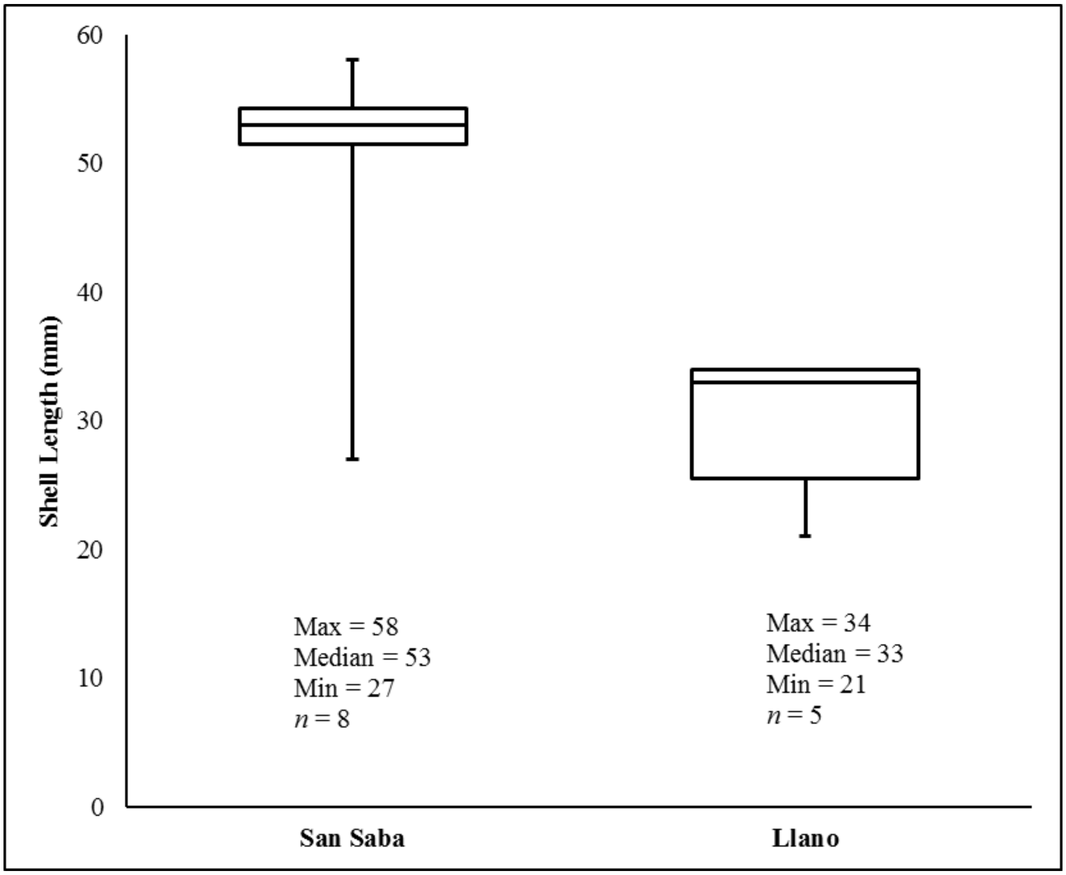

Figure 19. Shell length data of *Quadrula houstonensis* (smooth pimpleback) populations in the San Saba and Llano Rivers of the Colorado River drainage.

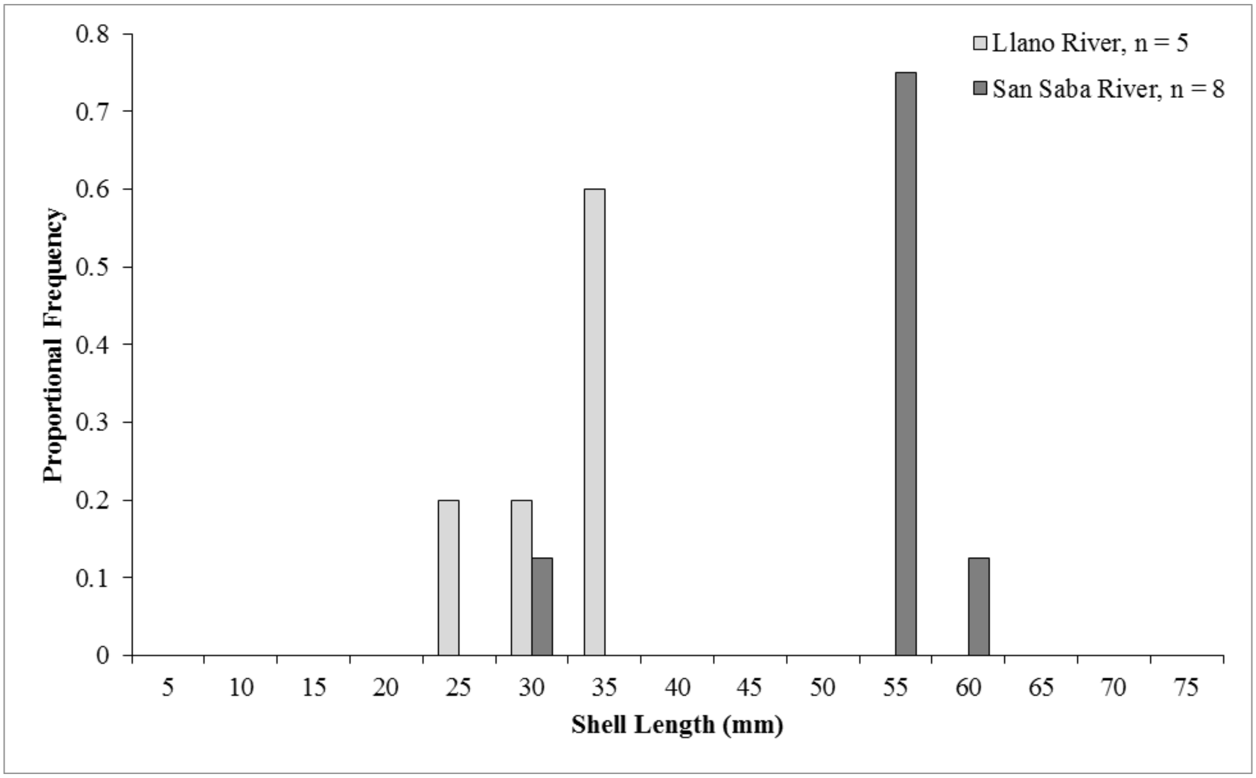

Figure 20. Proportional frequency of shell length of *Quadrula houstonensis* (smooth pimpleback) in the Llano and San Saba Rivers. Shell lengths are binned into 5 mm groups.

Distribution and Habitat Use for *Quadrula petrina* (Texas pimpleback)

Section Summary

The goal of this study was to determine the status, distribution, and mesohabitat associations for *Quadrula petrina* (Texas pimpleback), a candidate for protection under the U.S. Endangered Species Act, in the San Saba, Llano, and Pedernales rivers of the Colorado River drainage and the Guadalupe River between Gonzales and Victoria, TX. We used recent and historical data to inform a sampling program within the range of *Q. petrina.* In total, we surveyed 71 sites across the Colorado and Guadalupe River basins, and found 54 live individuals of *Q. petrina* from 12 of the 71 (or 17%) sites surveyed. In the Colorado River drainage, we surveyed 58 sites across the Llano, San Saba, and Pedernales rivers and found 24 live *Q. petrina*. The majority of live individuals found were from 3 sites on the Llano River (n = 23), upstream of the James River and Llano River confluence, occurring primarily in pool and pool/run habitats. A single live individual inhabiting a riffle was found at one site on the San Saba River near Menard, TX. No live individuals or shell material were observed from the Pedernales River. In the Guadalupe River basin, we found 41 live *Q. petrina* from 8 of the 13 (62%) sites surveyed. The majority of live individuals (n = 23) found occurred in pool habitat but not all habitat types were sampled equally due to persistent high flows throughout central Texas, which prevented sampling of riffle habitats, the optimal mesohabitat for this species. Within both drainages, size frequency distributions, using shell length as a proxy for age, suggest that some level of recruitment is occurring in both Llano River and Guadalupe River populations.

Introduction

Quadrula petrina (Gould 1855), Texas pimpleback, is known historically from the Colorado and San Antonio-Guadalupe River drainages of central Texas (Howells 2002, 2010). The type specimen was collected in the Llano River near present day U.S. Highway 87 (Mason County) by T. H. Webb in 1850 and described formally as *Unio petrinus* by Gould (1855). The species was subsequently grouped with *Margaron* and then assigned to *Quadrula* by Simpson (1900). Howells (2002, 2010) and Turgeon et al. (1998) assert *Q. petrina* is a taxonomically valid species.

The historical distribution of *Q. petrina* in the Guadalupe River drainage is known from observations in the Guadalupe and Blanco rivers. In the Guadalupe River, *Q. petrina* was collected from Comal/Guadalupe (A. L. Fitzpatrick, BU-MMC_MO 33308 -A-B), Kendall (J. K. Strecker, BU-MMC_MO 33667 -A-B), Kerr (Strecker 1931; J. Dobie, AUM_4155), and Victoria counties (J. D. Mitchell, Strecker 1931). In the Blanco River, a major tributary of the San Marcos River, *Q. petrina* has been observed at several localities (Horne and McIntosh 1979), including a single specimen collected in Hays County (W.J. Williams, BU-MMC_MO 34296 -A-B). In the San Antonio River drainage, historical records for *Q. petrina* (Strecker 1931) are limited to a single specimen (UMMZ 77200) purportedly collected from Salado Creek, a tributary of the San Antonio River. In the 1970s, Joseph Bergmann reported shell materials, of unknown condition, of this species from the Medina River near Von Ormy and Macdona, TX, and Salado Creek near Fort Sam Houston (Howells 2010), but these collections have since been lost and so the identifications cannot be verified. Around the same time P. Barker, an amateur naturalist, reported a single specimen of *Q. petrina* from Medina Reservoir (Howells 2010). This species is not known to occur in lakes or reservoirs so the true collection locality for this specimen remains in question. More recently, investigators reported finding shell fragments thought to be *Q. petrina* from the San Antonio River at the San Antonio River Walk (Howells 1995), but the weathered condition of these fragments precludes confident identification to any species.

In the Colorado River drainage, *Q. petrina* is known historically from observations made in the mainstem of the Colorado River and several tributaries in the upper basin. Singley (1893) collected specimens of *Q. petrina* in the Colorado River near Austin, and through secondhand observations, he reported several records from the Brazos and Trinity rivers. However, Singley (1893) and Frierson (1927) noted that investigators often confused *Q. petrina* with other pimpleback species (e.g., *Quadrula houstonensis*, smooth pimpleback), particularly older specimens that were indistinguishable from each other. Thus, specimens collected in the Brazos and Trinity Rivers attributed to *Q. petrina* were misidentified *Q. houstonensis* and *Quadrula mortoni* (western pimpleback), respectively. Others have reported *Q. petrina* from the Colorado River near Austin (J. K. Strecker, BU-MMC_MO 33291 -A-B) and from a number of major tributaries in the basin: Llano River in Llano (W. T. Little, BU-MMC_MO 32982 -A-B; J. Dobie, AUM_2944, AUM_2975, AUM_4022, AUM_4046), Mason (A.L. Fitzpatrick, BU-MMC_MO 33549 -A-B), and Kimble counties (J. Dobie, AUM_4076); San Saba River in Menard (Strecker 1931, Cheatum et al. 1972, FWMSH_94V 2704) and McCulloch counties (A.L. Fitzpatrick, Strecker 1931); South Concho River in Tom Green County (Williams, Strecker 1931); Onion Creek in Travis County (J. D. Mitchell, Strecker 1931), and Pedernales River in Blanco County (Howells 2010).

In recent years, investigators have suggested that *Q. petrina* has become increasingly rare throughout its range (Howells 2002, 2010). Howells (2010) in particular suggested that since 1992 *Q. petrina* was reduced to 4 known populations, including from its type locality in the Llano River (Howells et al. 1997). However, recent fieldwork in central Texas has led to the discovery of live individuals or very recently dead specimens of *Q. petrina* in the following rivers within the Guadalupe River drainage: San Marcos River in Caldwell, Guadalupe, Gonzales, and Hays counties; and Guadalupe River in Comal, Gonzales, Kerr, Kendall, and Victoria counties (Burlakova and Karatayev 2010, Howells 2010, Sowards et al. 2012, Randklev et al. 2013, Braun et al. 2014, Tsakiris and Randklev 2014). Live individuals or very recently dead specimens have also been reported recently from the following rivers in the Colorado River drainage: Elm Creek in Runnels County; Concho River in Concho County; Llano River in Mason County; San Saba River in Menard and San Saba counties; Colorado River in Colorado, San Saba, Mills, and Wharton counties. *Quadrula petrina* is currently listed as state threatened by the Texas Parks and Wildlife Department (TPWD 2010) and is under review for listing through the U.S. Endangered Species Act (USFWS 2011).

Little is known about the life history or reproductive requirements of *Q. petrina* (Howells 2010). Like other freshwater mussel species, it is an obligate ectoparasite on one or more host-fish species. Previous phylogenetic study revealed that *Q. petrina* belongs to the pimpleback lineage (i.e., the *pustulosa* clade), one of three distinct clades within the genus *Quadrula* (Serb et al. 2003), and closly related pimpleback species are short-term brooders and use mantle lures known as mantle magazines to attract host-fish species, such as members of the catfish family, Ictaluridae (Haag 2012, Sietman et al. 2012). *Quadrula petrina* is known to be reproductively active from April through August (Howells 2000; E.T. Tsakiris, *unpublished data*) and encysted glochidia have been observed on *Pylodictis olivaris* (flathead catfish), *Ameiurus natalis* (yellow bullhead), and *Lepomis macrochirus* (bluegill) (Howells 2010), though primary hosts for this species remain unconfirmed. Habitat preferences, based on recent field surveys, appear to include riffle and run mesohabitats with flowing water and sand, gravel, and cobble substrates, including gravel-filled cracks in bedrock slabs (Howells 2010, Tsakiris and Randklev 2014).

The objectives of this study were to assess the distribution, abundance, and habitat use for *Q. petrina* in selected rivers within the Colorado River and Guadalupe River basins. The resulting survey information was then used to develop Conservation Status Assessment Maps for this species within the Colorado and Guadalupe River drainages.

Methods

Study Area

The Colorado River originates in northeastern Dawson County, TX, runs approximately 1,040 km to the Gulf of Mexico, and drains an area of 100,000 km^2 (Huser 2000). The San Saba, Llano, Pedernales rivers are three major tributaries of the upper Colorado River that originate in the Edwards Plateau region (Higgins 2009). The San Saba River begins in Schleicher County and flows 225 km until its confluence with the Colorado River (Belisle and Josselet 1977). The Llano River originates in Kimble County and flows 161 km until emptying into Lake Lyndon B. Johnson, an impoundment on the Colorado River. The Pedernales River also originates in

Kimble County and flows approximately 170 km until emptying into Lake Travis, another major impoundment on the Colorado River (Perkin et al. 2010).

The Guadalupe River is spring fed, which originates on the Edwards Plateau in Kerr County, TX and drains an area of 15,539 km^2. The river runs 402 km in length to the Guadalupe and San Antonio Bays (Huser 2000). The major tributaries are the Blanco-San Marcos and the San Antonio rivers. The Guadalupe River has 10 main stem impoundments in its upper reaches with Canyon Lake in Comal County as the largest followed by Lake McQueeney in Guadalupe County (Huser 2000, Roach et al. 2014). Many small dams are located on the San Marcos and Guadalupe rivers with the most downstream dam occurring at the confluence near the city of Gonzales.

Sampling Methods

Survey sites in the San Saba, Llano, and Pedernales rivers (Figure 1) were selected using a random sampling design with the following strata: (1) upstream or downstream of a bridge crossing; (2) linear distance from the bridge; and (3) mesohabitat (banks, backwater, mid-channel, riffles, and pools). The locations of habitats were identified prior to field sampling. Specifically, mesohabitat within 2 km up-or downstream of a bridge crossing were identified and numbered on aerial imagery. A random number generator was then used to select a specific habitat type and distance from a given bridge. For locations where specific habitats could not be identified using satellite imagery (e.g., riffles), we modified our sampling design by surveying the first habitat encountered for that target habitat type. In total, 20 sites in the Llano, 19 sites in the Pedernales, and 19 sites in the San Saba rivers were selected for sampling.

Survey sites within the Guadalupe River were selected using a random sampling design. Specifically, we delineated the entire length of the Guadalupe between Cuero and Victoria, TX, into 10 km reaches and randomly chose a subset of those reaches to survey. Within each reach, sites were selected randomly by mesohabitat. For locations where habitats could not be identified using satellite imagery (e.g., riffles), we modified our sampling design by surveying the first habitat encountered for that target habitat type. In total, only 3 sites in DeWitt County and 10 sites in Victoria County were selected for sampling as a result of higher than normal flow conditions during 2015.

Qualitative surveys using the timed search method were performed at each site (i.e., mesohabitat type). The timed search method was chosen because it provides a more effective means of detecting rare species than quantitative sampling methodologies (Vaughn et al. 1997). At each site we confined the search boundaries within the randomly selected mesohabtiat and standardized the search area to 150 m^2, though in some cases the search area included multiple mesohabitat types (e.g., pool-run or riffle-run habitats). Each site was surveyed tactilely and visually for a total of 4 person-hours (p-h). However, because we are interested in the amount of effort needed to detect *Q. petrina* (which will be important for designing long-term monitoring programs), we divided the total search time into 4, 1 p-h intervals. At the end of each search interval, surveyors combined all live specimens into a mesh bag, which was kept submerged in water until completion of the survey. During each interval, surveyors were spread out in the search area and every effort was made to search all available microhabitats. Following completion of the survey, all live mussels from each time period were identified to species, counted, measured, checked for gravidity, and then returned back to the river into the appropriate habitat.

Quantitative surveys using the quadrat sampling method were performed in each mesohabitat in the Guadalupe River. The quadrat method provides a more effective means of detecting juveniles, and thus, can estimate demographic parameters in areas with high abundance more accurately (Vaughn et al. 1997). Each site was first surveyed quantitatively and then afterwards surveyed qualitatively using the timed-search method as explained above. For the quantitative sampling, we subdivided the 150 m^2 search area into a square meter grid and randomly selected 20 points for sampling using a random number generator. At each point, quadrats were sampled by excavating sediment up to 15 cm in depth using a modified Surber sampler with a 0.25 m^2 search area. Sediment was sieved through 3.175 mm mesh screen, and all live specimens from each quadrat were placed into a mesh bag, which was kept submerged in water until completion of the survey. Following completion of the survey, all live mussels from each quadrat were identified to species, counted, measured, checked for gravidity, and then returned back to the river into the appropriate habitat.

Data Analysis

Scatter plots of abundance (CPUE: number of individuals/total person-hours) vs. river kilometer (RKM) were used to examine the effect of stream position on total mussel and *Q. petrina* abundance in each river. Boxplots and length-frequency histograms were developed for *Q. petrina* to assess demographic patterns and population structure. Generally, multimodal size class distribution may indicate recruitment, whereas truncated distributions (absence of a particular age class, large, or small individuals) may indicate a lack of recent recruitment. Bar graphs were also used to visually represent presence of *Q. petrina* by mesohabitat type (i.e., riffle, mid-channel, pool, bank, and backwater).

Results/Discussion

In total, 232 person-hours (p-h) were spent surveying mussels at 58 sites in the San Saba, Llano, and Pedernales rivers of the Colorado River basin of Texas (Figure 1). Overall, we found 24 live individuals of *Q. petrina*, which were found at 1 of 19 (5.3%) sites in the San Saba River (n = 1 individual) and 3 of 20 (15.0%) sites in the Llano River (n = 23 individuals). No live individuals or shell material of *Q. petrina* were found in the Pedernales River. Catch-per-unit-effort (CPUE) of *Q. petrina* ranged from 0 to 3.3 mussels/p-h and averaged 0.01 ± 0.01 mussels/hr (± SE) in the San Saba River and 0.3 ± 0.2 mussels/p-h in the Llano River, respectively (Table 1). In comparison, CPUE measured for all mussels averaged 2.4 ± 0.7 and 1.0 ± 0.4 mussels/p-h in the San Saba and Llano rivers, respectively (Figures 2 and 3). Thus, relative abundance of *Q. petrina* was higher in the Llano River (7.9%) than San Saba River (0.3%) (Figures 2 and 3). Within the Llano River, the highest abundances (n = 8 at Site 1 and n = 13 at Site 2) were observed in pool and pool/run habitats located near Mason, TX, upstream from the James River and Llano River confluence (Table 1, Figure 4); whereas, the single live individual found in the San Saba River was observed in riffle habitat (Figure 5). Median shell length for San Saba and Llano River populations was 38 mm and minimum and maximum shell lengths were 22 mm and 49 mm, respectively (Figure 6 and 7).

Recent surveys conducted within the Colorado River drainage have also yielded several accounts of live individuals. Sowards et al. (2012) found 10 live *Q. petrina* in Llano River. In the Concho River, near Paint Rock, TX, Burlakova and Karatayev (2010) estimated a population size of *Q. petrina* to be 4,030 ± 498 individuals. In the lower San Saba River, Tsakiris et al. (2014) collected mussels from two sites, and average CPUE across both sites was 2.4 mussels/p-h for *Q. petrina* and 8.9 mussels/p-h for all mussels, which is higher than what we observed during this study. No live individuals or shell of *Q. petrina* were found in the Pedernales River, though we did observe live mussels of *Lampsilis bracteata* (Texas fatmucket) and several other mussel species presently considered common.

In total, 52 p-h were spent surveying mussels at 13 sites in the Guadalupe River (Figure 8). Overall, we found 41 live individuals of *Q. petrina*, which were found at 7 of 13 (53.8%) sites during qualitative sampling (*n* = 30 individuals) and 4 of 13 (30.8%) sites during quantitative sampling (*n* = 11 individuals). CPUE of *Q. petrina* ranged from 0 to 4.0 mussels/p-h and averaged 0.6 ± 0.3 mussels/p-h (Table 2), while CPUE averaged 54.1 ± 25.1 mussels/p-h for all mussels (Figure 9). Density of *Q. petrina* in the Guadalupe River ranged from 0 to 1.4 mussels/m^2 and averaged 0.2 ± 0.1 mussels/m^2 (Table 2). In comparison, density measured for all mussels averaged 5.4 ± 3.5 mussels/m^2 (Figure 10). The highest abundance (1.8 ± 1.1 mussels/p-h) and density (0.5 ± 0.4 mussels/m^2) of *Q. petrina* were observed in pool habitat (Figures 11 and 12). Median shell length for this population was 52 mm and minimum and maximum shell lengths were 26 mm and 85 mm, respectively (Figure 13).

In previous sampling of the Guadalupe River drainage, 2 live individuals had been reported near Victoria, TX in 2009 (N. A. Johnson, pers. comm. Howells 2010). Although the number of *Q. petrina* found in our study was small (*n* = 41 total), 52 sites were sampled in 2014 between Gonzales and Cuero, TX resulting in 852 *Q. petrina*. An Indicator Species Analysis (ISA) was done on the 2014 dataset and from this analysis (IV = 0.623, p-value = 0.001, and frequency = 42) we concluded that this species occurs primarily in riffle habitat (Randklev, unpublished data). Because we did not sample riffles in the Guadalupe River in 2015 due to elevated water levels and unsafe sampling conditions the number of *Q. petrina* occurring in the Guadalupe between Cuero and Victoria, TX, is likely much higher than what we observed during our survey. Despite difficulties in sampling riffles due to elevated flows, our survey results largely corroborate the ISA for *Q. petrina* as this species was found primarily in riffle and pool habitats across the Colorado and Guadalupe drainages (Figure 4, Figure 5, Figure 11, and Figure 12). Shell length frequency distribution of *Q. petrina* from both Colorado and Guadalupe river basins suggest some level of recruitment (Figure 7 and Figure 14).

In summary, our results demonstrate that *Q. petrina* continues to persist within the Llano and San Saba rivers in the Colorado River drainage and the mainstem of the Guadalupe River. Our results, along with observations within the past 20 years (Howells 2002, 2010, Burlakova and Karatayev 2010, Sowards et al. 2012, Tsakiris and Randklev 2014), indicate that *Q. petrina* occurs in low abundance in the Llano and San Saba rivers and moderate abundance in the Guadalupe River. For the Pedernales River, no live individuals or shell material of *Q. petrina* were observed despite it being collected from this river in the early 1970s. In our study, *Q. petrina* was most often found in pool and riffle habitat, though the different habitat types surveyed were not sampled equally (Randklev, unpublished data). Within both drainages, size frequency distributions using shell length as a proxy for age show recruitment is occurring in the

Llano and Guadalupe rivers, though it’s unknown whether it’s sufficient enough to ensure long-term persistence in either river.

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Table 1. Locality and collection information for mussel survey sites in the Colorado River drainage. CPUE = total number of *Q. petrina* encountered at each site divided by the number of person hours (4) searched at each site. Habitat key: BW = backwater, P = pool, R = riffle, B = Bank, PR = Pool/Run combined, RR = Riffle/Run combined, All = site encompassed multiple habitat types. Sites are ordered upstream to downstream in each river.

Site/ Reach	Habitat	Locality	County	Date of collection	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m2)
1A	B	San Saba	Menard	8/5/2015	0	0	-	4	150
2A	P	San Saba	Menard	8/5/2015	0	0	-	4	150
3A	P	San Saba	Menard	8/5/2015	0	0	-	4	150
4A	B	San Saba	Menard	8/3/2015	0	0	-	4	150
5A	P	San Saba	Menard	8/4/2015	0	0	-	4	150
6A	P	San Saba	Menard	8/4/2015	0	0	-	4	150
7A	BW	San Saba	Menard	8/4/2015	0	0	-	4	150
8A	B	San Saba	Menard	8/4/2015	0	0	-	4	150
9A	B	San Saba	Menard	8/5/2015	0	0	-	4	150
10A	P	San Saba	Menard	8/5/2015	0	0	-	4	150
11B	R	San Saba	Menard	8/6/2015	1	0.25	Y	4	150
12B	B	San Saba	Menard	8/6/2015	0	0	-	4	150
13B	BW	San Saba	Menard	8/6/2015	0	0	-	4	150
14B	P	San Saba	Menard	8/6/2015	0	0	-	4	150
15B	B	San Saba	Menard	8/6/2015	0	0	-	4	150
16C	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
17C	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
18C	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
19C	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
20D	P	Llano	Mason	7/30/2015	8	2	Y	4	150
21D	PR	Llano	Mason	7/30/2015	13	3.25	Y	4	150
22D	PR	Llano	Mason	7/30/2015	0	0	-	4	150
23D	PR	Llano	Mason	7/30/2015	2	0.5	Y	4	150
24D	B	Llano	Mason	7/30/2015	0	0	-	4	150
25D	PR	Llano	Mason	7/29/2015	0	0	-	4	150
26D	B	Llano	Mason	7/29/2015	0	0	-	4	150
27D	P	Llano	Mason	7/31/2015	0	0	-	4	150
28E	B	Llano	Llano	7/29/2015	0	0	-	4	150
29E	P	Llano	Llano	7/29/2015	0	0	-	4	150
30E	B	Llano	Llano	7/29/2015	0	0	-	4	150
31E	P	Llano	Llano	7/29/2015	0	0	-	4	150
32E	P	Llano	Llano	7/28/2015	0	0	-	4	150
33E	B	Llano	Llano	7/28/2015	0	0	-	4	150
34E	B	Llano	Llano	7/28/2015	0	0	-	4	150
35E	PR	Llano	Llano	7/28/2015	0	0	-	4	150
36F	P	Llano	Llano	7/27/2015	0	0	-	4	150
37F	B	Llano	Llano	7/28/2015	0	0	-	4	150
38F	PR	Llano	Llano	7/27/2015	0	0	-	4	150
39F	B	Llano	Llano	7/27/2015	0	0	-	4	150
40G	P	Live Oak Creek	Gillespie	6/24/2015	0	0	-	4	150
41G	P	Live Oak Creek	Gillespie	6/24/2015	0	0	-	4	150
42G	All	Live Oak Creek	Gillespie	6/23/2015	0	0	-	4	150
43G	All	Live Oak Creek	Gillespie	6/23/2015	0	0	-	4	150

Table 1. Continued.

Site Reach	Habitat	Locality	County	Date of collection	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m2)
44G	B	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
45G	P	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
46G	B	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
47G	B	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
48G	BW	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
49H	MC	Pedernales	Gillespie	6/23/2015	0	0	-	4	150
50H	B	Pedernales	Gillespie	6/23/2015	0	0	-	4	150
51H	RR	Pedernales	Blanco	6/23/2015	0	0	-	4	150
52H	B	Pedernales	Blanco	6/23/2015	0	0	-	4	150
53H	BW	Pedernales	Blanco	6/23/2015	0	0	-	4	150
54I	P	Pedernales	Blanco	6/22/2015	0	0	-	4	150
55I	RR	Pedernales	Blanco	6/22/2015	0	0	-	4	150
56I	P	Flat Creek	Blanco	6/25/2015	0	0	-	4	150
57I	BW	Pedernales	Travis	6/25/2015	0	0	-	4	150
58I	B	Pedernales	Travis	6/25/2015	0	0	-	4	150

Table 2. Locality and collection information for mussel survey sites in the Guadalupe River drainage. CPUE = total number of *Q. petrina* encountered at each site during qualitative sampling divided by the number of person hours (4) searched at each site. Density = mean number of *Q. petrina* per 0.25 m^2 quadrat. Habitat key: P = pool, BH = Bank, BW = backwater, R = riffle, FPB = front of point bar, BPB = behind point bar. Sites are ordered upstream to downstream.

Site/ Reach	Habitat	Locality	County	Date of collection	Number of live	CPUE	Density /m²	Sub adult	Effort		Area (m²)
									Hours	Quadrats	
1A	P	Guadalupe	DeWitt	8/27/2015	6	1.25	0.2	Y	4	20	150
2A	P	Guadalupe	DeWitt	8/27/2015	1	0.25	0	N	4	20	150
3A	BW	Guadalupe	DeWitt	8/27/2015	4	1	0	N	4	20	150
4B	BH	Guadalupe	Cuero	8/19/2015	2	0.25	0.2	N	4	20	150
5B	R	Guadalupe	Cuero	8/25/2015	2	0	0.4	N	4	20	150
6B	BW	Guadalupe	Cuero	8/19/2015	1	0.25	0	N	4	20	150
7B	FPB	Guadalupe	Cuero	8/19/2015	0	0	0	-	4	20	150
8B	P	Guadalupe	Cuero	8/20/2015	23	4	1.4	Y	4	20	150
9B	BH	Guadalupe	Cuero	8/18/2015	0	0	0	-	4	20	150
10B	FPB	Guadalupe	Cuero	8/18/2015	0	0	0	-	4	20	150
11B	BPB	Guadalupe	Cuero	8/25/2015	0	0	0	-	4	20	150
12B	BPB	Guadalupe	Cuero	8/25/2015	0	0	0	-	4	20	150
13B	BW	Guadalupe	Cuero	3/9/2015	2	0.5	0	N	4	20	150

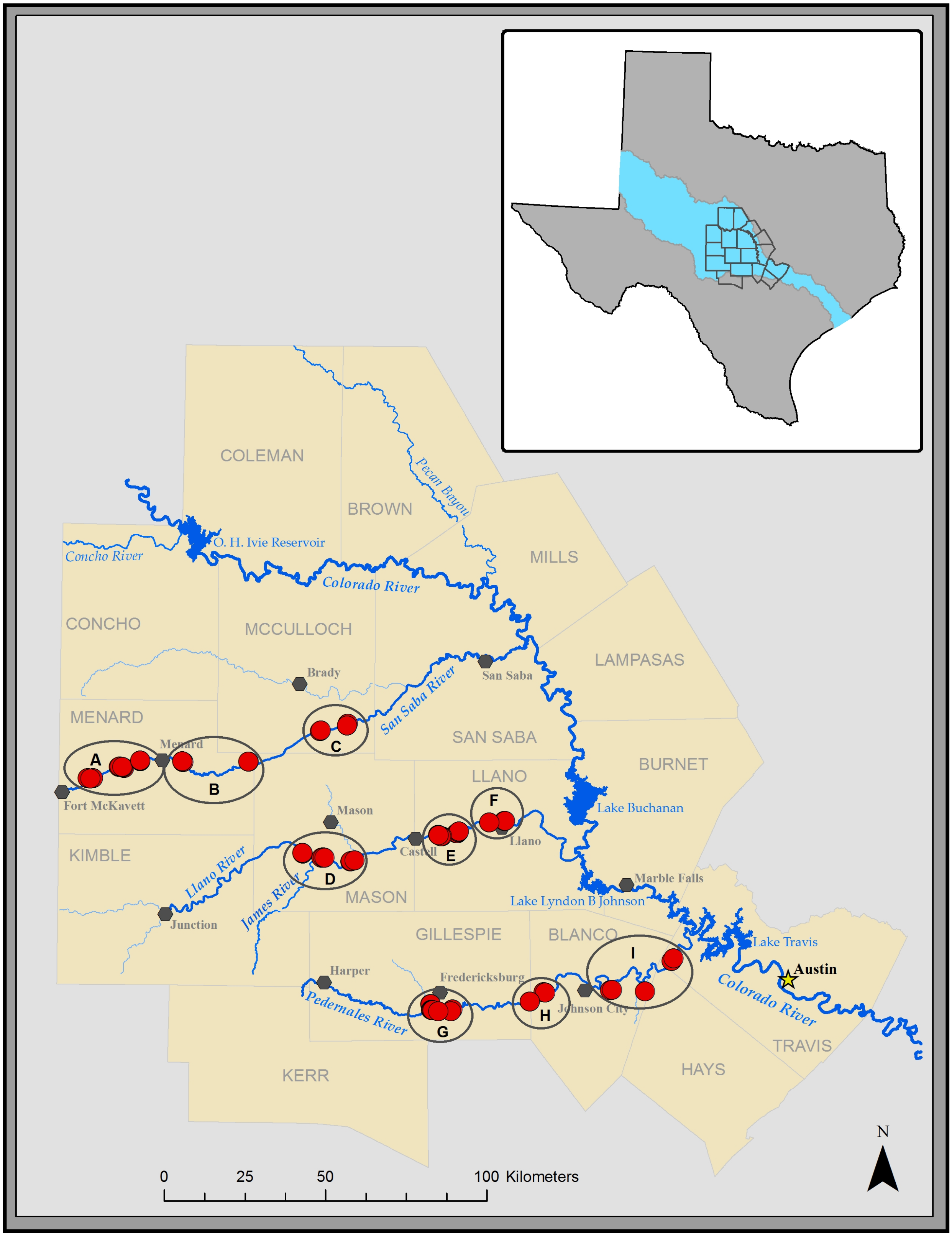

Figure 1. Map of Colorado drainage study area. Shaded circles denote sampling locations. Reaches are indicated by letter and correspond to Table 1.

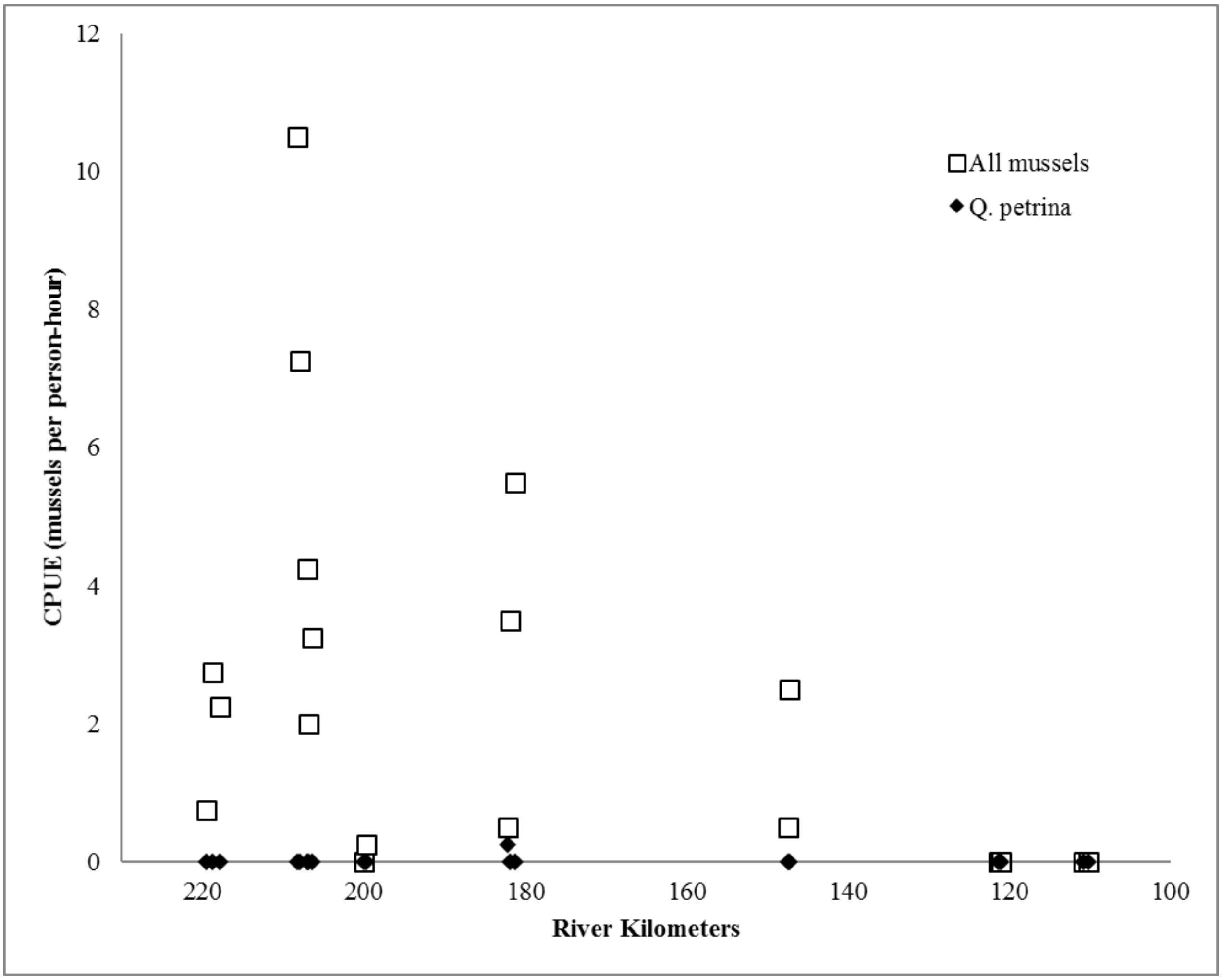

Figure 2. Catch-per-unit effort (CPUE) of *Quadrula petrina* (Texas pimpleback) and all mussel species (All mussels) in the San Saba River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Colorado River (0 River Kilometers). CPUE = total number of either *Q. petrina* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

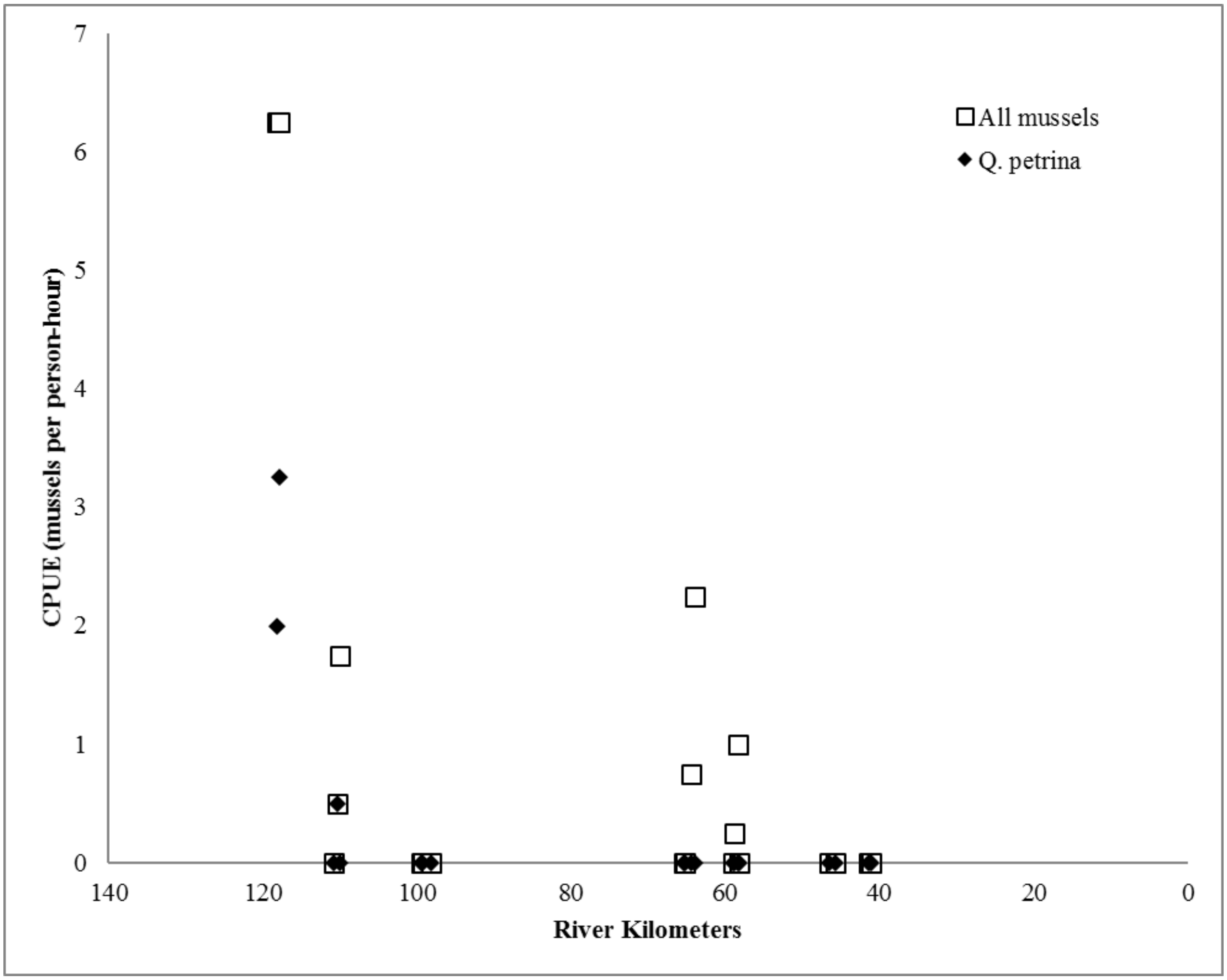

Figure 3. Catch-per-unit-effort (CPUE) of *Quadrula petrina* (Texas pimpleback) and all mussel species (All mussels) in the Llano River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Colorado River (0 River Kilometers). CPUE = total number of either *Q. petrina* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

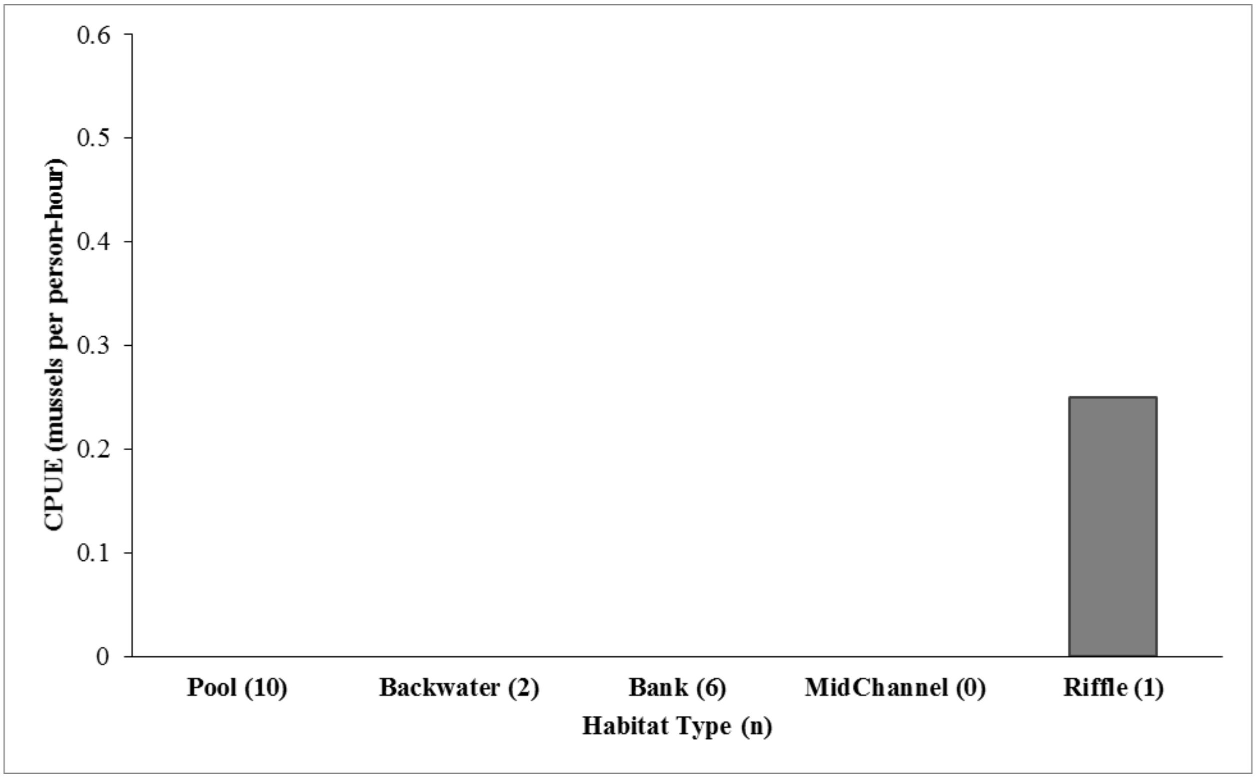

Figure 4. Catch-per-unit-effort (CPUE) of *Quadrula petrina* (Texas pimpleback) by mesohabitat type in the San Saba River. The total number of sites sampled at each habitat in the San Saba River are listed in parenthesis.

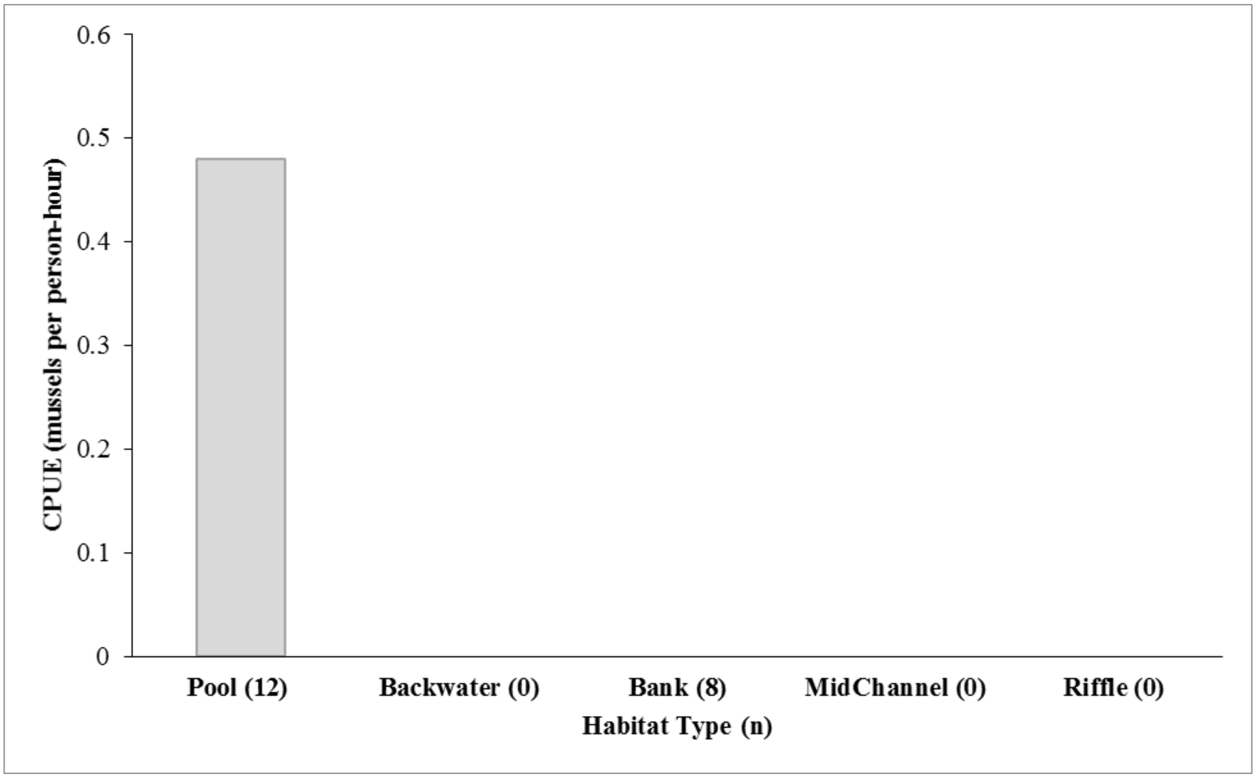

Figure 5. Catch-per-unit-effort (CPUE) of *Quadrula petrina* (Texas pimpleback) by mesohabitat type in the Llano River. The total number of sites sampled at each habitat in the Llano River are listed in parenthesis.

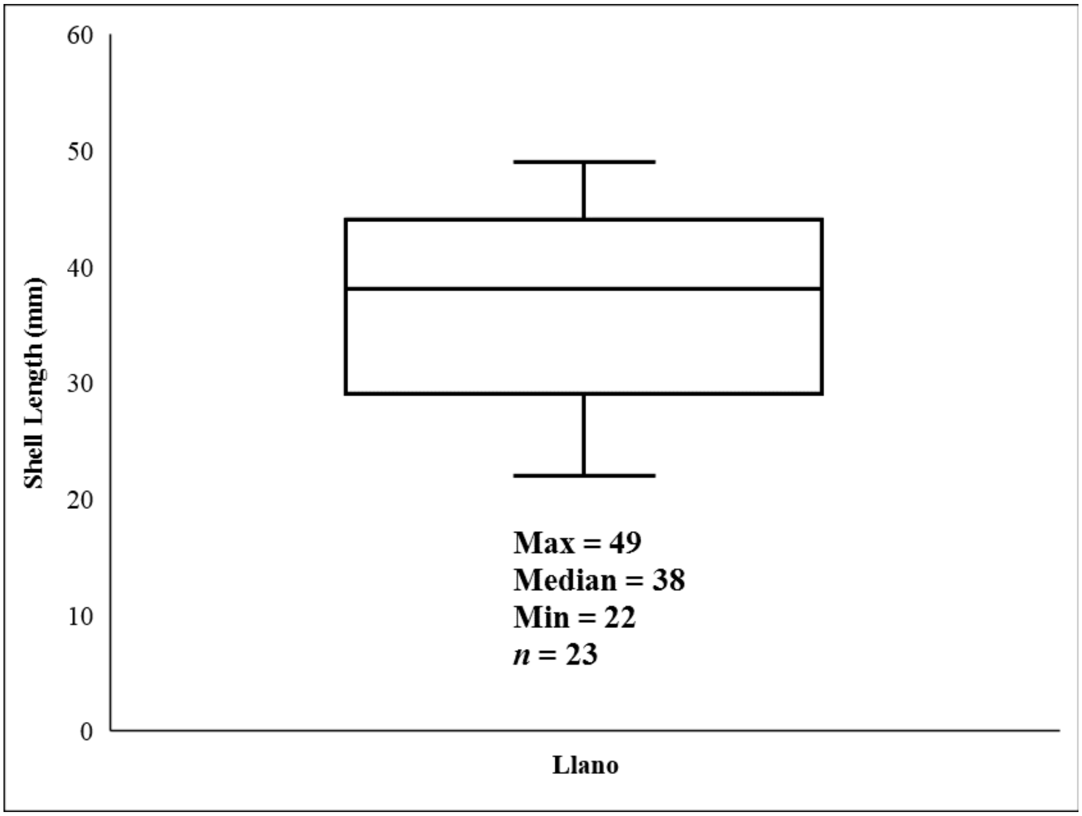

Figure 6. Shell length data of *Q.petrina* (Texas pimpleback) populations in the Llano River of the Colorado River drainage.

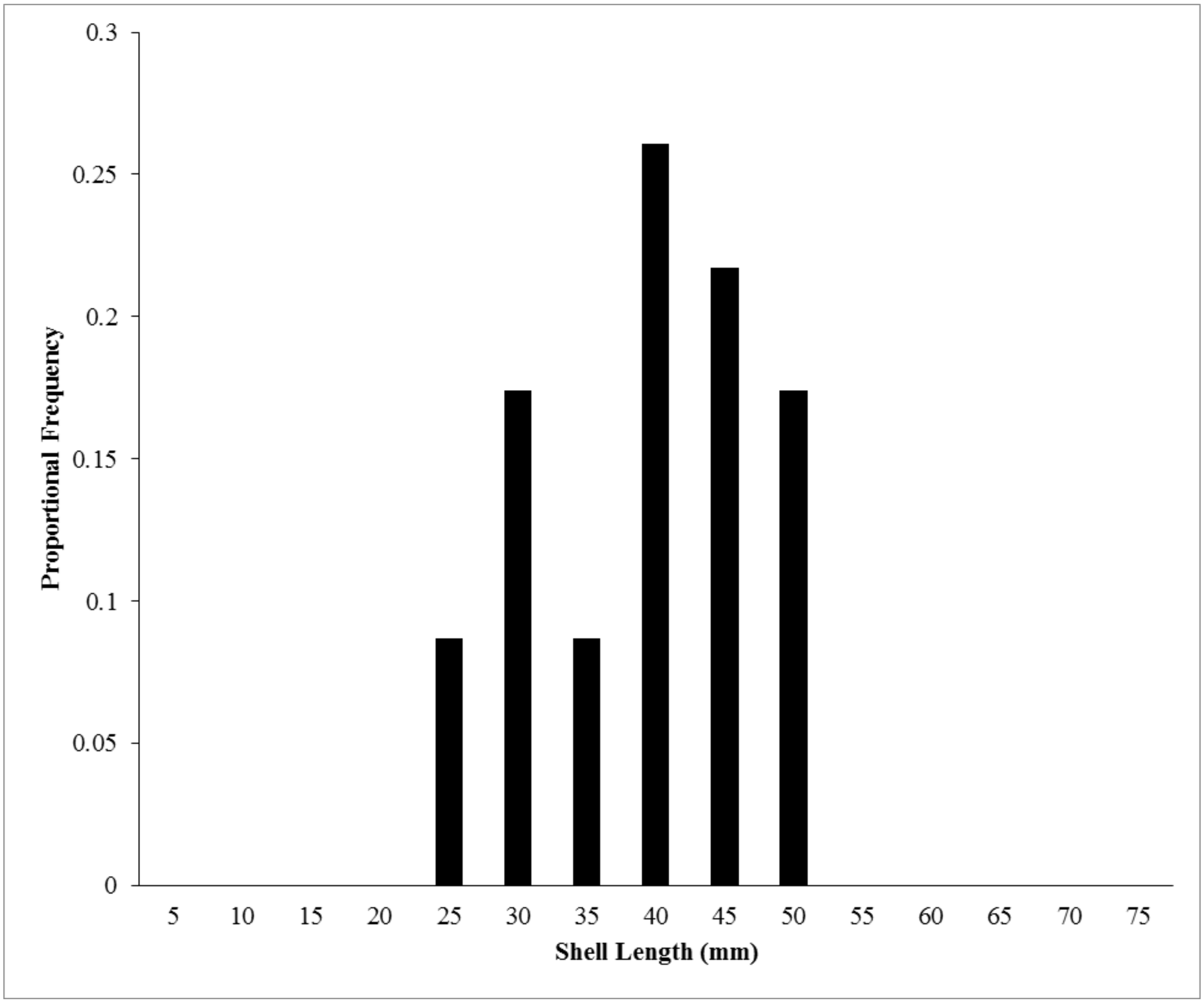

Figure 7. Proportional frequency of shell length of *Quadrula petrina* (Texas pimpleback) in the Llano River (*n* = 23). Shell lengths are binned into 5 mm groups.

Figure 8. Map of Guadalupe River study area. Shaded circles denote sampling locations. Reaches are indicated by letter and correspond to Table 2.

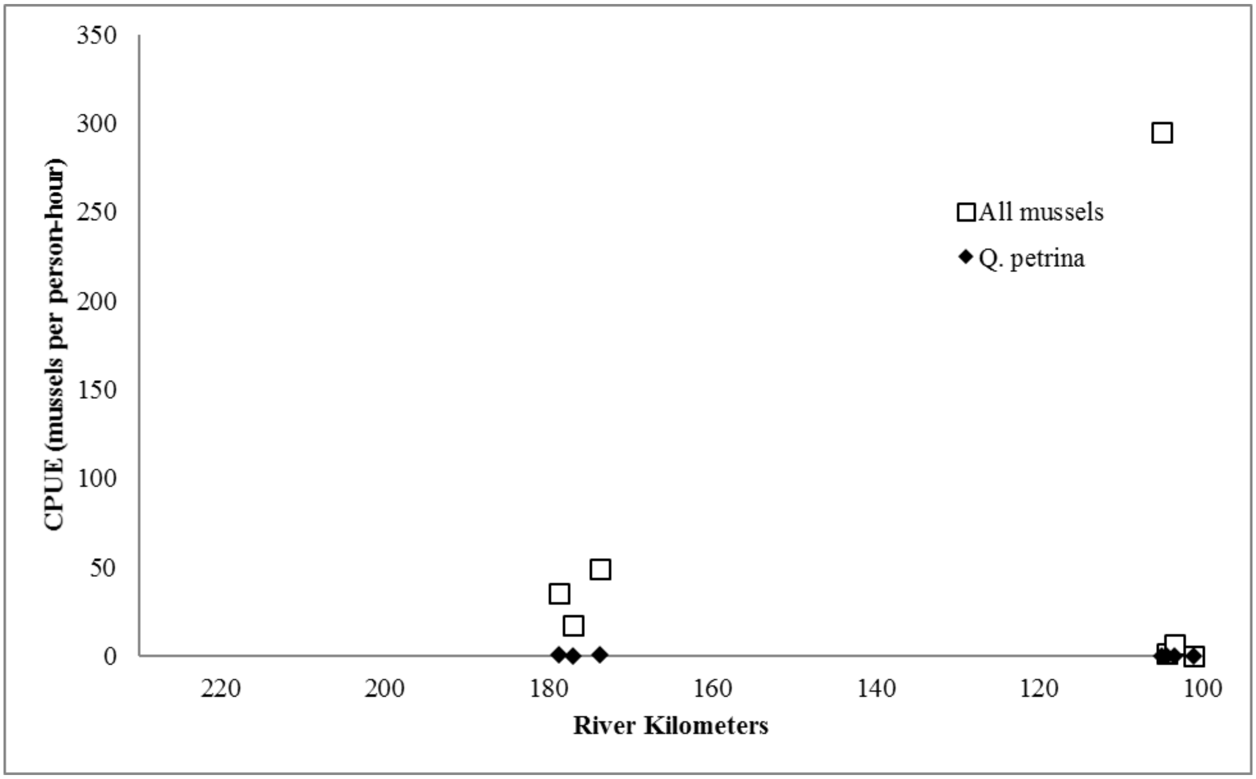

Figure 9. Catch-per-unit effort (CPUE) of *Quadrula petrina* (Texas pimpleback) and all mussel species (All mussels) in the Guadalupe River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the San Antonio Bay (0 River Kilometers). CPUE = total number of either *Q. petrina* or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

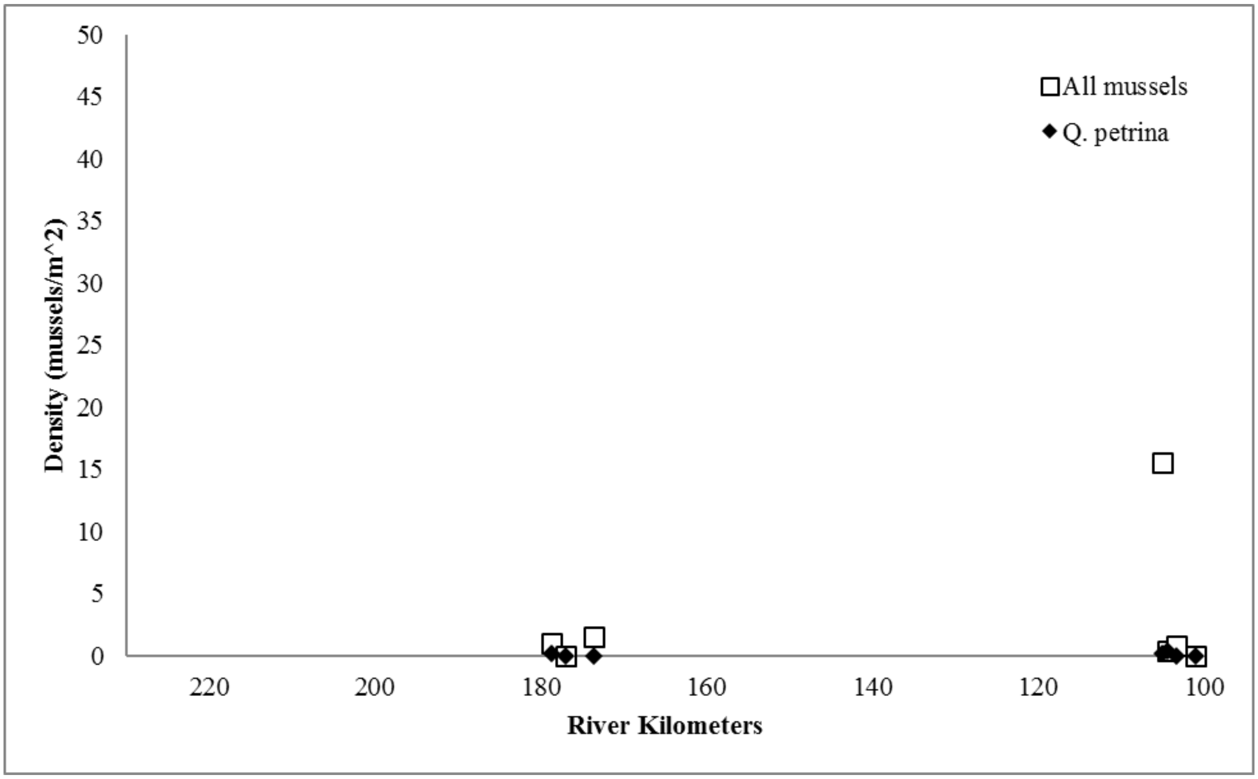

Figure 10. Densities of *Quadrula petrina* (Texas pimpleback) and all mussel species (All mussels) in the Guadalupe River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the San Antonio Bay (0 River Kilometers).

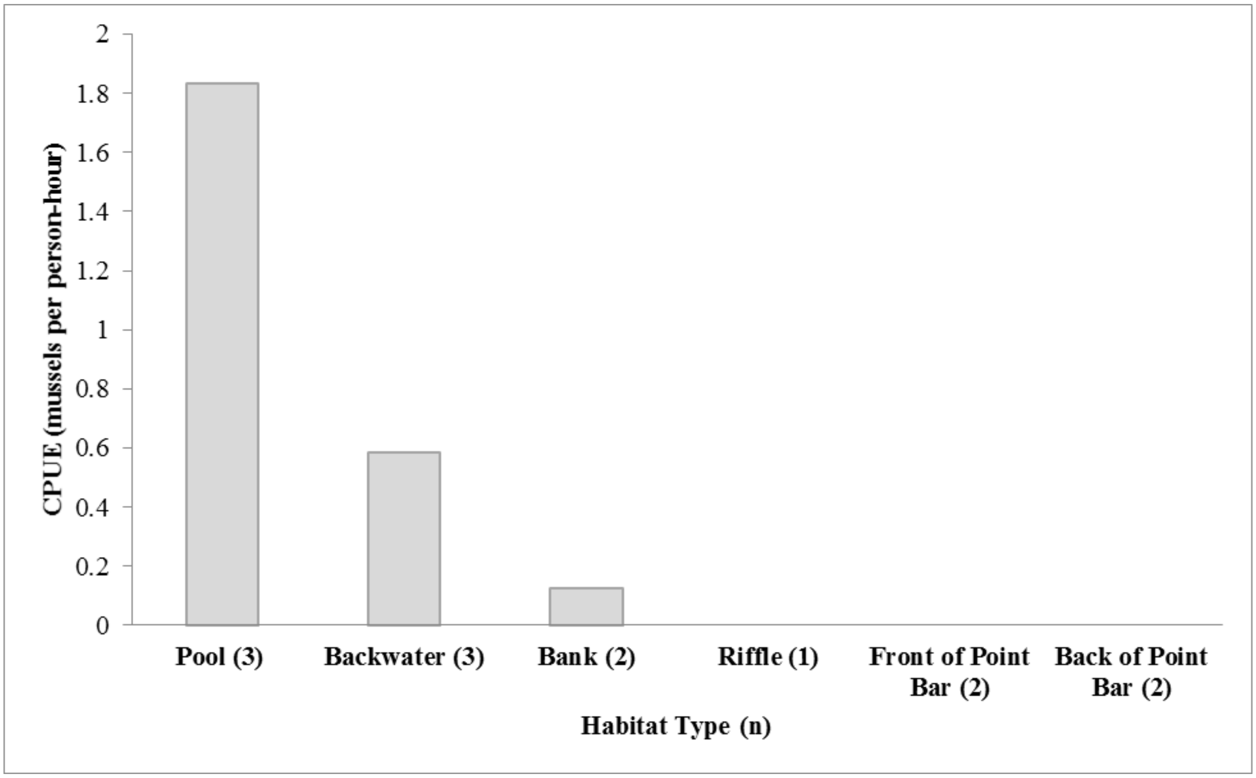

Figure 11. Catch-per-unit-effort (CPUE) of *Quadrula petrina* (Texas pimpleback) by mesohabitat type in the Guadalupe River. The number of sites sampled at each habitat are listed in parenthesis.

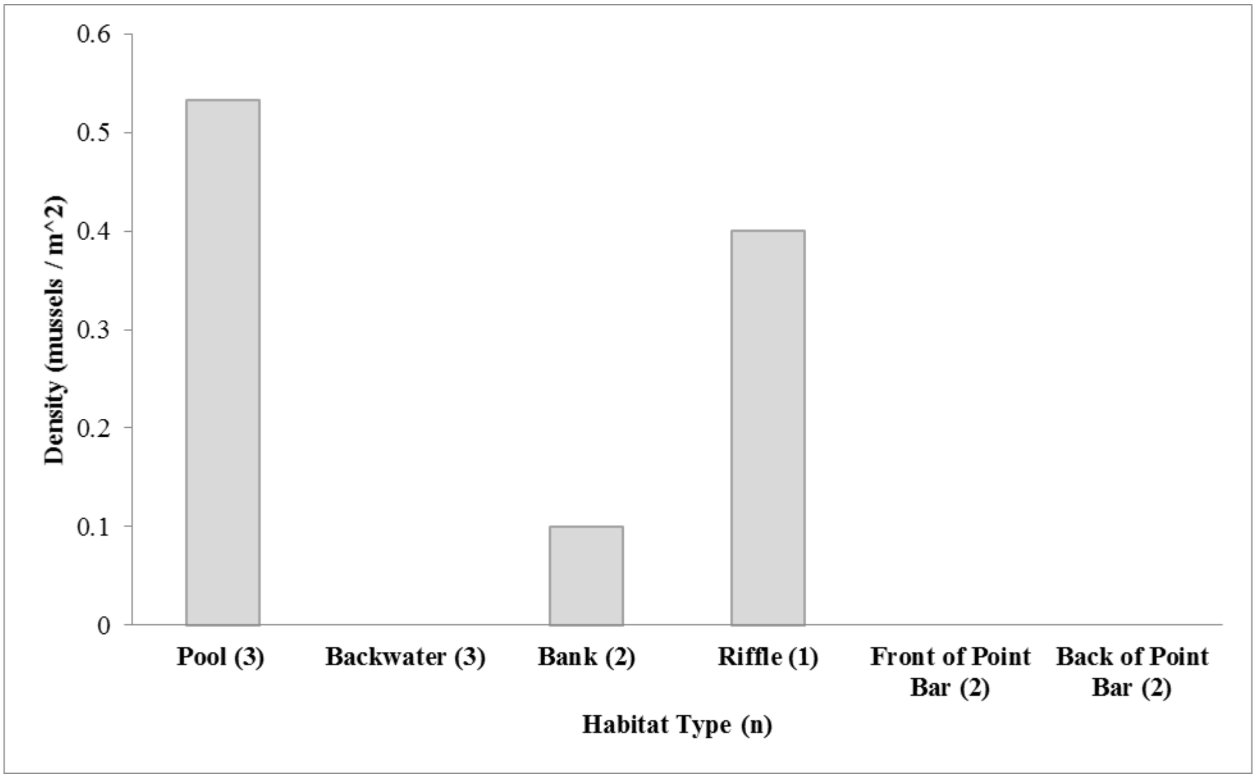

Figure 12. Density of *Quadrula petrina* (Texas pimpleback) by mesohabitat type in the Guadalupe River. The number of sites sampled at each habitat are listed in parenthesis. Density is calculated as the mean number of *Q. petrina* per 0.25 m² quadrat.

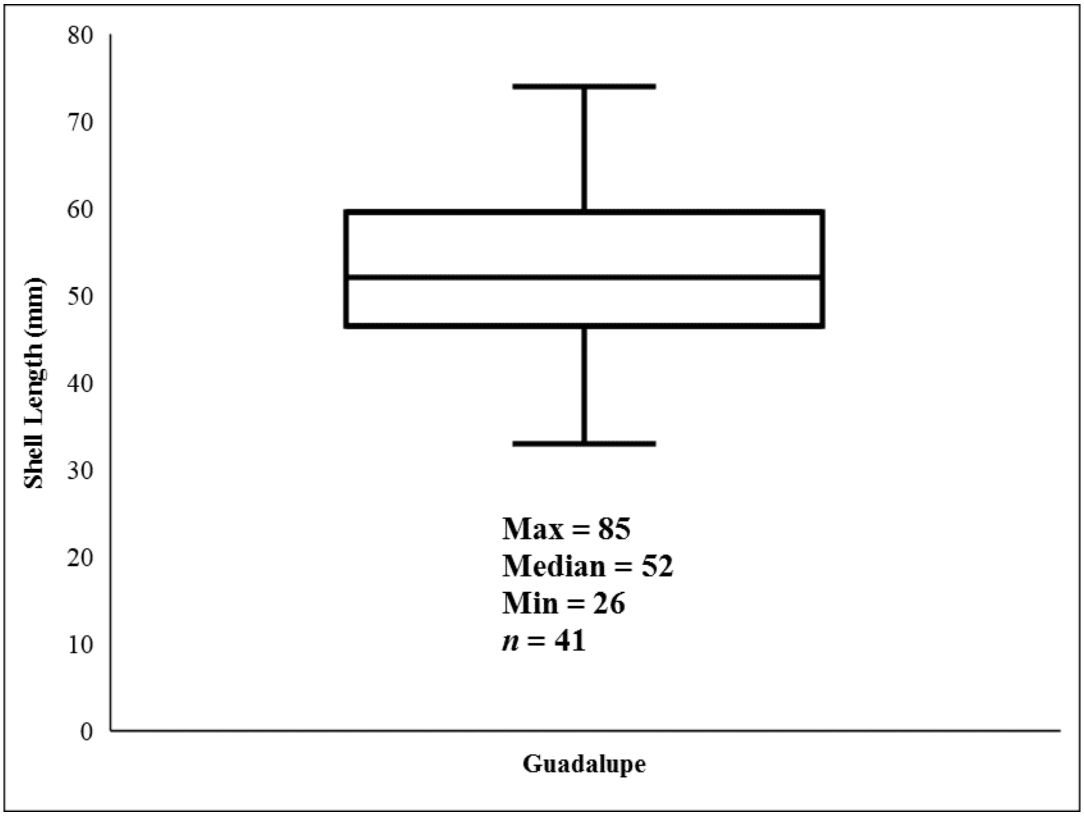

Figure 13. Shell length data for *Q. petrina* (Texas pimpleback) populations in the Guadalupe River drainage.

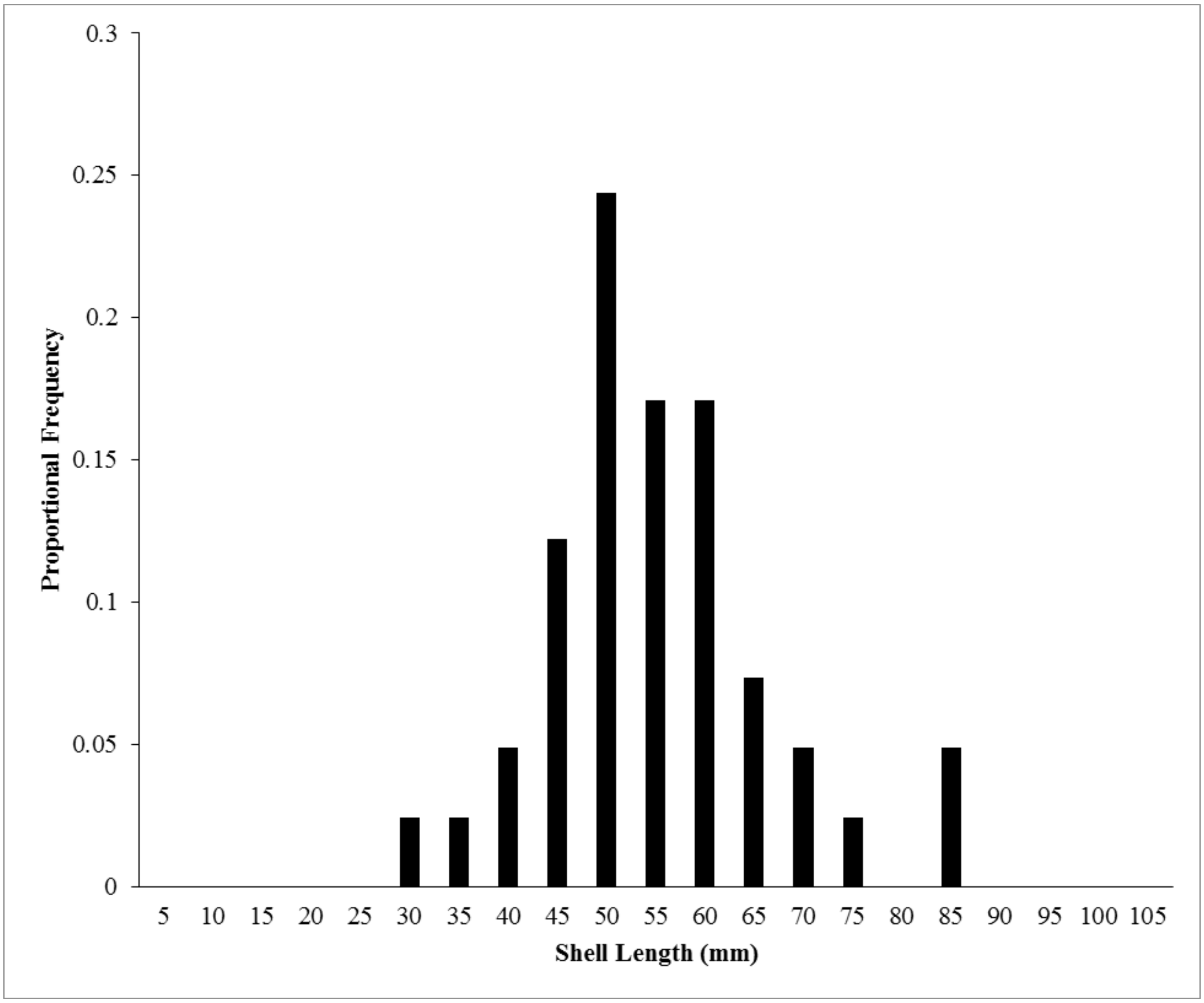

Figure 14. Proportional frequency of shell lengths for *Quadrula petrina* (Texas pimpleback) Guadalupe River (*n* = 41). Shell lengths are binned into 5 mm groups.

Distribution and Habitat Use for *Truncilla macrodon* (Texas Fawnsfoot)

Section Summary

The goal of this study was to determine the status, distribution, and mesohabitat associations for *Truncilla macrodon* (Texas fawnsfoot), a candidate for protection under the U.S. Endangered Species Act, in the Brazos, and Colorado River drainages. We used recent and historical data to inform a sampling program within the range of *T. macrodon.* In total, we surveyed 117 sites across 6 tributaries of the Brazos and Colorado rivers, and found 4 live *T. macrodon* from 2 of 117 (2%) sites. In the Brazos River drainage, we surveyed 59 sites across Brushy Creek and the San Gabriel and Little rivers and found 4 live *T. macrodon.* The live individuals were found at 2 of the 9 (22%) sites on the Little River. No live individuals or shell material were observed from Brushy Creek or the San Gabriel River. In the Colorado River drainage, we surveyed 58 sites across the Llano, San Saba, and Pedernales rivers and no live individuals or shell material were observed.

Introduction

Truncilla macrodon, Texas fawnsfoot, is known historically from the Brazos and Colorado River drainages of Central Texas (Howells 2010). The type specimen was collected from the Colorado River near Rutersville, Texas by C. G. Forshey and described by Isaac Lea (Lea 1859; USNM_25714). The species was initially placed into *Margaron* and *Plagiola*; however, it was ultimately moved into *Truncilla* by Rafinesque (1820). *Truncilla macrodon* is currently recognized as a taxonomically valid species (Howells et al. 1996, Turgeon et al. 1998).

In the Brazos River basin, historic records of *T. macrodon* have primarily come from the mainstem of the Brazos River, though several observations have been reported from its large tributaries. *Truncilla macrodon* has been reported in the Brazos River from Austin/Waller (C. M. Mather, CMM 3190), Bosque (D.H. Stansbery, OSUM_15742), Brazos (C. M. Mather, CMM 1123; Singley 1893; D.H. Stansbery, OSUM_52020; J. K. Strecker, BU-MMC_MO 32591-A-B), Burleson (D.H. Stansbery, OSUM_22486), Hood (L. Johnson, JBFMC_2402), Robertson (Singley 1893, D.H. Stansbery, OSUM_42848), Milam (D.H. Stansbery, OSUM_22486), Palo Pinto (C. M. Mather, CMM 2748) and Somervell (W. C. Starnes and L. B. Starnes, NCSM_7165) counties. Others have reported *T. macrodon* from a several tributaries of the Brazos River: Aquilla Creek in McLennan County (Strecker 1931); Big Creek in Fort Bend County (Athearn_19161); Brushy Creek in Milam County (H. G. Askew, UMMZ_71037); Clear Fork in Stephens (J. Bergman, TX0274 [R. G. Howells database]), Shackelford (N. F. Drake, JBFMC_1474) and Young counties (W. F. Cummings, JBFMC_1513); Leon River in Coryell County (Strecker 1931, BU-MMC_MO31987-A-B), North Bosque River in McLennan County (J. K. Strecker, BU-MMC_MO 31987-A-B); and Paluxy Creek from Somervell County (BU-MMC_MO31297-A-B). Live individuals have also been collected in Lake Brazos, Waco, TX (C.E. Fontanier, BU-MMC_MO36660-A-B), and weathered shells have been collected from the Navasota River in Brazos County (USAO_2739).

In the Colorado River basin, historic records of *T. macrodon* have come from the mainstem of the Colorado River and its major tributaries. *Truncilla macrodon* has been reported in the Colorado River from Bastrop (J. Bergman, JAMP TX0046), Burnet (C. F. Stapp, BU-MMC_MO 32673-A-B), Colorado (Burlakova & Karatayev 2010; N. Johnson, FLMNH_440984; Howells 1997; Strecker 1931), Coryell (Strecker 1931), Travis (Athearn_1417; J.K. Strecker, BU-MMC_MO 31918-A-B), and Wharton County (C. M. Mather, CMM 2863; J. A. Singley, FLMNH_269818; Strecker 1931). Other observations of *T. macrodon* have been reported from tributaries of the Colorado River: Little Colorado River in Wharton County (J. Bequaert, ANSP_132100); Llano River in Mason (A. L. Fitzpatrick, BU-MMC_MO 32728-A-B; Strecker 1931) and Llano counties (JBFMC_1493); San Saba River in San Saba County (C. R. Randklev, JBFMC_8002); South Concho River (W. J. Williams, BU-MMC_SM 2599). Specimens were also observed in an unnamed creek near Mertzon, TX (a tributary of the South Concho River) (Randklev et al. 2010b.).

Although Strecker (1931) originally remarked that *T. macrodon* was abundant in the Brazos and Colorado River drainages, based on the prevalence of shell material, until recently this species was considered to be extirpated from most of its range (Howells 2010, Randklev et al. 2010). As a result, *T. macrodon* is listed as state threatened by the Texas Parks and Wildlife Department (TPWD 2010) and a candidate for listing under the U.S. Endangered Species Act (USFWS 2011). Currently, the American Fisheries Society lists *T. macrodon* as endangered (Williams et al. 1993) and NatureServe ranks it as imperiled. Since the 1990s, live and recently dead individuals have been observed from the following rivers in the Brazos River drainage: Clear Fork (Shackelford, Stephens, and Young counties); upper Brazos River (Palo Pinto, Parker, and Somervell counties); central Brazos River (Brazos, Burleson, McClennan, Grimes, and Washington counties); lower Brazos River (Austin, Fort Bend, and Waller counties); Little River (Milam County); Navasota River (Brazos and Grimes counties); Yegua Creek (Burleson and Washington counties); Big Creek (Fort Bend County); and Deer Creek (Falls County) (Howells 1996, 1997, 2009, 2010, Karatayev and Burlakova 2008, Randklev et al. 2010, 2013a, 2013c, 2014, Tsakiris and Randklev 2016). In the Colorado River drainage live and recently dead individuals have been reported from the Colorado River (Colorado, Mills and San Saba counties) and San Saba River (San Saba County) (Howells 2000, 2009, 2010, Burlakova and Karatayev 2010, Randklev et al. 2013c, Sowards et al. 2013).

Currently, little is known about the life history or reproductive requirements of *T. macrodon* (Howells 2010). Like other freshwater mussel species, it is likely an obligate ectoparasite on one or more host-fish species, and its congeners appear to be long-term brooders that are host specialists of *Aplodinotus grunniens*, Freshwater Drum (Haag 2012). Based on recent observations from field surveys throughout *T. macrodon's* range, adults appear to occur most often in bank habitats and occasionally in backwater, riffle, and point bar habitats with low to moderate water velocities and fine or coarse sediments (Randklev et al. 2014). These mesohabitat types appear to serve as flow refuges, where near-bed shear stress remains low during high flow events.

The objectives of this study were to assess the distribution, abundance, and habitat use for *T. macrodon* in the Brazos and Colorado River drainages. The resulting survey information was then used to develop Conservation Status Assessment Maps for this species within the Brazos and Colorado River drainages**.**

Methods

Study Area

The Brazos River originates near the Texas-New Mexico border, runs southeast for approximately 1900 km into the Gulf of Mexico and drains a total of 118,000 km^2 (Kammerer 1990). The Little River, San Gabriel River, and Brushy Creek are all part of the Little River system, a tributary of the Brazos River, which drains the Edwards Plateau and Blackland Prairie regions of Central Texas (Rose and Echelle 1981). The Little River system transitions from high gradient streams in the upper San Gabriel watershed to low gradient streams in the confluence with the Brazos River. The sediment type of the lower

Little River includes clay, silt, and woody debris, and the land use types include urban areas and agricultural land (Labay 2010). The Little River is formed by the confluence of the Leon and Lampasas rivers in Bell County and flows for 258 km, draining an area of approximately 12,485 km^2 before emptying into the Brazos River in Milam County (Rose and Echelle 1981). The San Gabriel River begins in Georgetown where the North and South Forks converge, and flows east for roughly 80 km through Williamson and Milam counties until its confluence with the Little River (Belisle and Josselet 1977). The major impoundment of the San Gabriel River is Granger Lake in Williamson County, a 1619 hectare reservoir used primarily for flood control (Mcalister et al. 2013). Brushy Creek originates in Williamson County and flows east through Milam County for 111 km before emptying into the San Gabriel River near Rockdale (Belisle and Josselet 1977).

The Colorado River originates in northeastern Dawson County, Texas, runs approximately 1,040 km to the Gulf of Mexico and drains an area of 100,000 km^2 (Huser 2000). The San Saba, Llano, Pedernales rivers are three major tributaries of the upper Colorado River that originate in the Edwards Plateau region (Higgins 2009). The San Saba River begins in Schleicher County and flows 225 km until its confluence with the Colorado River (Belisle and Josselet 1977). The Llano River originates in Kimble County and flows 161 km until emptying into Lake Lyndon B. Johnson, an impoundment on the Colorado River. The Pedernales River also originates in Kimble County and flows approximately 170 km until emptying into Lake Travis, another major impoundment on the Colorado River (Perkin et al. 2010).

Sampling Methods

Survey sites within the Brazos and Colorado River drainages were selected using a stratified random sampling design with the following strata: (1) upstream or downstream of an access point (e.g., bridge crossing); (2) linear distance (river-kilometers) from an access point; and (3) mesohabitat (banks, backwater, mid-channel, riffles, and pools). We first used aerial imagery to delineate and assign numbers to mesohabitats between bridge crossings that could be accessed by canoe or motorboat. Random number generator was then used to select habitat type and distance from a bridge randomly. For locations where specific habitats could not be identified using satellite imagery (e.g., riffles), we modified our sampling design by surveying the first habitat encountered for that target habitat type. In total, 20 sites in the Llano River, 19 sites in the Pedernales River, and 19 sites in the San Saba River were selected for sampling in the Colorado River basin. In the Brazos River basin, we randomly selected 30 sites in Brushy Creek, 20 sites in the San Gabriel River, and 9 sites in the Little River for sampling. These sites include sites that have and have not been sampled historically.

Qualitative surveys using the timed search method were performed at each site (i.e., mesohabitat type). The timed search method was chosen because it provides a more effective means of detecting rare species than quantitative sampling methodologies (Vaughn et al. 1997). At each site we confined the search boundaries within the randomly selected mesohabtiat and standardized the search area to 150 m^2, though in some cases the search area included multiple mesohabitat types (e.g., pool-run or riffle-run habitats).

Each site was surveyed tactilely and visually for a total of 4 person-hours (p-h). Because our focus was to determine the amount of effort needed to detect *T. macrodon* (which will be important for designing long-term monitoring programs), we divided the total search time into 4, 1 p-h intervals. At the end of each search interval, surveyors combined all live specimens into a mesh bag, which was kept submerged in water until completion of the survey. During each interval, surveyors were spread out in the search area and every effort was made to search all available microhabitats. Following completion of the survey, all live mussels from each time period were identified to species, counted, measured, checked for gravidity, and then returned back to the river into the appropriate habitat.

Quantitative surveys using the quadrat sampling method were performed in each mesohabitat in the Guadalupe River. The quadrat method provides a more effective means of detecting juveniles, and thus, can estimate demographic parameters in areas with high abundance more accurately (Vaughn et al. 1997). For quantitative sampline, we subdivided the 150 m^2 search area into a square meter grid and 20 points were selected within the grid using a random number generator. At each randomly selected point, quadrats were sampled by excavating sediment up to 15 cm in depth using a modified Surber sampler with a 0.25 m2 search area. Sediment was sieved through 3.175 mm mesh screen, and all live specimens from each quadrat were placed into a mesh bag, which was kept submerged in water until completion of the survey. Following completion of the survey, all live mussels from each quadrat were identified to species, counted, measured, checked for gravidity, and then returned back to the river into the appropriate habitat. For sites where both sampling methods were used, quantitative sampling occurred prior to the timed-search method as explained above.

Data Analysis

Scatter plots of abundance (CPUE: number of individuals/total person-hours) vs. river kilometer (RKM) were used to examine the effect of stream position on total mussel and *T. macrodon* abundance in each river. Boxplots and length-frequency histograms were developed for *T. macrodon* to assess demographic patterns and population structure. Generally, multimodal size class distribution may indicate recruitment, whereas truncated distributions (absence of a particular age class, large, or small individuals) may indicate a lack of recent recruitment or a localized extinction event. Bar graphs were also used to visually represent presence of *T. macrodon* by mesohabitat type (i.e., riffle, mid-channel, pool, bank, and backwater).

Results/Discussion

In total, 236 p-h were spent surveying mussels at 59 sites in Brushy Creek, San Gabriel River, and Little River of the Brazos River drainage (Figure 1). Overall, we found 4 live individuals of *T. macrodon*, which were found at 2 of 9 (22.2%) sites in the Little River. No live individuals or shell material of *T. macrodon* were found in Brushy Creek (30 sites surveyed) or the San Gabriel River (20 sites surveyed). To our knowledge, since the last records by Strecker (1931), there are no records of occurrence from Brushy Creek and live individuals recorded only once in 2009 from the Little River (Jack Davis, pers. comm. Randklev et al. 2013a). Thus, these 4 individuals represent one of the few recent observations of live individuals in the Little River. Catch-per-unit-effort (CPUE) of *T. macrodon* ranged from 0 to 0.25 mussels/p-h and averaged 0.03 ± 0.03 mussels/hr (± SE) in the Little River (Table 1), while CPUE measured for all mussels averaged 7.7 ± 2.3 mussels/p-h (Figure 2). Density of *T. macrodon* in the Little River ranged from 0 to 0.4 mussels/m^2 and averaged 0.07 ± 0.05 mussels/m^2 (Table 1). In comparison, density measured for all mussels in the Little River averaged 1.2 ± 0.7 mussels/m^2 (Figure 3). As such, relative abundance of *T. macrodon* was low but nearly 20× greater for quantitative sampling (4.3%) than qualitative sampling (0.2%), owing to the higher detection of small-bodied mussel species (in this case, *T. macrodon*) typically observed in quadrat surveys (Vaughn et al. 1997). The highest average CPUE (0.125 mussels/p-h) and density (0.2 mussels/m^2) of *T. macrodon* were observed in backwater and riffle habitat, respectively (Figures 4 and 5). Habitat associations from the lower Brazos River indicate that *T. macrodon* prefers deep bank habitat, and occasionally point bar, riffle, or backwater habitats (Randklev et al. 2014). Preliminary results seem to support this trend (Tables 4 and 5). Median shell length for the Little River populations was 25.5 mm and minimum and maximum shell lengths were 17 mm and 49 mm, respectively (Figure 6).

In total, 232 p-h were spent surveying mussels at 58 sites in the San Saba, Llano, and Pedernales rivers of the Colorado River drainage (Figure 7). No live individuals or shell material of *T. macrodon* were found; however, recent surveys conducted within the Colorado River drainage have yielded live individuals. In the Colorado River, near Garwood, TX, Burlakova and Karatayev (2010) found an average density of 0.62 ± 1.99 mussels/m^2 (± SE) of *T. macrodon*. In the lower San Saba River, Randklev et al. (2013b) found a live individual in the San Saba River near the confluence with the Colorado River, and Sowards et al. (2013) found 3 live *T. macrodon* in the San Saba River in San Saba County. Tsakiris et al. (2014) found 7 live individuals from two sites in the lower San Saba River, and CPUE measured 0.04 mussels/p-h for *T. macrodon* and 8.9 mussels/p-h for all mussels.

In summary, our results indicate that *T. macrodon* occurs within the Little River (Brazos River drainage) in low densities, and if it occurs in the San Gabriel River or Brushy Creek, densities are too low to detect using conventional sampling methods. Because the majority of our sites occurred in a short segment of the Little River, large sections of river remain unsampled within that drainage. Within the Colorado River drainage, our results and those of recent surveys indicate that *T. macrodon* occurs in low densities in the lower San Saba River. There are no records of this species from the Pedernales River, and no recent records from the upper San Saba River or the Llano River since it was last recorded by Strecker (1931).

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Table 1. Locality and collection information for mussel survey sites in the Brazos River drainage. CPUE = total number of *T. macrodon* encountered at each site during qualitative sampling divided by the number of person hours (4) searched at each site. Density = mean number of *T. macrodon* per 0.25 m^2 quadrat. Habitat key: B = Bank, BW = Backwater, MC = Mid-Channel, P = Pool, R = Riffle. Sites are ordered upstream to downstream.

Site/ Reach	Habitat	Locality	County	Date of collection	Number of Live	CPUE	Density	Sub Adult	Effort		Area (m^2)
									Hours	Quadrats	
1A	B	Brushy Creek	Williamson	7/13/2015	0	0	n/a	-	4	0	150
2A	MC	Brushy Creek	Williamson	7/13/2015	0	0	n/a	-	4	0	150
3A	P	Brushy Creek	Williamson	7/14/2015	0	0	n/a	-	4	0	150
4A	MC	Brushy Creek	Williamson	7/14/2015	0	0	n/a	-	4	0	150
5A	P	Brushy Creek	Williamson	7/14/2015	0	0	n/a	-	4	0	150
6A	B	Brushy Creek	Williamson	7/14/2015	0	0	n/a	-	4	0	150
7A	MC	Brushy Creek	Williamson	7/16/2015	0	0	n/a	-	4	0	150
8A	BW	Brushy Creek	Williamson	7/16/2015	0	0	n/a	-	4	0	150
9A	R	Brushy Creek	Williamson	7/16/2015	0	0	n/a	-	4	0	150
10A	BW	Brushy Creek	Williamson	7/16/2015	0	0	n/a	-	4	0	150
11B	R	Brushy Creek	Williamson	7/15/2015	0	0	n/a	-	4	0	150
12B	B	Brushy Creek	Williamson	7/15/2015	0	0	n/a	-	4	0	150
13B	R	Brushy Creek	Williamson	7/15/2015	0	0	n/a	-	4	0	150
14B	MC	Brushy Creek	Williamson	7/15/2015	0	0	n/a	-	4	0	150
15B	P	Brushy Creek	Williamson	7/15/2015	0	0	n/a	-	4	0	150
16B	B	Brushy Creek	Milam	7/15/2015	0	0	n/a	-	4	0	150
17B	B	Brushy Creek	Milam	7/15/2015	0	0	n/a	-	4	0	150
18B	MC	Brushy Creek	Milam	7/16/2015	0	0	n/a	-	4	0	150
19B	P	Brushy Creek	Milam	7/16/2015	0	0	n/a	-	4	0	150
20B	B	Brushy Creek	Milam	7/16/2015	0	0	n/a	-	4	0	150
21C	R	Brushy Creek	Milam	7/7/2015	0	0	n/a	-	4	0	150
22C	P	Brushy Creek	Milam	7/7/2015	0	0	n/a	-	4	0	150
23C	R	Brushy Creek	Milam	7/10/2015	0	0	n/a	-	4	0	150
24C	B	Brushy Creek	Milam	7/7/2015	0	0	n/a	-	4	0	150
25C	BW	Brushy Creek	Milam	7/6/2015	0	0	n/a	-	4	0	100
26C	P	Brushy Creek	Milam	7/10/2015	0	0	n/a	-	4	0	150
27C	MC	Brushy Creek	Milam	7/7/2015	0	0	n/a	-	4	0	150
28C	BW	Brushy Creek	Milam	7/7/2015	0	0	n/a	-	4	0	150
29C	MC	Brushy Creek	Milam	7/10/2015	0	0	n/a	-	4	0	150
30C	B	Brushy Creek	Milam	7/6/2015	0	0	n/a	-	4	0	100
31F	BW	Little	Milam	4/28/2015	0	0	0	-	4	20	105
32F	R	Little	Milam	4/28/2015	2	0	0.4	Y	4	20	100
33F	BW	Little	Milam	4/29/2015	2	0.25	0.2	Y	4	20	140
34F	MC	Little	Milam	4/29/2015	0	0	0	-	4	20	100
35F	P	Little	Milam	4/29/2015	0	0	0	-	4	20	100
36F	R	Little	Milam	4/30/2015	0	0	0	-	4	20	104
37F	B	Little	Milam	4/30/2015	0	0	0	-	4	20	100
38F	MC	Little	Milam	4/30/2015	0	0	0	-	4	20	100
39F	B	Little	Milam	4/30/2015	0	0	0	-	4	20	100
40D	MC	San Gabriel	Williamson	7/8/2015	0	0	n/a	-	4	0	150
41D	B	San Gabriel	Williamson	7/8/2015	0	0	n/a	-	4	0	150
42D	BW	San Gabriel	Williamson	7/8/2015	0	0	n/a	-	4	0	150

Table 1. Continued.

Site	Habitat	Locality	County	Date of collection	Number of Live	CPUE	Density / m^2	Sub Adult	Effort		Area (m^2)
									Hours	Quadrats	
43D	P	San Gabriel	Williamson	7/9/2015	0	0	n/a	-	4	0	150
44D	MC	San Gabriel	Williamson	7/8/2015	0	0	n/a	-	4	0	150
45D	BW	San Gabriel	Williamson	7/9/2015	0	0	n/a	-	4	0	150
46D	R	San Gabriel	Williamson	7/9/2015	0	0	n/a	-	4	0	150
47D	B	San Gabriel	Williamson	7/8/2015	0	0	n/a	-	4	0	150
48D	R	San Gabriel	Williamson	7/9/2015	0	0	n/a	-	4	0	150
49D	P	San Gabriel	Williamson	7/9/2015	0	0	n/a	-	4	0	150
50E	BW	San Gabriel	Milam	4/27/2015	0	0	0	-	4	20	100
51E	P	San Gabriel	Milam	4/20/2015	0	0	0	-	4	20	90
52E	MC	San Gabriel	Milam	4/20/2015	0	0	0	-	4	20	100
53E	B	San Gabriel	Milam	4/21/2015	0	0	0	-	4	20	90
54E	R	San Gabriel	Milam	4/21/2015	0	0	0	-	4	20	100
55E	R	San Gabriel	Milam	4/22/2015	0	0	0	-	4	20	100
56E	MC	San Gabriel	Milam	4/23/2015	0	0	0	-	4	20	90
57E	BW	San Gabriel	Milam	4/22/2015	0	0	0	-	4	20	90
58E	P	San Gabriel	Milam	4/27/2015	0	0	0	-	4	20	100
59E	B	San Gabriel	Milam	4/23/2015	0	0	0	-	4	20	100

Table 2. Locality and collection information for mussel survey sites in the Colorado River drainage. CPUE = total number of *T. macrodon* encountered at each site divided by the number of person hours (4) searched at each site. Habitat key: BW = Backwater, P = Pool, R = riffle, B = Bank, MC = Mid-Channel, PR = Pool/Run combined, RR = Riffle/Run combined, All = site encompassed multiple habitat types. Sites are ordered upstream to downstream in each river.

Site	Habitat	Locality	County	Date of collection	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
1A	B	San Saba	Menard	8/5/2015	0	0	-	4	150
2A	P	San Saba	Menard	8/5/2015	0	0	-	4	150
3A	P	San Saba	Menard	8/5/2015	0	0	-	4	150
4A	B	San Saba	Menard	8/3/2015	0	0	-	4	150
5A	P	San Saba	Menard	8/4/2015	0	0	-	4	150
6A	P	San Saba	Menard	8/4/2015	0	0	-	4	150
7A	BW	San Saba	Menard	8/4/2015	0	0	-	4	150
8A	B	San Saba	Menard	8/4/2015	0	0	-	4	150
9A	B	San Saba	Menard	8/5/2015	0	0	-	4	150
10A	P	San Saba	Menard	8/5/2015	0	0	-	4	150
11B	R	San Saba	Menard	8/6/2015	0	0	-	4	150
12B	B	San Saba	Menard	8/6/2015	0	0	-	4	150
13B	BW	San Saba	Menard	8/6/2015	0	0	-	4	150
14B	P	San Saba	Menard	8/6/2015	0	0	-	4	150
15B	B	San Saba	Menard	8/6/2015	0	0	-	4	150
16C	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
17C	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
18C	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
19C	P	San Saba	McCulloch	8/7/2015	0	0	-	4	150
20D	P	Llano	Mason	7/30/2015	0	0	-	4	150
21D	PR	Llano	Mason	7/30/2015	0	0	-	4	150
22D	PR	Llano	Mason	7/30/2015	0	0	-	4	150
23D	PR	Llano	Mason	7/30/2015	0	0	-	4	150
24D	B	Llano	Mason	7/30/2015	0	0	-	4	150
25D	PR	Llano	Mason	7/29/2015	0	0	-	4	150
26D	B	Llano	Mason	7/29/2015	0	0	-	4	150
27D	P	Llano	Mason	7/31/2015	0	0	-	4	150
28E	B	Llano	Llano	7/29/2015	0	0	-	4	150
29E	P	Llano	Llano	7/29/2015	0	0	-	4	150
30E	B	Llano	Llano	7/29/2015	0	0	-	4	150
31E	P	Llano	Llano	7/29/2015	0	0	-	4	150
32E	P	Llano	Llano	7/28/2015	0	0	-	4	150
33E	B	Llano	Llano	7/28/2015	0	0	-	4	150
34E	B	Llano	Llano	7/28/2015	0	0	-	4	150
35E	PR	Llano	Llano	7/28/2015	0	0	-	4	150
36F	P	Llano	Llano	7/27/2015	0	0	-	4	150
37F	B	Llano	Llano	7/28/2015	0	0	-	4	150
38F	PR	Llano	Llano	7/27/2015	0	0	-	4	150
39F	B	Llano	Llano	7/27/2015	0	0	-	4	150
40G	P	Live Oak Creek	Gillespie	6/24/2015	0	0	-	4	150
41G	P	Live Oak Creek	Gillespie	6/24/2015	0	0	-	4	150
42G	All	Live Oak Creek	Gillespie	6/23/2015	0	0	-	4	150
43G	All	Live Oak Creek	Gillespie	6/23/2015	0	0	-	4	150

Table 2. Continued.

Site	Habitat	Locality	County	Date of collection	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
44G	B	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
45G	P	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
46G	B	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
47G	B	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
48G	BW	Pedernales	Gillespie	6/24/2015	0	0	-	4	150
49H	MC	Pedernales	Gillespie	6/23/2015	0	0	-	4	150
50H	B	Pedernales	Gillespie	6/23/2015	0	0	-	4	150
51H	RR	Pedernales	Blanco	6/23/2015	0	0	-	4	150
52H	B	Pedernales	Blanco	6/23/2015	0	0	-	4	150
53H	BW	Pedernales	Blanco	6/23/2015	0	0	-	4	150
54I	P	Pedernales	Blanco	6/22/2015	0	0	-	4	150
55I	RR	Pedernales	Blanco	6/22/2015	0	0	-	4	150
56I	P	Flat Creek	Blanco	6/25/2015	0	0	-	4	150
57I	BW	Pedernales	Travis	6/25/2015	0	0	-	4	150
58I	B	Pedernales	Travis	6/25/2015	0	0	-	4	150

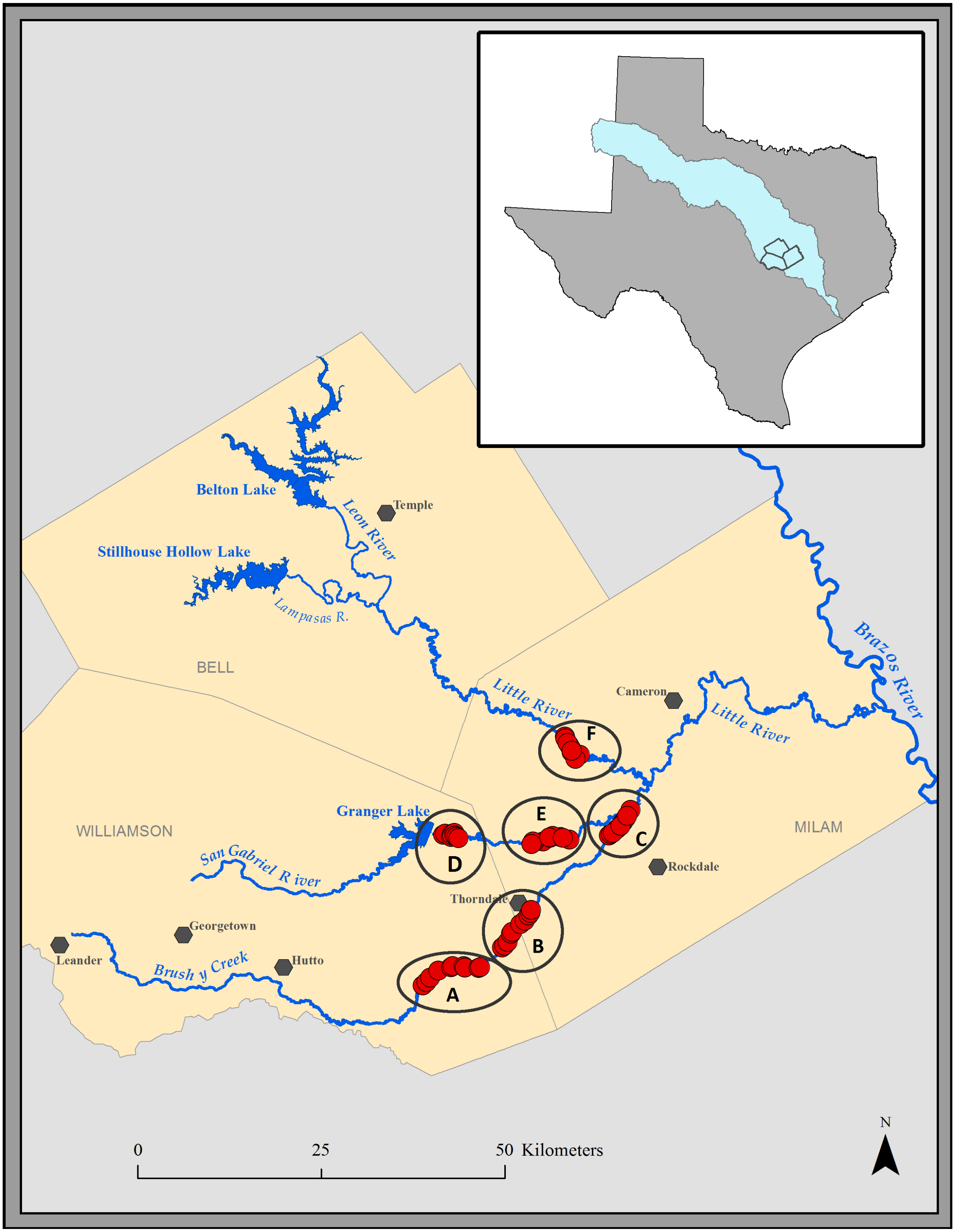

Figure 1. Map of Brazos River drainage with shaded (red) circles denoting sampling locations. Reaches are indicated by letter and correspond to Table 1.

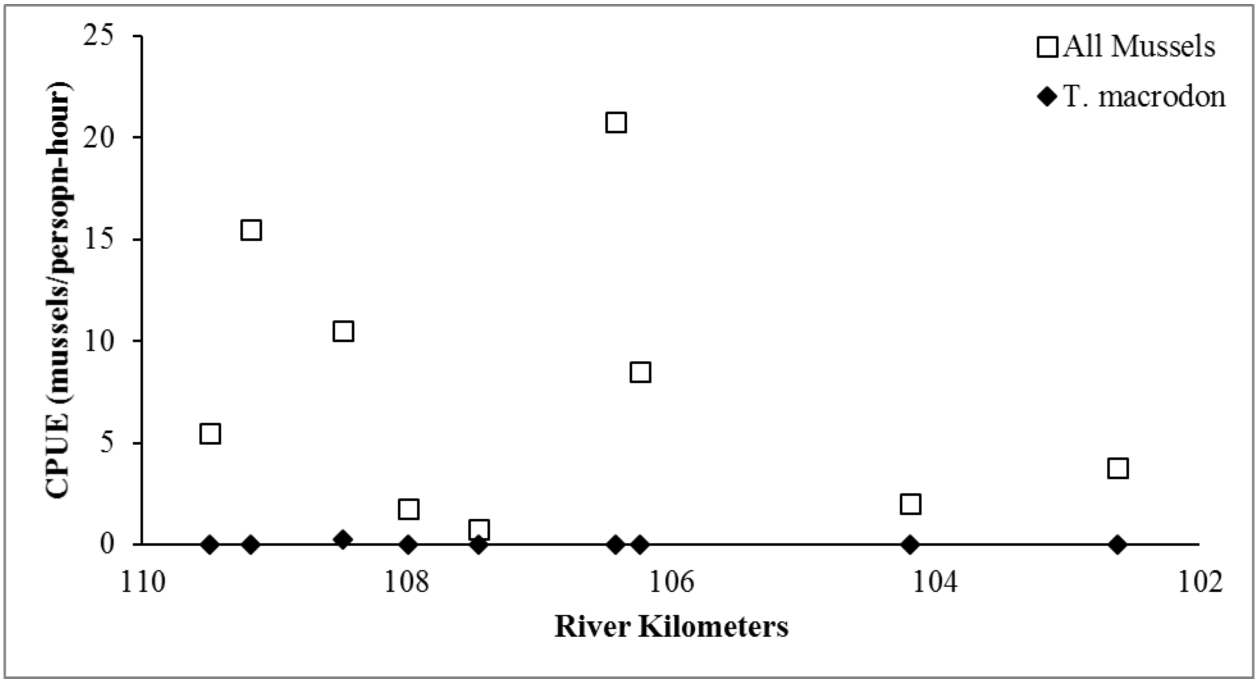

Figure 2. Catch-per-unit effort (CPUE) of *Truncilla macrodon* (Texas fawnsfoot) and all mussel species (All mussels) in the Little River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Brazos River (0 River Kilometers). CPUE = total number of either *T. macrodon* or all mussels encountered at each site divided by the number of person hours (4) searched at each site.

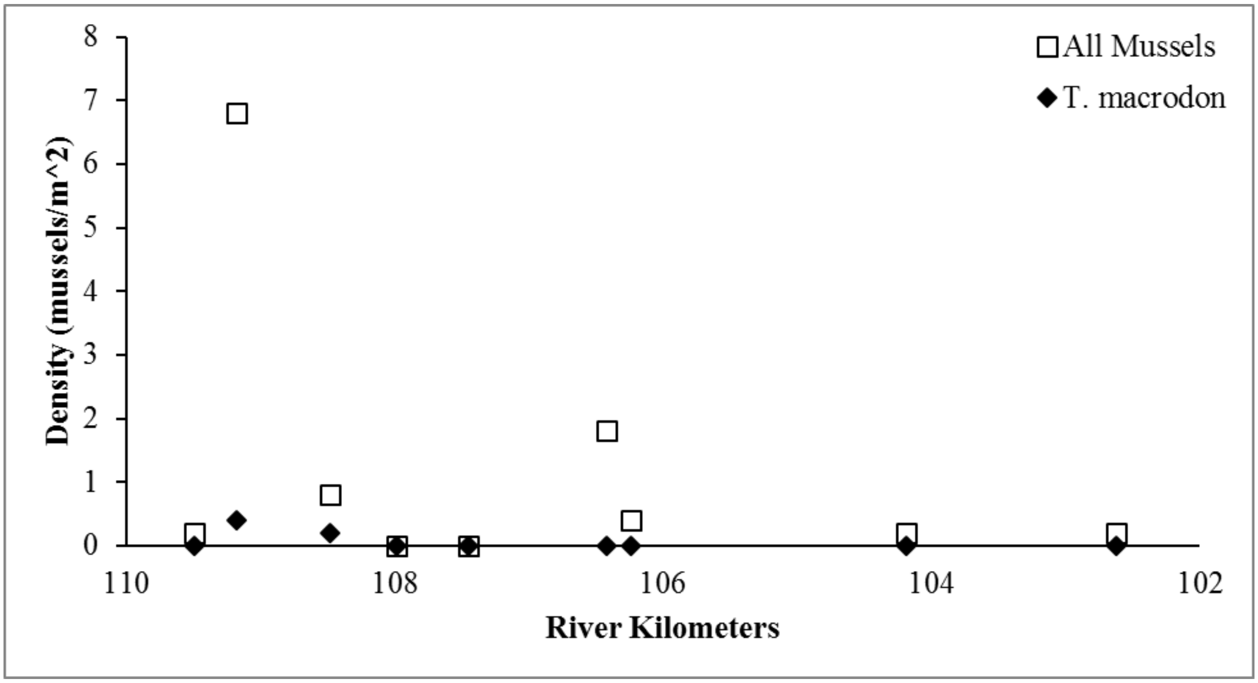

Figure 3. Densities of *Truncilla macrodon* (Texas fawnsfoot) and all mussel species (All mussels) in the Little River. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the confluence with the Brazos River (0 River Kilometers). Density = total number of either *T. macrodon* or all mussels encountered at each site divided by the total area of quadrats searched at each site.

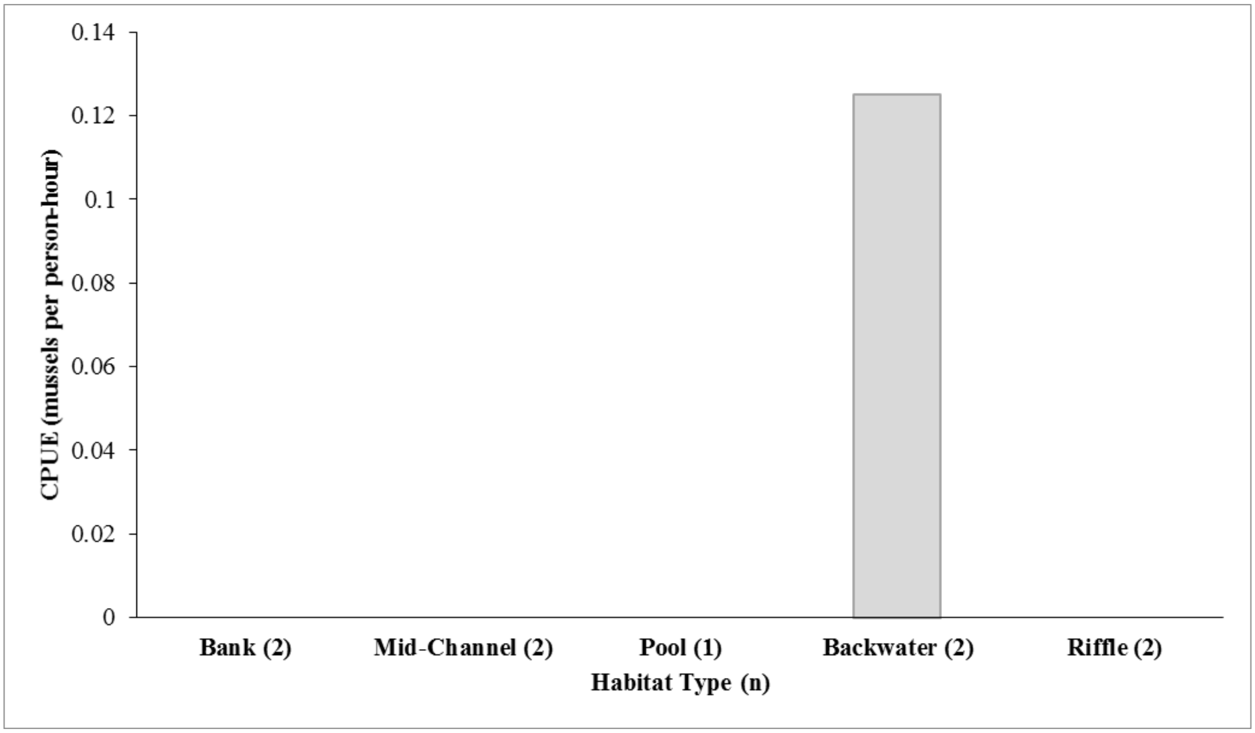

Figure 4. Catch-per-unit-effort (CPUE) of *Truncilla macrodon* (Texas fawnsfoot) by mesohabitat type in the Little River. The total number of sites sampled for each habitat are listed in parenthesis.

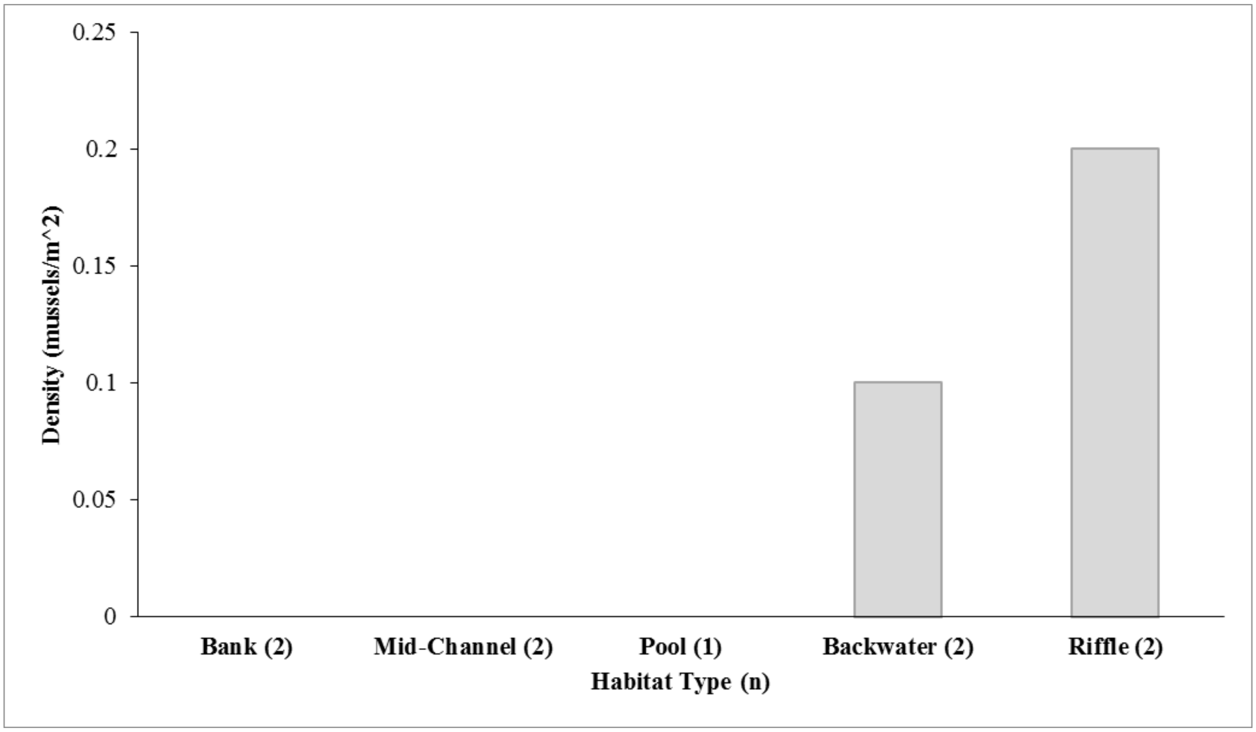

Figure 5. Density of *Truncilla macrodon* (Texas fawnsfoot) by mesohabitat type in the Little River. The total number of sites sampled for each habitat are listed in parenthesis.

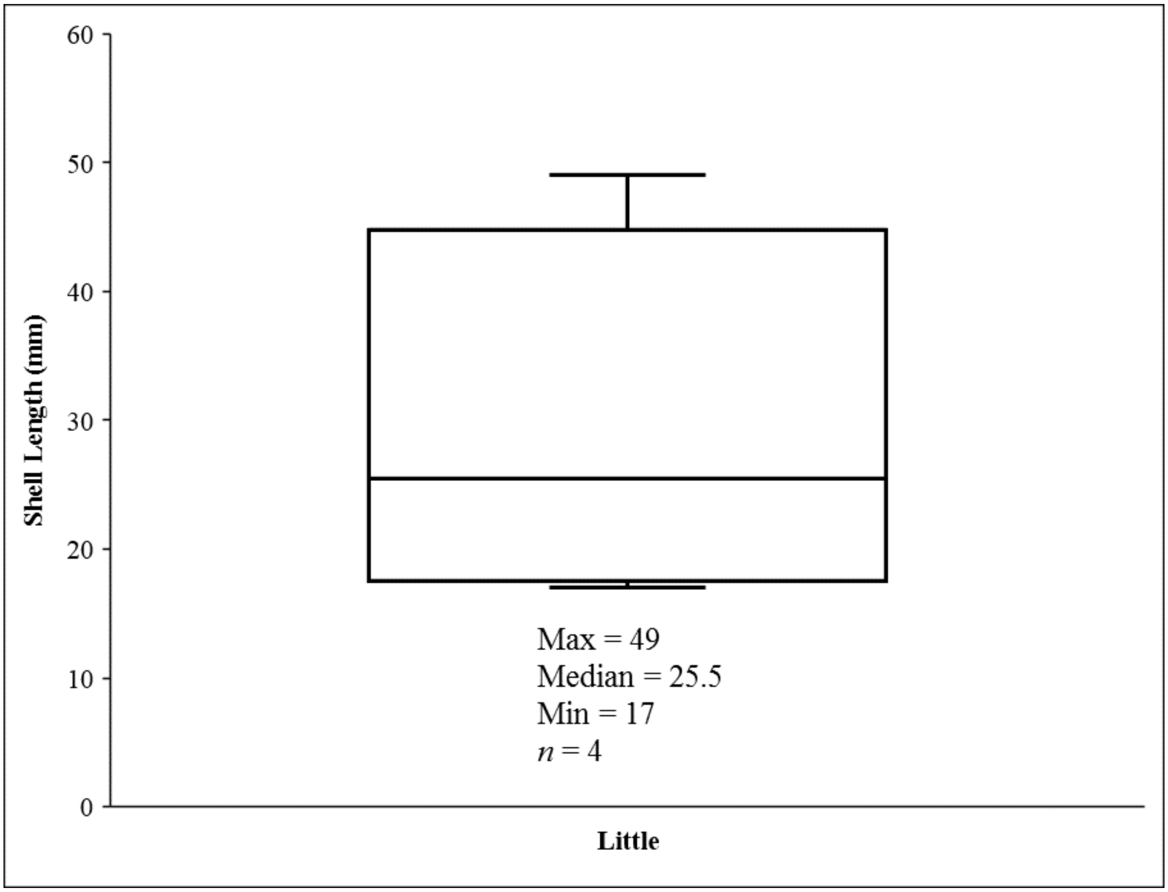

Figure 6. Shell length data of *Truncilla macrodon* (Texas fawnsfoot) populations in the Little River of the Brazos River drainage.

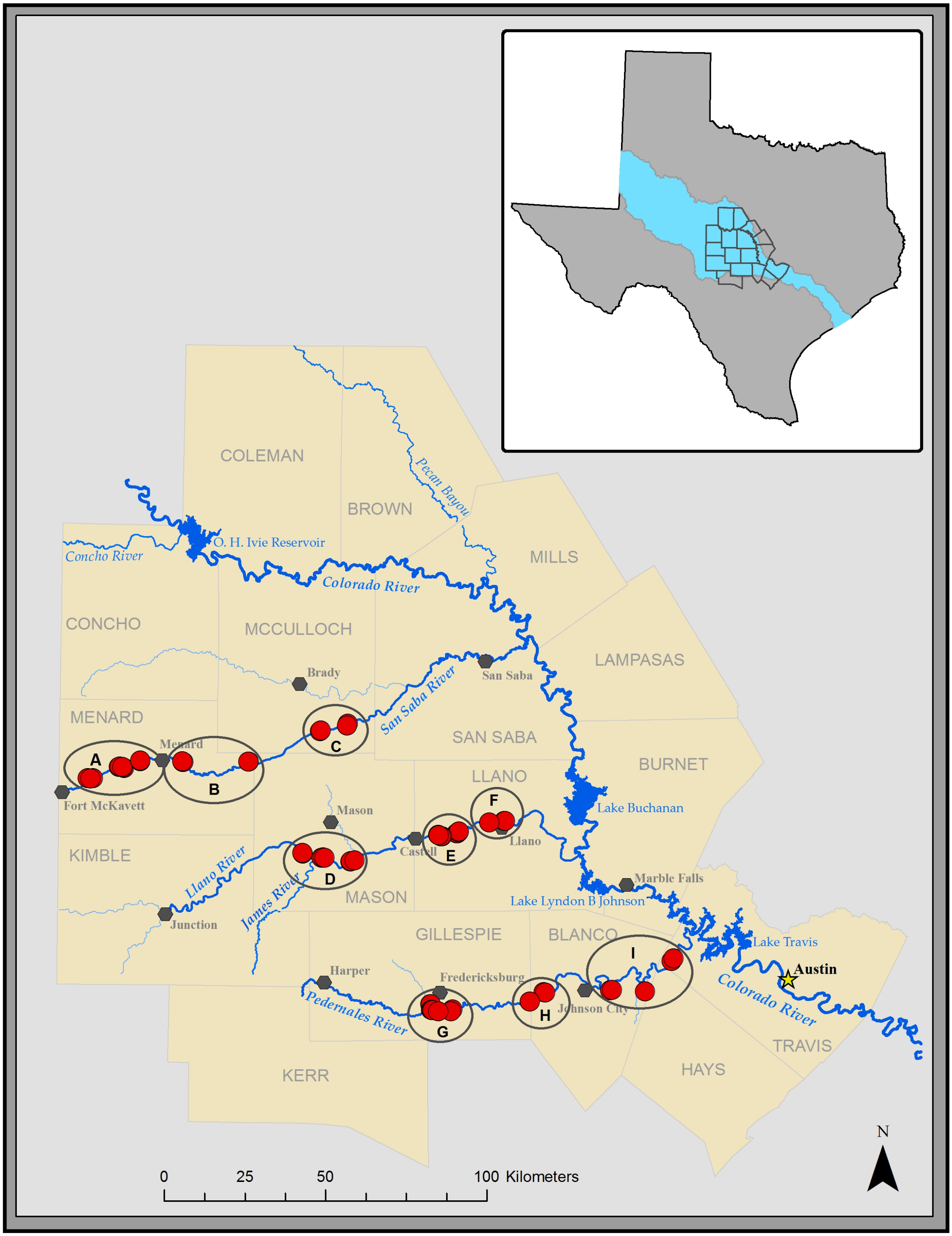

Figure 7. Map of Colorado drainage study area. Shaded circles denote sampling locations. Reaches are indicated by letter and correspond to Table 2.

Task 2: Comprehensive surveys the Rio Grande Basin

Distribution and Habitat Use for *Potamilus metnecktayi* (Salina mucket)

Section Summary

The goal of this study was to determine the status, distribution, and mesohabitat associations for *Potamilus metnecktayi* (Salina mucket), a species currently under review for protection under the Endangered Species Act, in the Rio Grande drainage. We used recent and historical data to inform a sampling program within the range of *P. metnecktayi.* In total, we surveyed 196 sites in the Rio Grande, Devils River, and Pecos River, and found 92 live *P. metnecktayi* from 22 of 196 (11.2%) sites. In the Rio Grande, we surveyed 114 sites, and found all live individuals in the upper Rio Grande, above Lake Amistad (*n* = 92). The majority of live individuals were found in rocky habitat, such as canyon walls. Size frequency distributions, using shell length as a proxy for age, suggest that some level of recruitment is occurring in the Rio Grande. We surveyed 39 sites in the Devils River and 43 sites in the Pecos River, and found no live individuals or shell material of *P. metnecktayi*.

Introduction

Potamilus metnecktayi, (Salina mucket), is known historically from the Rio Grande system and southward into Mexico (Neck 1984; Johnson 1998). The holotype specimen was collected and described formally by Richard I. Johnson from the Rio Salado near Nueva Laredo, Tamaulipas, Mexico (Johnson 1998).

In the Rio Grande basin, historic records of *P. metnecktayi* have come from the mainstem of the Rio Grande and its major tributaries. In the late 60s and early 70s, live specimens were collected from the Pecos River (1.28 km above the confluence of the Rio Grande at the former crossing of US Hwy 90) in 1968 and from the Rio Grande (9.7 km west of Del Rio) in 1972 (Metcalf 1982; Johnson 1999). However, only dead shells were found from these rivers in the mid 1970s (Howells 1994; Howells et al., 1997; Howells 1999; 2000). In the late 90s and early 2000s, live and recently dead *P. metnecktayi* were reported from the upper Rio Grande between Big Bend National Park and Lake Amistad (Howells 2006) and from the Lower Canyons of the Rio Grande near Dryden (Howells 2004; Burlakova and Karatayev 2008). To date, no live or dead individuals have been reported in the middle or lower Rio Grande, though long-dead/subfossil shell material has been collected within these reaches (Karatayev et al. 2012). *Potamilus metnecktayi* is listed as state threatened by the Texas Parks and Wildlife Department (TPWD 2010) and is being reviewed for listing under the U.S. Endangered Species Act (USFWS 2011).

Currently, little is known about the life history or reproductive requirements of *P. metnecktayi* (Howells 2002). Like other freshwater mussel species, it is an obligate ectoparasite on one or more host-fish species. Similar to other *Potamilus* species, it is likely *P. metnecktayi* is a long-term brooder and uses freshwater drum, *Aplodinotus grunniens* as a host (Surber 1915). Based on recent observations from field surveys throughout *P. metnecktayi*'s range, sub-adults and adults appear to occur most often in rock crevices, travertine shelves, and under large boulders, where small-grained material, such as clay, silt, or sand gathers (Burlakova and Karatayev 2008; 2010; Randklev et al. unpublished data).

The objectives of this study were to assess the distribution, abundance, and habitat use for *P. metnecktayi* in the Rio Grande. The resulting survey information was then used to develop Conservation Status Assessment Maps for this species within the Rio Grande and major tributaries**.**

Methods

Study Area

The Rio Grande originates in Colorado and is considered the 4th largest river in the United States, with an approximate length of 3,050 km and draining a totaling 870,236 km^2 (Kammerer 1990). The river flows from San Juan County, Colorado, through New Mexico and into Texas where it forms the shared border between Texas and Mexico before emptying into to the Gulf of Mexico near Brownsville, TX (Benke and Cushing 2011). Throughout its length the Rio Grande flows through arid and semiarid desert scrubland and grassland habitats (Dahm et al. 2005). Flow in the Rio Grande is regulated by two large reservoirs (Falcon and Amistad reservoirs) and a number of small low-head dams. The World Wildlife Fund currently ranks the Rio Grande as the most imperiled river in the United States due to water over-extraction and over-appropriation by human populations along the river (Wong et al. 2007).

The Devils River is a pristine tributary to the Rio Grande originating in Sutton County, TX, and flows intermittently southward into Val Verde County, TX, where it becomes perennial. Flow is unregulated and provided from groundwater seepage and springs. The river lies within the Edwards Plateau region and drains an approximate area of 10,000 km^2, which is sparsely populated (Cantu and Winemiller 1997).

The Pecos River is the largest tributary to the Rio Grande from the North and originates in New Mexico, draining approximately 115,000 km^2. This river is highly saline in Texas due to saline aquifer input as well as anthropogenic impacts such as groundwater extraction and irrigation; and has experienced a dramatic shift in fish fauna as well as harmful algal blooms from golden alga (*Prymnesium parvum*) since the 1980s (Southard 2010).

Sampling Methods

Survey sites were selected following methods outlined by Albanese et al. (2007). Specifically, species occurrence data from previous sampling efforts were used to determine the following: 1) HUC watersheds where live individuals of species of concern had been reported; 2) HUC watersheds that had been surveyed, but no live individuals were found; and 3) HUC watershed that had not been surveyed. The resulting map was then used to prioritize survey needs by focusing on areas that have not been surveyed (UNS_HUCs) or in areas where past surveys failed to detect species of concern (ND_HUCs). For a subset of HUCs that met these criteria and could be accessed safely, we delineated the entire length of the river into 10 km reaches. Within each reach specific sites were selected using a random sampling design with 2 strata: river left or river right (except for midchannel habitats) and 2) mesohabitat: (banks, backwater, midchannel, riffles, rock slabs, canyon walls (only for reaches located in the upper Rio Grande and lower Pecos River), pools (only for reaches in the Devils River), and boulder fields (only for reaches in the Pecos River). Within each sampling reach, at least 2 sites per available habitat type were selected.

Qualitative surveys using the timed search method were performed at each site (i.e., mesohabitat type). The timed search method was chosen because it provides a more effective means of detecting rare species than quantitative sampling methodologies (Vaughn et al. 1997). At each site, we confined the search boundaries within the randomly selected mesohabitat and standardized the search area to 150 m^2. Each site was surveyed tactilely and visually for a total of 4 person-hours (p-h). However, because we were interested in the amount of effort needed to detect *P. metnecktayi* (which will be important for designing long-term monitoring programs), we divided the total search time into 4, 1 p-h intervals. At the end of each search interval, surveyors combined all live specimens into a mesh bag, which was kept submerged in water until completion of the survey. During each interval, surveyors were spread out in the search area and every effort was made to search all available microhabitats. Following completion of the survey, all live mussels from each time period were identified to species, counted, measured, checked for gravidity, and then returned back to the river into the appropriate habitat.

Data Analysis

Scatter plots of abundance (CPUE: number of individuals/total person-hours) vs. river kilometer (RKM) were used to examine the effect of stream position on total mussel and *P. metnecktayi* abundance in each river. Boxplots and length-frequency histograms were developed for *P. metnecktayi* to assess demographic patterns and population structure. Generally, multimodal size class distribution may indicate recruitment, whereas truncated distributions (absence of a particular age class, large, or small individuals) may indicate a lack of recent recruitment or a localized extinction event. Bar graphs were also used to visually represent presence of *P. metnecktayi* by mesohabitat type (i.e., riffle, mid-channel, pool, bank, and backwater).

Results/Discussion

In total, 456 person-hours (p-h) were spent surveying mussels at 114 sites in the Rio Grande (Figure 1). Overall, we found 92 live individuals of *P. metnecktayi*, which were found at 22 of 114 (19.3%) sites. Catch-per-unit-effort (CPUE) ranged from 0 to 6 mussels/p-h and averaged 0.2 ± 0.1 mussels/p-h (± SE) for *P. metnecktayi* (Table 1), while CPUE averaged 5.8 ± 1.5 mussels/p-h for all mussels (Figure 2). Relative abundance of *P. metnecktayi* was 3.5% of all mussels collected within the Rio Grande. The highest abundance (1.2 ± 0.2 mussels/p-h) of *P. metnecktayi* by habitat type was observed in rock wall habitat (Figure 3). The highest abundance (1.2 ± 0.4 mussels/p-h) of *P. metnecktayi* by reach was observed in Reach 3 (Table 1). Live individuals of *P. metnecktayi* were found only in the upper Rio Grande in the reaches above Lake Amistad (Reaches 1, 2, and 3). Previous studies reported similar results, with live individuals found above Lake Amistad in Terrell and Val Verde counties (Howells 2004, Burlakova and Karatayev 2010, 2013, 2014). Shell materials had previously been reported in Brewster County (Howells 1999, 2000, 2004, 2005), but, to our knowldege, our results represent the first record of live individuals in Brewster County and thus the most upstream observation of live individuals. Median shell length for *P. metnecktayi* in the Rio Grande was 80 mm and minimum and maximum shell lengths were 31 mm and 137 mm, respectively (Figure 4). Shell length distributions were right skewed, although the presence of smaller size-classes indicates recruitment in recent years (Figure 5).

In total, 156 person-hours were spent surveying mussels at 39 sites in the Devils River of the Rio Grande drainage (Figure 6). No live individuals or shell material of *P. metnecktayi* were found. In the lower Pecos River, a total of 172 person-hours were spent surveying mussels at 43 sites downstream from Independence Creek to the confluence of the Rio Grande (Figure 7). No live individuals or shell material of *P. metnecktayi* were found. To our knowledge, no recent or historical records of *P. metnecktayi* occurrence exist from the Devils River, and in the Pecos River, only shell material has been collected within the last 50 years (Burlakova and Karatayev 2013).

In summary, our results indicate that *P. metnecktayi* occurs within the upper Rio Grande above Lake Amistad at low abundances, and if it occurs in the Devils or Pecos rivers, densities are too low to detect using conventional sampling methods. Previous studies reported similar results, with live individuals found only above Lake Amistad (Howells 2004, Burlakova and Karatayev 2013, 2014).

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Table 1. Locality and collection information for mussel survey sites in the Rio Grande. CPUE = total number of *P. metnecktayi* encountered at each site during qualitative sampling divided by the number of person hours (4) searched at each site. Habitat key: BH = Bank, BW = Backwater, MC = Mid-Channel, R = Riffle, RS = Rock Slab, RW = Rock Wall. Sites are ordered upstream to downstream.

Site	Reach	Habitat	Locality	County	Date of collection	Number of Live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
1	1	MC	La Linda	Brewster	6/8/2015	0	0	0	4	150
2	1	BW	La Linda	Brewster	6/8/2015	0	0	0	4	150
3	1	RW	La Linda	Brewster	6/8/2015	1	0.25	0	4	150
4	1	BH	La Linda	Brewster	6/8/2015	0	0	0	4	150
5	1	R	La Linda	Brewster	6/8/2015	0	0	0	4	150
6	1	RS	La Linda	Brewster	6/8/2015	0	0	0	4	150
7	1	BW	La Linda	Brewster	6/9/2015	1	0.25	0	4	150
8	1	BH	La Linda	Brewster	6/9/2015	1	0.25	0	4	150
9	1	R	La Linda	Brewster	6/9/2015	0	0	0	4	150
10	1	RW	La Linda	Brewster	6/9/2015	4	1	0	4	150
11	1	MC	La Linda	Brewster	6/9/2015	0	0	0	4	150
12	1	RS	La Linda	Brewster	6/9/2015	1	0.25	0	4	150
13	2	R	Black Gap	Brewster	6/11/2015	0	0	0	4	150
14	2	RW	Black Gap	Brewster	6/11/2015	6	1.5	0	4	150
15	2	RW	Black Gap	Brewster	6/11/2015	3	0.75	0	4	150
16	2	BW	Black Gap	Brewster	6/11/2015	0	0	0	4	150
17	2	RS	Black Gap	Brewster	6/11/2015	0	0	0	4	150
18	2	MC	Black Gap	Brewster	6/11/2015	0	0	0	4	150
19	2	R	Black Gap	Brewster	6/11/2015	0	0	0	4	150
20	2	BH	Black Gap	Brewster	6/10/2015	0	0	0	4	150
21	2	BH	Black Gap	Brewster	6/10/2015	2	0.5	0	4	150
22	2	RW	Black Gap	Brewster	6/10/2015	3	0.75	0	4	150
23	2	RS	Black Gap	Brewster	6/10/2015	3	0.75	0	4	150
24	2	BW	Black Gap	Brewster	6/10/2015	1	0.25	0	4	150
25	3	RW	John's Marina	Terrell	9/8/2015	6	1.5	0	4	150
26	3	RW	John's Marina	Terrell	5/16/2015	8	2	0	4	150
27	3	RS	John's Marina	Terrell	9/8/2015	1	0.25	0	4	150
28	3	BH	John's Marina	Terrell	5/16/2015	3	0.75	0	4	150
29	3	RW	John's Marina	Terrell	5/16/2015	3	0.75	0	4	150
30	3	BW	John's Marina	Terrell	5/14/2015	24	6	0	4	150
31	3	RW	John's Marina	Terrell	5/14/2015	7	1.75	0	4	150
32	3	R	John's Marina	Terrell	5/14/2015	0	0	0	4	150
33	3	BH	John's Marina	Terrell	5/15/2015	0	0	0	4	150
34	3	BW	John's Marina	Terrell	5/15/2015	0	0	0	4	150
35	3	R	John's Marina	Terrell	5/14/2015	1	0.25	1	4	150
36	3	BH	John's Marina	Terrell	5/14/2015	4	1	0	4	150
37	3	RW	John's Marina	Terrell	5/15/2015	8	2	0	4	150
38	3	RS	John's Marina	Terrell	5/15/2015	1	0.25	0	4	150
39	4	RS	Del Rio	Val Verde	5/13/2015	0	0	0	4	150
40	4	BW	Del Rio	Val Verde	5/12/2015	0	0	0	4	150
41	4	BH	Del Rio	Val Verde	5/13/2015	0	0	0	4	150
42	4	R	Del Rio	Val Verde	5/12/2015	0	0	0	4	150

Table 1. Continued.

Site	Reach	Habitat	Locality	County	Date of collection	Number of Live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
43	4	BW	Del Rio	Val Verde	5/12/2015	0	0	0	4	150
44	4	R	Del Rio	Val Verde	5/12/2015	0	0	0	4	150
45	4	MC	Del Rio	Val Verde	5/12/2015	0	0	0	4	150
46	4	BH	Del Rio	Val Verde	5/12/2015	0	0	0	4	150
47	4	RS	Del Rio	Val Verde	5/12/2015	0	0	0	4	150
48	5	RS	Eagle Pass	Maverick	4/10/2015	0	0	0	4	150
49	5	BH	Eagle Pass	Maverick	4/11/2015	0	0	0	4	150
50	5	MC	Eagle Pass	Maverick	4/10/2015	0	0	0	4	150
51	5	R	Eagle Pass	Maverick	4/10/2015	0	0	0	4	150
52	5	BW	Eagle Pass	Maverick	4/11/2015	0	0	0	4	150
53	5	BW	Eagle Pass	Maverick	4/11/2015	0	0	0	4	150
54	5	R	Eagle Pass	Maverick	4/10/2015	0	0	0	4	150
55	5	MC	Eagle Pass	Maverick	5/11/2015	0	0	0	4	150
56	5	BH	Eagle Pass	Maverick	5/11/2015	0	0	0	4	150
57	5	RS	Eagle Pass	Maverick	4/10/2015	0	0	0	4	150
58	6	R	El Indio	Maverick	4/9/2015	0	0	0	4	150
59	6	BW	El Indio	Maverick	4/9/2015	0	0	0	4	150
60	6	MC	El Indio	Maverick	4/9/2015	0	0	0	4	150
61	6	BW	El Indio	Maverick	4/9/2015	0	0	0	4	150
62	6	RS	El Indio	Maverick	4/9/2015	0	0	0	4	150
63	6	MC	El Indio	Maverick	4/9/2015	0	0	0	4	150
64	6	BH	El Indio	Maverick	4/9/2015	0	0	0	4	150
65	6	BH	El Indio	Maverick	4/8/2015	0	0	0	4	150
66	6	RS	El Indio	Maverick	4/8/2015	0	0	0	4	150
67	6	R	El Indio	Maverick	4/8/2015	0	0	0	4	150
68	7	R	Apache	Webb	2/24/2015	0	0	0	4	150
69	7	RS	Apache	Webb	2/25/2015	0	0	0	4	150
70	7	R	Apache	Webb	2/24/2015	0	0	0	4	150
71	7	BW	Apache	Webb	2/25/2015	0	0	0	4	150
72	7	BW	Apache	Webb	2/25/2015	0	0	0	4	150
73	7	RS	Apache	Webb	2/24/2015	0	0	0	4	150
74	8	BW	Columbia	Webb	2/20/2015	0	0	0	4	150
75	8	RS	Columbia	Webb	2/19/2015	0	0	0	4	150
76	8	MC	Columbia	Webb	2/20/2015	0	0	0	4	150
77	8	R	Columbia	Webb	2/19/2015	0	0	0	4	150
78	8	BH	Columbia	Webb	2/20/2015	0	0	0	4	150
79	8	RS	Columbia	Webb	9/6/2015	0	0	0	4	150
80	8	RS	Columbia	Webb	2/20/2015	0	0	0	4	150
81	8	BW	Columbia	Webb	11/20/2014	0	0	0	4	150
82	8	R	Columbia	Webb	2/19/2015	0	0	0	4	150
83	8	RS	Columbia	Webb	9/6/2015	0	0	0	4	150
84	8	BH	Columbia	Webb	11/20/2014	0	0	0	4	150
85	9	RS	La Bota	Webb	9/5/2015	0	0	0	4	150
86	9	RS	La Bota	Webb	2/21/2015	0	0	0	4	150
87	9	BH	La Bota	Webb	11/18/2014	0	0	0	4	150
88	9	R	La Bota	Webb	2/22/2015	0	0	0	4	150
89	9	MC	La Bota	Webb	2/21/2015	0	0	0	4	150
90	9	BW	La Bota	Webb	11/16/2014	0	0	0	4	150
91	9	MC	La Bota	Webb	2/22/2015	0	0	0	4	150
92	9	R	La Bota	Webb	2/21/2015	0	0	0	4	150

Table 1. Continued.

Site	Reach	Habitat	Locality	County	Date of collection	Number of Live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
93	9	BW	La Bota	Webb	2/22/2015	0	0	0	4	150
94	9	BH	La Bota	Webb	11/16/2014	0	0	0	4	150
95	9	RS	La Bota	Webb	2/22/2015	0	0	0	4	150
96	9	BW	La Bota	Webb	11/18/2014	0	0	0	4	150
97	9	RS	La Bota	Webb	9/5/2015	0	0	0	4	150
98	9	R	La Bota	Webb	4/7/2015	0	0	0	4	150
99	9	RS	La Bota	Webb	4/7/2015	0	0	0	4	150
100	9	BW	La Bota	Webb	4/7/2015	0	0	0	4	150
101	9	R	La Bota	Webb	4/7/2015	0	0	0	4	150
102	10	RS	San Ygnacio	Zapata	2/26/2015	0	0	0	4	150
103	10	BH	San Ygnacio	Zapata	11/14/2014	0	0	0	4	140
104	10	BW	San Ygnacio	Zapata	11/13/2014	0	0	0	4	150
105	10	R	San Ygnacio	Zapata	2/18/2015	0	0	0	4	150
106	10	R	San Ygnacio	Zapata	2/18/2015	0	0	0	4	150
107	10	RS	San Ygnacio	Zapata	2/18/2015	0	0	0	4	150
108	10	MC	San Ygnacio	Zapata	11/15/2014	0	0	0	4	150
109	10	RS	San Ygnacio	Zapata	2/18/2015	0	0	0	4	150
110	10	MC	San Ygnacio	Zapata	11/15/2014	0	0	0	4	150
111	10	BH	San Ygnacio	Zapata	11/13/2014	0	0	0	4	150
112	10	BW	San Ygnacio	Zapata	2/26/2015	0	0	0	4	150
113	11	BH	Salenino	Starr	11/19/2014	0	0	0	4	150
114	11	BW	Salenino	Starr	11/19/2014	0	0	0	4	150

Table 2. Locality and collection information for mussel survey sites in the Devils River. CPUE = total number of *P. metnecktayi* encountered at each site during qualitative sampling divided by the number of person hours (4) searched at each site. Habitat key: BH = Bank, BW = Backwater, MC = Mid-Channel, R = Riffle, RS = Rock Slab, P = Pool. Sites are ordered upstream to downstream.

Site	Reach	Habitat	County	Date of collection	Number of Live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
1	A	R	Val Verde	5/11/2013	0	0	-	4	150
2	A	R	Val Verde	5/11/2016	0	0	-	4	150
3	A	BW	Val Verde	5/11/2016	0	0	-	4	150
4	A	R	Val Verde	5/11/2016	0	0	-	4	150
5	A	P	Val Verde	9/15/2015	0	0	-	4	150
6	A	R	Val Verde	5/12/2016	0	0	-	4	150
7	A	R	Val Verde	5/12/2016	0	0	-	4	150
8	A	R	Val Verde	5/12/2016	0	0	-	4	150
9	A	P	Val Verde	9/15/2015	0	0	-	4	150
10	B	MC	Val Verde	9/15/2015	0	0	-	4	150
11	B	P	Val Verde	9/16/2015	0	0	-	4	150
12	B	R	Val Verde	4/28/2016	0	0	-	4	150
13	B	BH	Val Verde	4/28/2016	0	0	-	4	150
14	B	BH	Val Verde	4/28/2016	0	0	-	4	150
15	B	MC	Val Verde	4/28/2016	0	0	-	4	150
16	B	MC	Val Verde	9/16/2015	0	0	-	4	150
17	B	R	Val Verde	6/13/2016	0	0	-	4	150
18	B	R	Val Verde	6/13/2016	0	0	-	4	150
19	B	BW	Val Verde	6/13/2016	0	0	-	4	150
20	C	RS	Val Verde	9/17/2015	0	0	-	4	150
21	C	MC	Val Verde	9/17/2015	0	0	-	4	150
22	C	BW	Val Verde	9/17/2015	0	0	-	4	150
23	C	R	Val Verde	9/17/2015	0	0	-	4	150
24	C	R	Val Verde	6/13/2016	0	0	-	4	150
25	C	R	Val Verde	6/13/2016	0	0	-	4	150
26	C	R	Val Verde	4/27/2016	0	0	-	4	150
27	C	MC	Val Verde	4/26/2016	0	0	-	4	150
28	C	BH	Val Verde	4/27/2016	0	0	-	4	150
29	C	R	Val Verde	4/27/2016	0	0	-	4	150
30	D	RS	Val Verde	4/26/2016	0	0	-	4	150
31	D	BH	Val Verde	4/26/2016	0	0	-	4	150
32	D	R	Val Verde	4/26/2016	0	0	-	4	150
33	D	R	Val Verde	4/26/2016	0	0	-	4	150
34	D	BW	Val Verde	4/26/2016	0	0	-	4	150
35	E	BH	Val Verde	9/18/2015	0	0	-	4	150
36	E	BW	Val Verde	9/18/2015	0	0	-	4	150
37	E	R	Val Verde	5/10/2016	0	0	-	4	150
38	E	R	Val Verde	5/10/2016	0	0	-	4	150
39	E	R	Val Verde	5/10/2016	0	0	-	4	150

Table 3. Locality and collection information for mussel survey sites in the lower Pecos River. CPUE = total number of *P. metnecktayi* encountered at each site during qualitative sampling divided by the number of person hours (4) searched at each site. Habitat key: BH = Bank, BF = Boulder Field, BW = Backwater, MC = Mid-Channel, R = Riffle, RS = Rock Slab, RW = Rock Wall, P = Pool. Sites are ordered upstream to downstream.

Site	Reach	Habitat	County	Date of collection	Number of Live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
1	A	R	Terrell/Crockett	3/17/2016	0	0	-	4	150
2	A	RS	Terrell/Crockett	3/17/2016	0	0	-	4	150
3	A	BH	Terrell/Crockett	3/17/2016	0	0	-	4	150
4	A	R	Terrell/Crockett	3/17/2016	0	0	-	4	150
5	A	P	Terrell/Crockett	3/17/2016	0	0	-	4	150
6	B	R	Val Verde	3/18/2016	0	0	-	4	150
7	B	RS	Val Verde	3/18/2016	0	0	-	4	150
8	B	BF	Val Verde	3/18/2016	0	0	-	4	150
9	B	BH	Val Verde	3/19/2016	0	0	-	4	150
10	B	R	Val Verde	3/19/2016	0	0	-	4	150
11	B	RS	Val Verde	3/19/2016	0	0	-	4	150
12	B	RW	Val Verde	5/20/2016	0	0	-	4	150
13	B	BF	Val Verde	5/20/2016	0	0	-	4	150
14	B	BF	Val Verde	5/20/2016	0	0	-	4	150
15	B	RW	Val Verde	5/20/2016	0	0	-	4	150
16	B	R	Val Verde	5/21/2016	0	0	-	4	150
17	B	BW	Val Verde	5/21/2016	0	0	-	4	150
18	B	RW	Val Verde	5/21/2016	0	0	-	4	150
19	C	RW	Val Verde	5/21/2016	0	0	-	4	150
20	C	BF	Val Verde	5/21/2016	0	0	-	4	150
21	C	BF	Val Verde	5/21/2016	0	0	-	4	150
22	C	RW	Val Verde	5/22/2016	0	0	-	4	150
23	C	BF	Val Verde	5/22/2016	0	0	-	4	150
24	C	BW	Val Verde	5/22/2016	0	0	-	4	150
25	C	BF	Val Verde	5/22/2016	0	0	-	4	150
26	C	R	Val Verde	5/22/2016	0	0	-	4	150
27	C	RW	Val Verde	5/22/2016	0	0	-	4	150
28	D	RW	Val Verde	5/23/2016	0	0	-	4	150
29	D	RW	Val Verde	5/23/2016	0	0	-	4	150
30	D	BF	Val Verde	5/23/2016	0	0	-	4	150
31	D	BF	Val Verde	5/23/2016	0	0	-	4	150
32	D	BW	Val Verde	5/23/2016	0	0	-	4	150
33	D	R	Val Verde	5/23/2016	0	0	-	4	150
34	E	BF	Val Verde	5/23/2016	0	0	-	4	150
35	E	RW	Val Verde	5/24/2016	0	0	-	4	150
36	E	R	Val Verde	5/24/2016	0	0	-	4	150
37	E	BF	Val Verde	5/24/2016	0	0	-	4	150
38	E	BW	Val Verde	5/24/2016	0	0	-	4	150
39	E	RW	Val Verde	5/24/2016	0	0	-	4	150
40	F	BF	Val Verde	3/20/2016	0	0	-	4	150
41	F	RS	Val Verde	3/21/2016	0	0	-	4	150
42	F	RS	Val Verde	3/20/2016	0	0	-	4	150
43	F	RS	Val Verde	3/21/2016	0	0	-	4	150

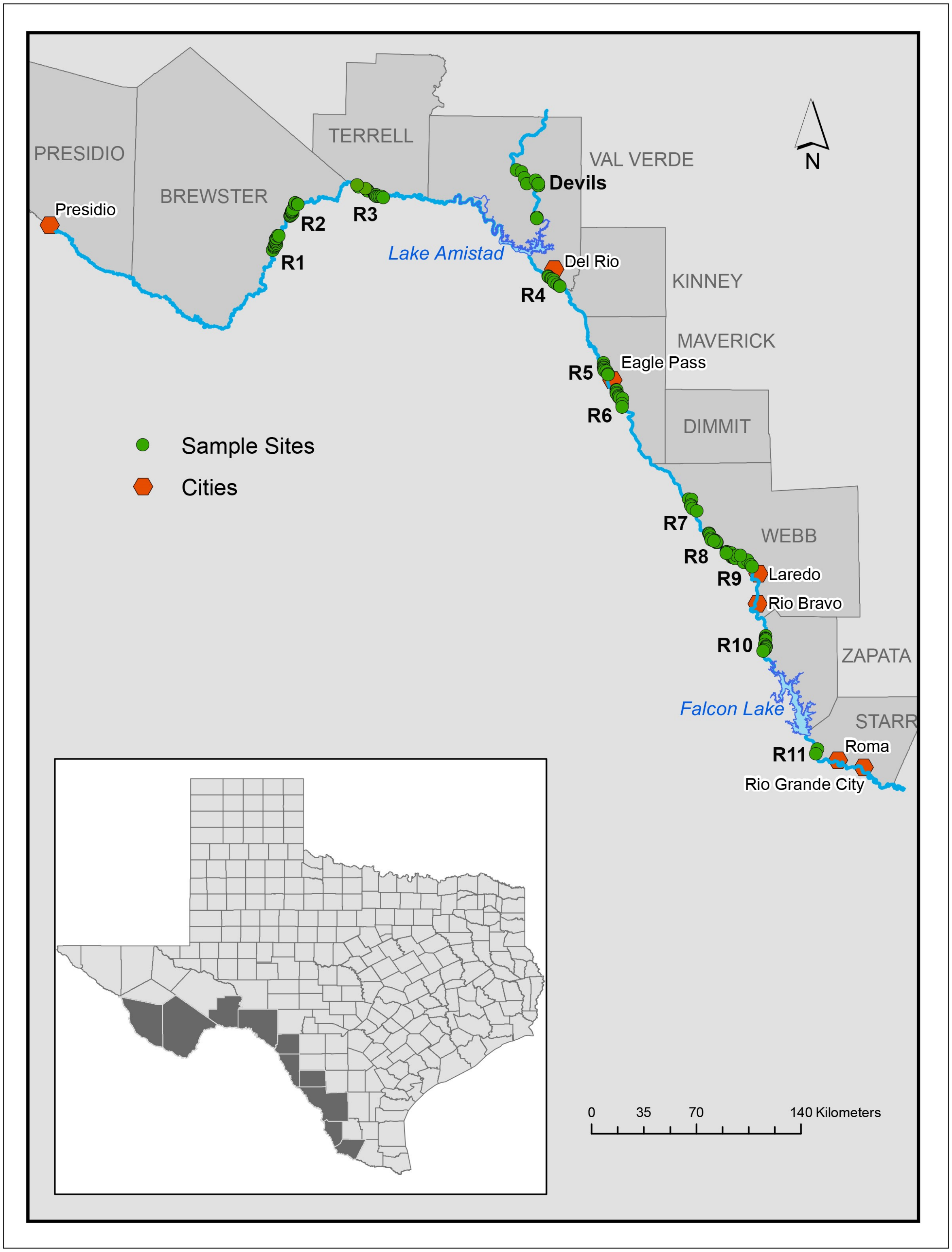

Figure 1. Map of Rio Grande drainage with shaded (green) circles denoting sampling locations. Reaches are indicated by number and correspond to Table 1.

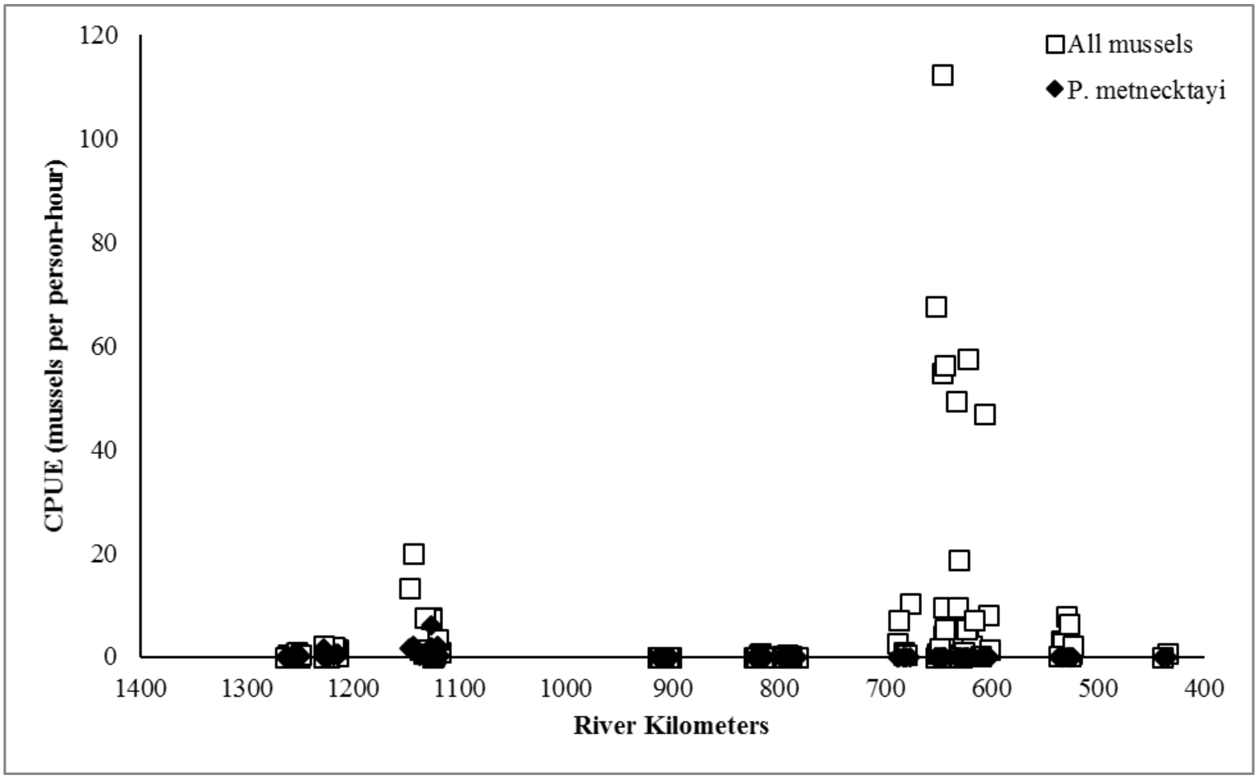

Figure 2. Catch-per-unit effort (CPUE) of *Potamilus metnecktayi* (Salina mucket) and all mussel species (All mussels) in the Rio Grande. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the most downstream site (0 River Kilometers). CPUE = total number of either *P. metnecktayi* or all mussels encountered at each site divided by the number of person hours (4) searched at each site.

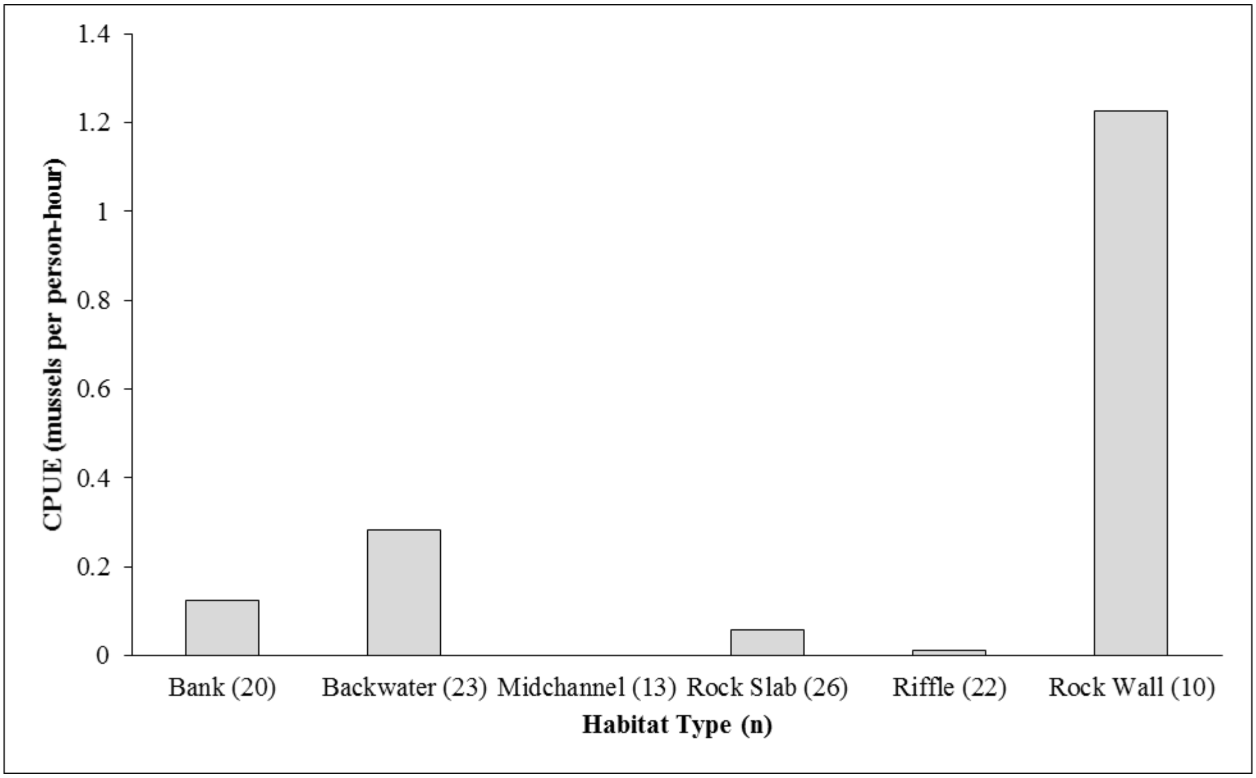

Figure 3. Catch-per-unit-effort (CPUE) of *Potamilus metnecktayi* (Salina mucket) by mesohabitat type in the Rio Grande. The total number of sites sampled for each habitat are listed in parenthesis.

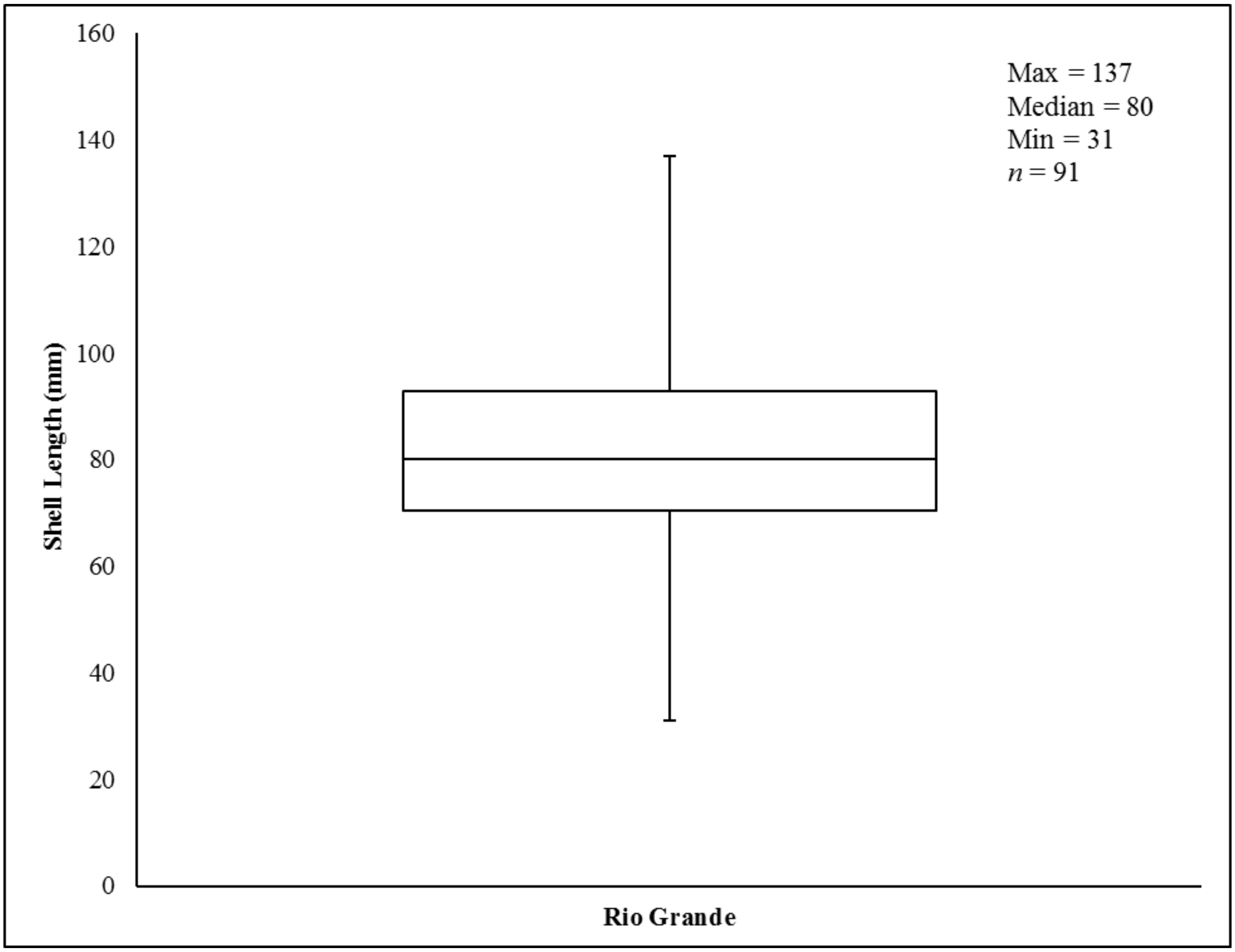

Figure 4. Shell length data of *Potamilus metnecktayi* (Salina mucket) populations in the Rio Grande.

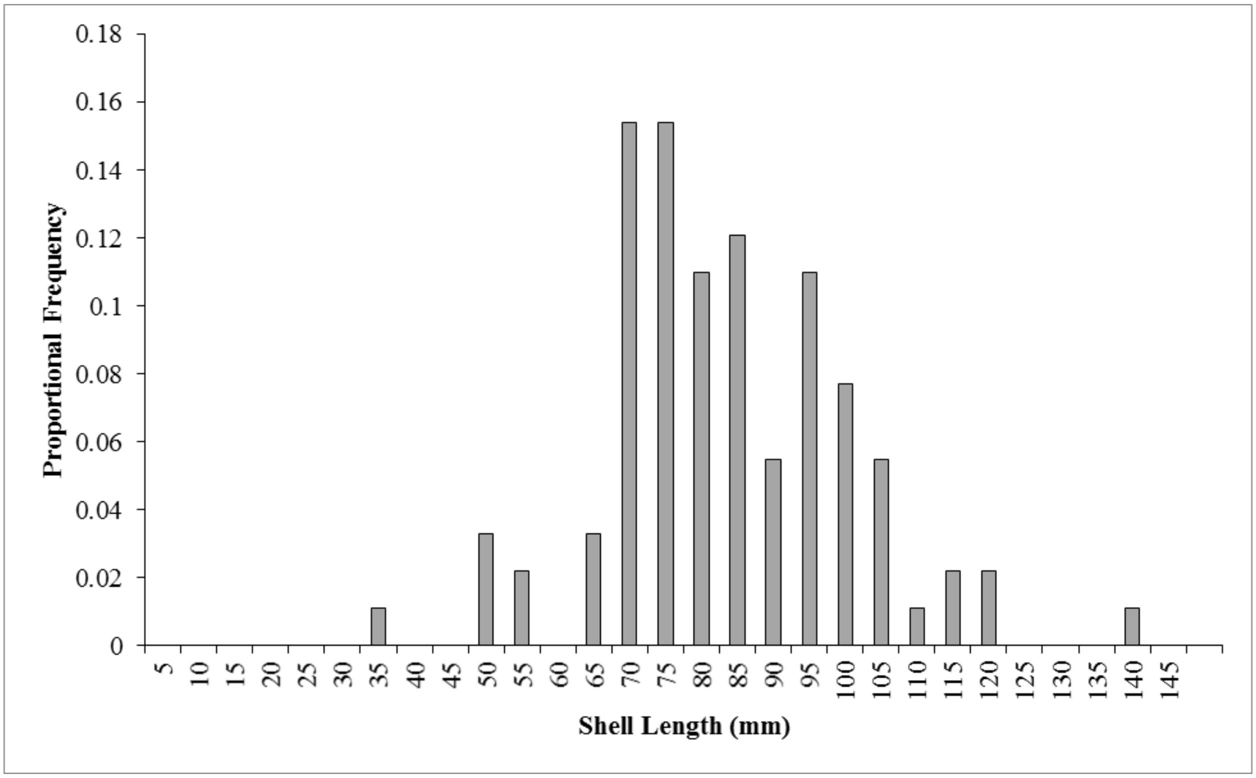

Figure 5. Proportional frequency of shell length of *Potamilus metnecktayi* (Salina mucket) in the Rio Grande. Shell lengths are binned into 5 mm groups.

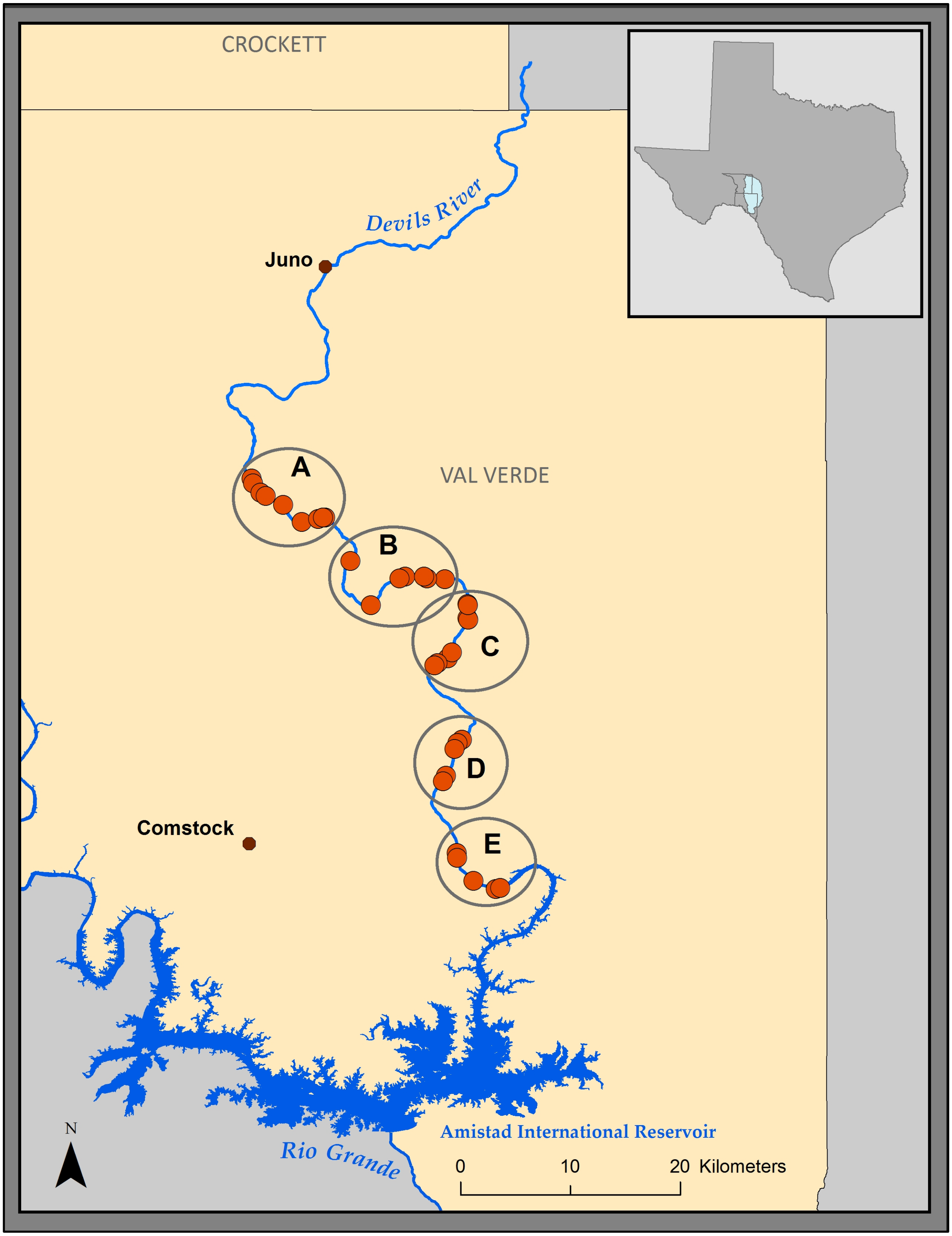

Figure 6. Devils River study area. Reaches correspond to table 1.

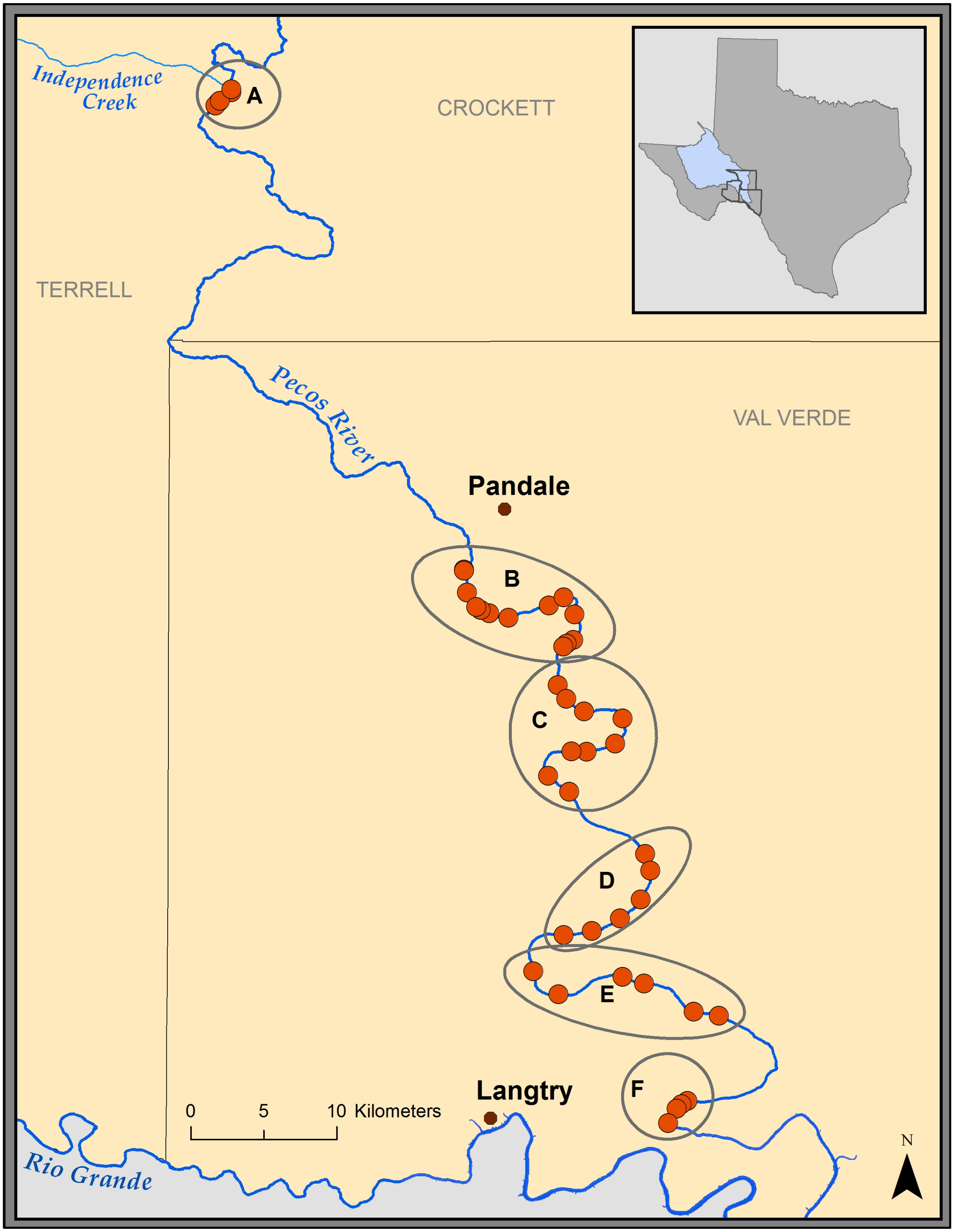

Figure 7. Pecos River study area. Reaches correspond to table 2.

Distribution and Habitat Use for *Popenaias popeii* (Texas hornshell)

Section Summary

The goal of this study was to determine the status, distribution, and mesohabitat associations for *Popenaias popeii* (Texas hornshell), a candidate for protection under the Endangered Species Act, in the upper and middle Rio Grande and the Devils and Pecos Rivers. We used recent and historical data to inform a year-long sampling program across the range of *P. popeii* in Texas. In total, we surveyed 114 sites in the Rio Grande and collected 2,063 individuals of *P. popeii* from 29 of the 114 sites surveyed. *Popenaias popeii* was less abundant (*n* = 189 live individuals) but more prevalent (37% or 14/38 sites) in the upper Rio Grande (*n* = 189 live individuals) compared to the middle Rio Grande (*n* = 1,874 live individuals; 20% or 11/74 sites). For the Devils River, we surveyed 11 sites and collected 35 live individuals across two different locations. For the Pecos River, we surveyed 15 sites across three different reaches located near the confluence with Independence Creek, at Pandale Road Crossing, and between Paint Rock Rapids and the weir dam near the reservoir interface with Lake Amistad. A single live individual was found near Pandale, TX, which represents an important find as the last observation of live *P. popeii* in the Pecos was ~ 47 years ago. Results from our Indicator Species Analysis indicate that *P. popeii* primarily occurs in rocky-type habitats (rock slabs, canyon walls with crevices, boulders, and large cobble). Comparing our results to previous studies in the Rio Grande, we found *P. popeii* occupying more sites (i.e., number of sites *P. popeii* was detected) and at higher abundance near Laredo, TX, than previous efforts had suggested. For the Rio Grande near Del Rio and Eagle Pass, TX, we did not find *P. popeii*, which corroborates previous findings in these areas. We also discovered a new population for *P. popeii* upstream from Lake Amistad in the Lower Canyons of the Rio Grande Wild and Scenic River and the Pecos River near Pandale, TX, and confirmed the presence of a population in the Devils River. For the Rio Grande, population size frequency distributions, using shell length as a proxy for age, suggest that recruitment is occurring. We also observed reproductively active females (gravid, i.e., gills containing a brood of either developing eggs or viable larvae) in the upper and lower Rio Grande and Devils River.

Introduction

Popenaias popeii, Texas hornshell, is known in Texas historically from the Rio Grande and the following tributaries: Pecos River, Devils River, and Las Moras Creek (references in Howells 1999). Type specimens of the species were collected from the Devils River, Val Verde County, Texas by Captain John Pope (~1853) and Rio Salado, Nuevo Leon, Mexico by Jean Louis Berlandier (~1828). Records of questionable validity exist from the Colorado watershed in Central Texas; one specimen from the Llano River collected in 1972 and identified by Stansbery in 1973 (OSUM 1972:365) and one valve from the South Concho River collected in 1991 after a flood (Strenth et al. 2004). These are the only records from central Texas and are likely spurious; either misidentified false spike (*Fusconaia mitchelli*) which tend to be elongated in the upper Colorado basin and look more like Texas hornshell than elsewhere, or misplaced shells from the Rio Grande or Pecos River drainages.

Within the Rio Grande proper, the upstream and downstream limits for *P. popeii* are not well supported. The downstream range limit for *P. popeii* is based on specimens collected by R. D. Camp, a naturalist and purveyor of biological specimens in the early 20th century, and are attributed to the Keller Resaca, an oxbow of the Rio Grande near Brownsville, TX. However, *P. popeii* is not known to occur in lentic habitats such as oxbows as determined by recent sampling (Karateyev et al. 2015), and our own analyses. Camp's collection is currently housed at the Corpus Christi Museum, Corpus Christi, TX, and was inventoried by Raymond Neck, a biologist and malacologist for TPWD, in the early 1980s Neck (1987). In his inventory, Neck provided a list and notes on Camp's molluscan collection, which included comments on the overall condition of the collection and that a number of specimens were either missing or had questionable labels. For the *P. popeii* specimens, Neck (1987) observed calcium carbonate residue on the posterior margin of the specimens, which led him to question whether these specimens actually originated from the Brownsville area. In the Rio Grande, shells that show precipitated calcium carbonate are usually found in spring-fed, hard water, flowing environments like that of the Devils River not slackwater habitats like oxbows. To confirm Neck's (1987) observation, we visited the Corpus Christi Museum in November 2015. During our visit, we found that the identification of these specimens was correct, but the original locality information was missing, and calcium carbonate residue was present on the specimens. Given that the locality for these specimens cannot be confirmed, then the most downstream record of confirmed *P. popeii* in the Lower Rio Grande is at Chapeño, Starr County, TX, which is located immediately downstream of the present Falcon Dam, prior to the dam's completion in 1954 (Neck 1987; Neck and Metcalf 1988). This location is approximately 350 river kilometers upstream of Brownsville. The upstream range limit in the Rio Grande proper prior to data presented herein was thought to be at Bullis Fold (Dean Canyon), downstream of Big Bend National Park in Brewster County where Howells (1999) collected recently and long dead specimens. The most upstream collection of live material prior to our surveys was near Langtry, Val Verde County, Texas (Karatayev et al. 2012).

In tributaries, Texas hornshell is known to have existed in the Devils River from the confluence with the Rio Grande upstream to Finnegan Springs, Val Verde County at present (Strecker 1931, data herein). Las Moras creek from the Rio Grande to the source at Fort Clark Springs, Kinney County (USNM_01 (1898) E. A. Mearns). The Pecos River, from Barstow, TX, Ward County (J.D. Mitchell, ~1890, USNM 464732) to the mouth of the Pecos at the Rio Grande (Metcalf 1974, 1982; A.L. Metcalf 1974, USNM 709228). For Las Moras Creek, live *P. popeii* were collected in 1898 by E. A. Mearns, a physician and naturalist stationed at Fort Clark (USNM 01, 130175,151538, 308943; MCZ 295007). In 1902, the U.S. Army walled in the spring and in 1939 a concrete swimming pool was constructed next to the spring head by the Works Progress Administration (Haen 2002). The springs temporarily ceased to flow in 1964 and 1971 (Brune 1981). From 1971-75, Harold D. Murray, a Professor at Trinity University, surveyed 48 km of Las Moras Creek in search of *P. popeii* but was unable to locate live individuals or shell (Murray 1975). He also observed workers removing heavy plant growth from the creek, which according to local landowners was repeated 2 to 3 times a year starting in 1971 (Murray 1975). It is unknown if this practice continues today. Based on this observation and the lack of live individuals, Murray (1975) argued that *P. popeii* had been extirpated from Las Moras Creek. Portions of Las Moras Creek were informally resurveyed in 1995 by Texas Parks and Wildlife (Howells et al 1997) and Karateyev et al in 2000s. To date, no live or dead *P. popeii* have been collected from Las Moras Creek since Mearns' collections in 1898 (Karatayev et al. 2015).

For reaches of the Pecos that flow through Texas, weathered shell material for *P. popeii* has been collected near Barstow, TX, Ward County (J.D. Mitchell, ~1890, USNM 464732), though Karatayev et al. (2012), Burlakova and Karatayev (2014) and Karatayev et al. (2015) incorrectly reported these individuals as live at time of collection. Downstream of this location, a single fragment of a *P. popeii* shell has been found from the Pecos River near Iraan, TX (Pecos/Crockett Counties) and long dead shells from an ~ 8 km stretch in the lower Pecos, just upstream from the confluence with the Rio Grande. Between these locations, live *P. popeii* have been collected near Pandale, Val Verde County, Texas downstream of the Independence Creek confluence in 1973 (A.L. Metcalf 1974, USNM 709228).

In New Mexico, *P. popeii* was recorded historically from recent shell material on the Pecos River at Carlsbad (R.J. Drake, 1948 USNM 758208) and live individuals were found in its tributary the North Spring Creek near Roswell (Cockerell 1902). The species is only known currently in the Black River near Malaga (Neck 1984; Lang 2001; Strenth et al. 2004; Carman 2007) but long dead shells were found in the Pecos proper downstream from the Black's confluence and in the neighboring Delaware River in the 1990s (Lang 2001). Reintroduction efforts into the Delaware River using adults from the Black River have recently started (2015 Fisheries Management Plan, New Mexico Game and Fish).

The current status of Texas hornshell in Mexico is unknown. Records exist from the Rio Salado watershed which flows into the Rio Grande at Falcon Lake, as well as other drainages to the south that flow to the Gulf of Mexico: the Soto de Marina, Tamesi, Panuco, Cazones, and Tamul watersheds (Hinkley 1907). However, the genetic identity of the specimens from outside of the Rio Grande watershed have not been confirmed as *P. popeii* and may represent other species, e.g., *Popenaias metallica*.

Until recently Texas hornshell was considered extremely rare. Singley (1893) recorded *P. popeii* from very few locations (in the Devils and Pecos Rivers) and commented that this species was rare. Neck (1982) suggested considering this species for listing by the USFWS. Williams et al. (1993) listed the species as threatened and more recently elevated it to endangered (Williams et al. in review). NatureServe ranks *P. popeii* as critically imperiled across its range and this species is currently listed as a candidate for protection under the U.S. Endangered Species Act (USFWS 2001). Surveys by Miller et al. (unpublished data), Karatayev et al. (2012), and Burlakova and Karatayev (2014) have reported live individuals or recently dead specimens for this species from the Devils River (Val Verde Co.), Rio Grande near John's Marina (Terrell Co.), Del Rio, TX (Val Verde Co.), and Laredo, TX (Webb Co.). These surveys were not initially designed to detect species with low abundance, assess evidence of recruitment, or provide population estimates. Thus, the conservation status of this species throughout the Rio Grande is still uncertain.

Popenaias popeii is considered a valid species (Chapman et al. 2008) and like other freshwater mussels species is an obligate ectoparasite on one or more host-fish species. Reproductive information for this species has been collected from studies focused on a small, disjunct population from the Black River in New Mexico. There, *P. popeii* was considered a short-term brooder, spawning during the early summer months of May and June (Smith et al. 2003). Potential host fishes identified through artificial inoculations in the laboratory include: Longnose gar, Gizzard shad, Mexican tetra, Red shiner, Common carp, Roundnose shiner, Plains minnow, Speckled chub, Rio Grande shiner, Flathead minnow, Central stoneroller, River carpsucker, Blue sucker, Grey Redhorse, Yellow bullhead, Channel catfish, Plains killifish, Rainwater killifish, Western mosquitofish, Rio Grande cichlid, Green sunfish, Bluegill, Longear sunfish, Largemouth bass, and Greenthroat darter (references in Carman 2007). These results suggest that *P. popeii* lacks specialization for attracting specific host species (host generalist – see Barnhart et al. 2008) and employs an opportunistic strategy for host infection, including the free release of glochidia with larval threads that can attach to both skin and gills of hosts (Carman 2007). However, only a small number of these fishes have been observed to harbor parasitic larvae from *P. popeii* in the river (Levine et al. 2012). This indicates that most of the hosts identified during laboratory testing may not be effective hosts for *P. popeii* in the wild. To date, reproductive timing and host-fish relationships for populations in the Rio Grande or Devils River have not been assessed. This information is important for determining which factors (biotic, abiotic, or both) may be responsible for the decline of this species.

Popenaias popeii have been reported to reside in rock crevices, travertine shelves, and under large boulders, where small-grained material, such as clay, silt, or sand gathers (references in Carman 2007; Howells 2010). Karatayev et al. (2012) and Burlakova and Karatayev (2013) performing surveys in portions of the upper and middle Rio Grande reported similar observations, however, their findings were also anecdotal as they primarily focused on habitats that were known or suspected to harbor *P. popeii* populations (i.e., rock slabs and boulders). Other habitats that may be suitable for mussels (e.g., banks or backwater areas) were not surveyed. Thus, habitat associations for this species remain untested and for juveniles, undescribed.

The objectives of this study were to assess the distribution, abundance, and habitat use for *P. popeii* in the Rio Grande. The resulting survey information was then used to develop Conservation Status Assessment Maps for this species within the Rio Grande and major tributaries.

Methods

Study Area

The Rio Grande originates in Colorado and is considered the 4th largest river in the United States, with an approximate length of 3,050 km and draining a totaling 870,236 km^2 (Kammerer 1990). The river flows from San Juan County, Colorado, through New Mexico and into Texas where it forms the shared border between Texas and Mexico before emptying into to the Gulf of Mexico near Brownsville, TX (Benke and Cushing 2011). Throughout its length the Rio Grande flows through arid and semiarid desert scrubland and grassland habitats (Dahm et al. 2005). Flow in the Rio Grande is regulated by two large reservoirs (Falcon Reservoir and Lake Amistad) and a number of small low-head dams. The World Wildlife Fund currently ranks the Rio Grande as the most imperiled river in the United States due to water over-extraction and over-appropriation by human populations along the river (Wong et al. 2007). The Devils River is a pristine tributary to the Rio Grande originating in Sutton County, TX, and flows intermittently southward into Val Verde County, TX, where it becomes perennial. Flow is unregulated and provided from groundwater seepage and springs. The river lies within the Edwards Plateau region and drains an approximate area of 10,000 km^2, which is sparsely populated (Cantu and Winemiller 1997). The Pecos River flows from the Rocky Mountains in north-central New Mexico south to Texas through arid landscapes where much of its water is captured by impoundments for agricultural, municipal, and industrial use. Below Red Bluff Dam near the Texas-New Mexico border the river flows freely, but suffers from elevated salinity levels until receiving groundwater input, most notably at Independence Creek. The river then flows through remote desert before it empties into Amistad Reservoir, where it joins the Rio Grande above Del Rio, Texas.

The present study was located primarily in the upper (upstream of Lake Amistad) and middle (between Lake Amistad and Falcon Reservoir) portions of the Rio Grande in Texas, although several sites were surveyed immediately downstream of Lake Falcon. For the Devils River, sampling was conducted between Baker's Crossing at the Highway 163 bridge and the Big Satan (South) unit of the Devils River State Natural Area (Figure 1). For the Pecos, a reconnaissance sampling trip was performed during March 16 – 21, 2016 near the confluence of Independence Creek, at Pandale Road Crossing, and between Paint Rock Rapids and the weir dam near the reservoir interface with Lake Amistad.

Sampling Methods:

Survey sites within the Rio Grande were selected following methods outlined by Albanese et al. (2007). Specifically, 10-digit HUC watersheds were used to delineate the entire length of Rio Grande within our study area. Species occurrence data from previous sampling efforts in the Rio Grande were then used to determine the following: 1) HUC watersheds where live individuals for *P. popeii* have been reported; 2) HUC watersheds that have been surveyed, but *P. popeii* was not found; and 3) HUC watershed that have not been surveyed. The resulting map was then used to prioritize survey needs by focusing on areas that have not been surveyed (UNS_HUCs) or in areas where past surveys failed to detect *P. popeii* (ND_HUCs). For a subset of HUCs that met these criteria and could be accessed safely using a motorized boat, we delineated the entire length of the river into 10 km reaches. Within each reach specific sites were selected using a random sampling design with 2 strata: river left or river right (except for midchannel habitats) and 2) mesohabitat: (banks, backwater, midchannel, riffles, rock slabs, canyon walls (only for reaches located in the upper Rio Grande), and pools (only for reaches in the Devils River). In total, 10 sites in the middle and 12 sites in the upper Rio Grande, 2 per habitat type, were selected within each reach for sampling. Sites in the Devils River were selected randomly from available mesohabitats, with a focus on deep water habitats that had not been surveyed during previous efforts, and habitats similar to those where *P. popeii* occurs in the Rio Grande. For the Pecos, sites were selected by distance from access point, then by presence of habitat typical of occupancy by Texas hornshell as found in the Rio Grande, Black, and Devils Rivers. Specifically, sites were sampled between ~ 0.1 and 2 km downstream from the confluence of Independence Creek, within ~2 km upstream and ~2 km downstream of the Pandale Road Crossing, and between Paint Rock Rapids and the weir dam near the reservoir interface with Lake Amistad. All sites were 150 m^2 in area and were searched for 4 person-hours visually and tactilely either by snorkel or SCUBA.

Qualitative surveys using the timed search method were performed in each randomly selected mesohabitat type. The timed search method was chosen because it provides a more effective means of detecting rare species than quantitative sampling methodologies (Vaughn et al., 1997). At each site (i.e., mesohabitat type), we confined the search boundaries to the specific habitat type, ensuring that the search area did not exceed 150 m^2. Each site was surveyed tactilely and visually for a total of 4 person-hours (p-h). However, because we are interested in the amount of effort needed to detect *P. popeii* (which will be important for designing long-term monitoring programs), we divided the total search time into 4, 1 p-h intervals. At the end of each search interval, surveyors combined all live specimens into a mesh bag, which was kept submerged in water until completion of the survey. During each interval, surveyors were spread out in the search area and every effort was made to search all available microhabitats. Following completion of the survey, all live mussels from each time period were identified to species, counted, measured, checked for gravidity, and then returned back to the river into the appropriate habitat.

Data analysis:

Bar graphs were used to visually explore relationships between *P. popeii* and total mussel abundance (converted to log scale) and relative abundance (CPUE: number of individuals/total person-hours) by sampling reach. Scatter plots of abundance and relative abundance (CPUE: number of individuals/total person-hours) vs. river kilometer (RKM) were used to examine the effect of stream position on total mussel and *P. popeii* abundance. Boxplots and length-frequency histograms were developed for *P. popeii* to assess demographic patterns and population structuring within populations. Generally, multimodal size class distribution may indicate recruitment, whereas truncated distributions (absence of a particular age class, large, or small individuals) may indicate a lack of recent recruitment or a localized extinction event. For the Pecos population, no analyses were performed because sample size (number of individuals and number of sites) is too small to draw any conclusions.

Indicator Species Analysis (ISA - Dufrêne and Legendre, 1997) was used to test the affinities of *P. popeii* to different habitat types. ISA identifies species or assemblages that are indicative of groups of sites, which have some ecological, conservation or management meaning (Dufrêne and Legendre, 1997). In the present study, we defined groups by mesohabitat type (i.e., riffle, midchannel, pool, backwater, rock slab, and rock face). ISA as proposed by Dufrêne and Legendre (1997) assigns an indicator value (IV) to each taxon by calculating the product of the relative frequency and relative average abundance of each species to a mesohabitat type. The probability of achieving an equal or larger IV value among groups (p) is then estimated based on 999 random permutations of the original data (Dufrêne and Legendre, 1997). ISA was performed with the INDICSPECIES package in R version 3.02 (R Foundation for Statistical Computing, Vienna, Austria), and we considered p-values ≤ 0.10 to be significant.

Results/Discussion

A total of 456 person-hours was spent surveying 114 sites located in the Rio Grande (Figure 1). A total of 2,063 live individuals of *P. popeii* were found across 29 sites in the upper (n = 189 individuals) and middle (n = 1,874 individuals) Rio Grande. Total abundance was greatest in Reach 3 (upper Rio Grande, n = 183 individuals), Reach 8 (middle Rio Grande near Colombia, n = 1,155 individuals), and Reach 9 [middle Rio Grande near La Bota, n = 652 individuals] (Figure 2). Relative abundance in the upper and middle portions of the river ranged from 0 to 111 mussels/hr with the highest occurring in Terrell (3 ± 5 mussels/hr; mean ± SD) and Webb (14 ± 27 mussels/ph; mean ± SD) counties (Table 1; Figure 3). These results indicate that *P. popeii* is more abundant in reaches downstream of Lake Amistad. However, the prevalence (i.e., number of sites *P. popeii* was detected) of this species upstream of Lake Amistad was greater (37% or 14/38 sites) than downstream (20% or 15/74), which indicates *P. popeii* may be more widely distributed in the upper Rio Grande. For the Devils River, a total of 44 hrs. across 11 sites was spent searching for mussels. A total of 35 live individuals were collected across 2 of the 11 sites. One site with 34 individuals was located within the Texas Nature Conservancy's (TNC) Dolan Falls Preserve while the site with a singleton was located in the Devils River SNA Big Satan (South) Unit (Table 1). For the Pecos, surveys near Independence Creek yielded no live or dead *P. popeii* from 5 sites. At the reach near Pandale Road Crossing, 1 live and 37 shells were found at 3 of 6 sites. The majority of these (1 live and 28 shells) were found at a single rock wall. At the most downstream reach near the weir dam 46 shells were found from 4 of 4 sites with the majority of shells (27) found in a boulder field approximately 1 m deep.

Prior to this study a significant population of *P. popeii* was known to occur in the Rio Grande immediately upstream of Laredo (Karatayev et al. 2012; Burlakova and Karatayev 2013). Our findings support this observation, but we found *P. popeii* to be more abundant and prevalent within this area than previous efforts had suggested. In the present survey, we examined ~ 40 river kilometers (rkm) spread across 90 rkm between Apache Ranch and La Bota, TX. Within this area, we found a total of 1,874 live individuals (55 ± 108 individuals/site; mean ± SD) across 15 sites or 44% (15/34) of all sites/habitats surveyed. However, for habitats consisting only of rock slabs and boulders, the presumed habitat for *P. popeii*, we collected 1,867 individuals (170 ± 131 individuals/site; mean ± SD) and found this species at 100% (11/11) of those randomly selected sites. In contrast, Karatayev et al. (2012) surveyed ~ 30 rkm between Santa Isabel Creek and the railroad bridge near the Convent Avenue border crossing (located within our study area) and reported 690 live *P. popeii* (12 ± 19 individuals/site; mean ± SD) at 38% (9/24) of all sites surveyed. However, ~ 60% (or 409) of these individuals were from a single mark-recapture site. In a subsequent survey, Burlakova and Karatayev (2013) examining 27 sites (mostly rock-type habitats) across 90 rkm from Apache Ranch to Laredo and documented 334 live individuals (25 ± 84 individual/site; mean ± SD) at 56% (15/27) of all sites surveyed.

For reaches near Del Rio, Eagle Pass, and downstream of Laredo our results corroborate findings by Karatayev et al. (2012) and Burlakova and Karatayev (2013) indicating that *P. popeii* is either extirpated or occurs in extremely low numbers that preclude detection using standard sampling methods.

For the upper Rio Grande, we found *P. popeii* to be more abundant and widely distributed than previously reported. Burlakova and Karatayev (2013) surveying near Langtry, TX, located downstream from our study area, but above Lake Amistad in the Amistad National Recreational Area, found only one live individual. In the upper portion of our study area in the lower Canyons, between La Linda and El Recodo Canyon, Brewster County (~ 50 rkms), we collected 6 individuals (0.25 ± 0.61; mean ± SD) across 17% (4/24) of the sites surveyed, which indicates that this stretch of the lower Canyons likely represents the upper distribution limit for *P. popeii* in the lower Canyons and mostly likely the Rio Grande (Figure 7). Downstream from the upper reach, between Bone Watering [Paso Colorado Crossing] and Sanderson Canyon Crossing, Terrell County (~ 13 rkms), we found 149 individuals (30 ± 30; mean ± SD) across 80% (4/5) of the sites surveyed, which represents a hotspot of *P. popeii* abundance in the lower Canyons. Finally, in the most downstream surveyed reach of the lower Canyons, between John's Marina and Arroyo El Zacate, Terrell County (~ 7 rkms), we found 34 individuals (4 ± 7; mean ± SD) across 67% (6/9) of the sites surveyed. The reduction in abundance, compared to the middle reach, indicates that this section of the lower Canyons is not a hotspot of *P. popeii* abundance. However, the fact that this species is present at a majority of rock-type habitats surveyed indicates this reach is likely not the lower end of *P. popeii's* range within the lower Canyons. Similar to reaches near Laredo, TX, rocky-type habitats were the most productive for this species; a total of 183 (11 ± 21; mean ± SD) individuals were collected across 63% (10/16) of sites consisting of rock slabs and boulders as the dominant substrate type.

For the Devils River, previous surveys reported a total of 11 individuals over a 12-year period (Burlakova and Karatayev, 2014) and most of these accounts were from downstream of Baker's Crossing to the Devils River SNA (North unit; above Dolan Falls) and one within the Devils River SNA (South unit; below Dolan Falls). Recent surveys by TPWD biologists in 2014 found a total of 13 live individuals across two sites within the TNC Dolan Falls Preserve. In the present study, we observed 35 individuals across two sites (a total of 11 sites were surveyed), which represents the largest number of live *P. popeii* collected to date from the Devils River. Our results combined with those from recent surveys by TPWD indicate that *P. popeii* may be more abundant in the Devils River than previous efforts have suggested.

For the Pecos River, previous researchers considered *P. popeii* to have been extirpated from this river (Burlakova and Karatayev 2013). The collection of a single live individual near Pandale, TX, plus shell indicates this species continues to persist in the lower Pecos. However, further sampling is needed to determine the exact distribution, abundance, and habitat associations of *P. popeii* in the lower Pecos River.

In the Rio Grande, *P. popeii* was the dominant species when present (Figure 2) and was occasionally found with *Cyrtonaias tampicoensis* (Tampico pearlymussel), *Lampsilis teres* (yellow sandshell), *Megalonaias nervosa* (washboard), *Potamilus metnecktayi* (Salina mucket), *Quadrula apiculata* (southern mapleleaf), *Truncilla cognata* (Mexican fawnsfoot), and *Utterbackia imbecillis* (paper pondshell). In the Devils River, *P. popeii* was the only mussel species collected, though specimens resembling *Potamilus purpuratus* (bleufer) have been found live in the lower reaches of the Devils River SNA. Similar to the Devils River, *P. popeii* was the only species collected in the Pecos, though shell for *C. tampicoensis* was collected near the confluence of Independence Creek.

In the upper and middle Rio Grande, adult and juvenile *P. popeii* were primarily found in rocky-type habitats containing large rocks, boulders, or in crevices along canyon walls (Figure 4), but in the Devils River live individuals were found at the heads of riffles and rapids or in clean-swept pools with bedrock (Figure 4). Indicator species analysis for *P. popeii* from the upper and middle portions of the Rio Grande supported our observation that this species primarily occurs in rocky-type habitats (which includes rock slabs, boulders, crevices along canyon walls) and the results were significant (IV = 0.70, *P*-value = 0.0003). This finding supports observations reported by Carman (2007), Howells (2010), Karatayev et al. (2012), and Burlakova and Karatayev (2014). These results also indicate that the absence of this species from reaches in Del Rio, Eagle Pass, and downstream of Laredo is not the result of sampling bias (i.e., surveyors not examining the correct habitat) as rock slabs and boulders, which are known to support this species in Laredo, John's Marina, and Black Gap WMA, were present and sampled in these reaches. Habitat associations for *P. popeii* from the Devils and Pecos were not tested because our sample size was too small (i.e., number of individuals collected and number of sites surveyed).

Size frequency distributions for the three populations were similar. Median shell length for populations in the upper and middle Rio Grande and Devils River was approximately 71 mm and the minimum and maximum shell lengths were 9.8 mm, 20.8 mm, 45 mm and 104.6 mm, 91.0 mm, and 84.5 mm, respectively (Figure 5). The shape of shell length distributions for all three populations resembles an "inverted teardrop" (*sensu* Miller and Payne 1993), which can be indicative of consistent annual recruitment (Figure 8). It is important to note that for the population upstream of Lake Amistad there appears to be a second mode around 35 mm, which may indicate recent recruitment. However, these results should be viewed with caution as *P. popeii* occupies a unique habitat type (under rock slabs and within rock crevices) that may preclude consistent detection of small individuals. That said, subadults (< 30 mm) were found at 12 of the 31 sites containing *P. popeii*. Three of these sites were from the lower Canyons and the remainder was from upstream of Laredo, TX. Generally, the population upstream of Laredo, TX, appears to have a greater abundance of subadults than the populations in the Lower Canyons or in the Devils River. The largest individuals occur in the upstream population, with several individuals exceeding 100 mm in shell length (Figure 6).

During the course of this study, we observed reproductively active females (gravid, i.e., gills containing a brood of either developing eggs or viable larvae) during summer and fall sampling events that coincide with the reproductive season of that in Black River population (Smith 2003). Specifically, we observed gravid individuals on April 7, 2015 at La Bota, on May 14-15, 2015 at John's Marina, and at Columbia, John's Marina, and La Bota on September 5-8, 2015. The smallest gravid individual observed was 38 mm long, suggesting early sexual maturation. During the September 5-8, 2015 sampling trip, 355 of 904 (39%) mussels observed were gravid. For the Devils River, gravid individuals were collected on September 17, 2015 and only 5 of 34 (15%) were gravid, but sample size was small. These results suggest that timing of spawning in the Devils River may not match that in the Rio Grande populations, which is not unexpected given the cooler water temperatures in the Devils River. Generally, cooler water temperatures may slow the maturation of fertilized eggs in the marsupium, which can reduce or postpone the period of host infection (Heinricher and Layzer, 1999). The occurrence of gravid females at La Bota in early April, John's Marina in mid-May, and both reaches again in early September suggests that *P. popeii* broods mature larvae for an extended period during summer months, and there may be asynchrony in spawning events within the population, or multiple broods are produced seasonally. The latter has been documented for members of Lampsilini (Parker et al, 1994) and Pleurobemini (Price and Eads, 2011) occurring on the coastal plain. An extended brooding period is likely, but unusual in that *Popenaias* has the ability to utilize many host species (Levine et al 2012), whereas most other "long term brooders" have specialized mechanism to attract a specific host.

Conservation maps populated with presence/absence records from 2000 to present show that recent accounts of *P. popeii* are within the last 5 years, presumably the result of increased sampling effort by qualified surveyors in the Rio Grande. The maps also show that the spatial extent of *P. popeii's* distribution has increased over the last 5 years (Figures 7 & 8). Presence records for *P. popeii* from the past 20 years show that there are 4 disjunct populations within the Rio Grande and tributaries within the United States. Amistad Reservoir is a physical barrier between these populations, and thus preventing gene flow by impeding the movement of fishes bearing *P. popeii* glochidia. Based on the total number of presence records for *P. popeii* within the last 20 years it appears the population just upstream of Laredo, TX, is the largest, but populations in the Lower Canyons and Devils and Pecos Rivers are recent discoveries and located in remote areas and as such have not been sampled as extensively as the Laredo population (Figure 9).

In summary, our results indicate that there are two disjunct populations for *P. popeii* in the Rio Grande: one in the Lower Canyons of the Rio Grande Wild and Scenic River, and the second upstream from Laredo (Figures 2 & 3). Our results also indicate that a third population does occur in the Devils River and a fourth in the Pecos River, but the status of both are still uncertain because overall sampling effort within either river remains limited and lacks sufficient spatial scale to characterize trends and identify environmental factors responsible for patterns in mussel assemblage structure. To date, the Rio Grande downstream of Falcon remains largely unsurveyed due to safety concerns stemming from drug cartel activity. In the present study, several sites were examined downstream of Falcon Lake and live unionid mussels were found, but none were *P. popeii*. Unfortunately, sampling in this portion of the Rio Grande was prematurely suspended after survey crews were temporarily detained by the Mexican Army. In general, habitat immediately downstream of Falcon Lake appears degraded, which is likely the result of frequent impoundment releases (i.e., pulsing) to support hydropower operations. Since dam-induced impacts attenuate with distance from the point of impact it is likely that instream habitat may improve further downstream. However, there are several large urban centers located along the river that discharge effluent of varying levels of pre-release treatment, which may offset any improvements to habitat associated with increased distance from Falcon Lake by degrading water quality.

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Table 1. Locality and collection information for survey sites on the Rio Grande and Devils and Pecos Rivers. Habitat abbreviations correspond to the following: BH = banks; BW = backwater; MC = midchannel; R = riffles; RS = rock slabs; and RW = canyon walls (only for reaches located in the upper Rio Grande). Reach number and corresponding locality are depicted in Figure 1. For the Pecos, the reach abbreviations correspond to the following: ICP = confluence of Independence Creek; PDL = Pandale Bridge Crossing; and WER = Weir). CPUE = mussel abundance per site divided by 4 person-hours. Subadults are defined as individuals less than 30 mm in shell length.

Site	Habitat	Reach	Locality	County	Date of collection	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
1	MC	1	La Linda	Brewster	6/8/15	0	0	-	4	150
2	BW	1	La Linda	Brewster	6/8/15	0	0	-	4	150
3	RW	1	La Linda	Brewster	6/8/15	0	0	-	4	150
4	BH	1	La Linda	Brewster	6/8/15	0	0	-	4	150
5	R	1	La Linda	Brewster	6/8/15	0	0	-	4	150
6	RS	1	La Linda	Brewster	6/8/15	0	0	-	4	150
7	BW	1	La Linda	Brewster	6/9/15	0	0	-	4	150
8	BH	1	La Linda	Brewster	6/9/15	0	0	-	4	150
9	R	1	La Linda	Brewster	6/9/15	0	0	-	4	150
10	RW	1	La Linda	Brewster	6/9/15	0	0	-	4	150
11	MC	1	La Linda	Brewster	6/9/15	0	0	-	4	150
12	RS	1	La Linda	Brewster	6/9/15	0	0	-	4	150
13	R	2	Black Gap	Brewster	6/11/15	0	0	-	4	150
14	RW	2	Black Gap	Brewster	6/11/15	1	0.25	N	4	150
15	RW	2	Black Gap	Brewster	6/11/15	0	0	-	4	150
16	BW	2	Black Gap	Brewster	6/11/15	0	0	-	4	150
17	RS	2	Black Gap	Brewster	6/11/15	0	0	-	4	150
18	MC	2	Black Gap	Brewster	6/11/15	0	0	-	4	150
19	R	2	Black Gap	Brewster	6/11/15	0	0	-	4	150
20	BH	2	Black Gap	Brewster	6/10/15	0	0	-	4	150
21	BH	2	Black Gap	Brewster	6/10/15	1	0.25	N	4	150
22	RW	2	Black Gap	Brewster	6/10/15	2	0.50	N	4	150
23	RS	2	Black Gap	Brewster	6/10/15	2	0.50	N	4	150
24	BW	2	Black Gap	Brewster	6/10/15	0	0	-	4	150
114	RW	3	John’s Marina	Terrell	9/8/2015	47	11.75	N	4	150
25	RW	3	John’s Marina	Terrell	5/16/15	72	18.00	Y	4	150
113	RS	3	John’s Marina	Terrell	9/8/2015	4	1.00	N	4	150
26	BH	3	John’s Marina	Terrell	5/16/15	0	0	-	4	150
27	RW	3	John’s Marina	Terrell	5/16/15	26	6.50	Y	4	150
28	BW	3	John’s Marina	Terrell	5/14/15	2	0.50	N	4	150
29	RW	3	John’s Marina	Terrell	5/14/15	22	5.50	N	4	150
30	R	3	John’s Marina	Terrell	5/14/15	0	0	-	4	150
31	BH	3	John’s Marina	Terrell	5/15/15	1	0.25	N	4	150
32	BW	3	John’s Marina	Terrell	5/15/15	0	0	-	4	150
33	R	3	John’s Marina	Terrell	5/14/15	2	0.50	Y	4	150
34	BH	3	John’s Marina	Terrell	5/14/15	0	0	-	4	150
35	RW	3	John’s Marina	Terrell	5/15/15	5	1.25	N	4	150
36	RS	3	John’s Marina	Terrell	5/15/15	2	0.50	N	4	150
37	RS	4	Del Rio	Val Verde	5/13/15	0	0	-	4	150
38	BW	4	Del Rio	Val Verde	5/12/15	0	0	-	4	150
39	BH	4	Del Rio	Val Verde	5/13/15	0	0	-	4	150
40	R	4	Del Rio	Val Verde	5/12/15	0	0	-	4	150
41	BW	4	Del Rio	Val Verde	5/12/15	0	0	-	4	150

Table 1. Continued.

Site	Habitat	Reach	Locality	County	Date of collection	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
42	R	4	Del Rio	Val Verde	5/12/15	0	0	-	4	150
43	MC	4	Del Rio	Val Verde	5/12/15	0	0	-	4	150
44	BH	4	Del Rio	Val Verde	5/12/15	0	0	-	4	150
45	RS	4	Del Rio	Val Verde	5/12/15	0	0	-	4	150
46	RS	5	Eagle Pass	Maverick	4/10/15	0	0	-	4	150
47	BH	5	Eagle Pass	Maverick	4/11/15	0	0	-	4	150
48	MC	5	Eagle Pass	Maverick	4/10/15	0	0	-	4	150
49	R	5	Eagle Pass	Maverick	4/10/15	0	0	-	4	150
50	BW	5	Eagle Pass	Maverick	4/11/15	0	0	-	4	150
51	BW	5	Eagle Pass	Maverick	4/11/15	0	0	-	4	150
52	R	5	Eagle Pass	Maverick	4/10/15	0	0	-	4	150
53	MC	5	Eagle Pass	Maverick	5/11/15	0	0	-	4	150
54	BH	5	Eagle Pass	Maverick	5/11/15	0	0	-	4	150
55	RS	5	Eagle Pass	Maverick	4/10/15	0	0	-	4	150
56	R	6	El Indio	Maverick	4/9/15	0	0	-	4	150
57	BW	6	El Indio	Maverick	4/9/15	0	0	-	4	150
58	MC	6	El Indio	Maverick	4/9/15	0	0	-	4	150
59	BW	6	El Indio	Maverick	4/9/15	0	0	-	4	150
60	RS	6	El Indio	Maverick	4/9/15	0	0	-	4	150
61	MC	6	El Indio	Maverick	4/9/15	0	0	-	4	150
62	BH	6	El Indio	Maverick	4/9/15	0	0	-	4	150
63	BH	6	El Indio	Maverick	4/8/15	0	0	-	4	150
64	RS	6	El Indio	Maverick	4/8/15	0	0	-	4	150
65	R	6	El Indio	Maverick	4/8/15	0	0	-	4	150
66	R	7	Apache	Webb	2/24/15	0	0	-	4	150
67	RS	7	Apache	Webb	2/25/15	27	6.75	Y	4	150
68	R	7	Apache	Webb	2/24/15	0	0	-	4	150
69	BW	7	Apache	Webb	2/25/15	0	0	-	4	150
70	BW	7	Apache	Webb	2/25/15	0	0	-	4	150
71	RS	7	Apache	Webb	2/24/15	40	10	Y	4	150
72	BW	8	Columbia	Webb	2/20/15	0	0	-	4	150
73	RS	8	Columbia	Webb	2/19/15	269	67.25	Y	4	150
74	MC	8	Columbia	Webb	2/20/15	0	0	-	4	150
75	R	8	Columbia	Webb	2/19/15	0	0	-	4	150
76	BH	8	Columbia	Webb	2/20/15	0	0	-	4	150
112	RS	8	Columbia	Webb	9/6/2015	444	111.00	N	4	150
77	RS	8	Columbia	Webb	2/20/15	215	53.75	Y	4	150
78	BW	8	Columbia	Webb	11/20/14	0	0	-	4	150
79	R	8	Columbia	Webb	2/19/15	0	0	-	4	150
111	RS	8	Columbia	Webb	9/6/2015	225	56.25	N	4	150
80	BH	8	Columbia	Webb	11/20/14	2	0.50	Y	4	150
81	RS	9	La Bota	Webb	2/21/15	28	7.00	N	4	150
82	BH	9	La Bota	Webb	11/18/14	0	0	-	4	150

Table 1. Continued.

Site	Habitat	Reach	Locality	County	Date of collection	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
83	R	9	La Bota	Webb	2/22/15	3	0.75	Y	4	150
84	MC	9	La Bota	Webb	2/21/15	0	0	-	4	150
85	BW	9	La Bota	Webb	11/16/14	0	0	-	4	150
86	MC	9	La Bota	Webb	2/22/15	0	0	-	4	150
87	R	9	La Bota	Webb	2/21/15	1	0.25	N	4	150
88	BW	9	La Bota	Webb	2/22/15	0	0	-	4	150
89	BH	9	La Bota	Webb	11/16/14	0	0	-	4	150
90	RS	9	La Bota	Webb	2/22/15	224	56.00	Y	4	150
91	BW	9	La Bota	Webb	11/18/14	0	0	-	4	150
109	RS	9	La Bota	Webb	9/5/2015	27	6.75	N	4	150
110	RS	9	La Bota	Webb	9/5/2015	184	46.00	Y	4	150
92	R	9	La Bota	Webb	4/7/15	0	0	-	4	150
93	RS	9	La Bota	Webb	4/7/15	184	46.00	Y	4	150
94	BW	9	La Bota	Webb	4/7/15	1	0.25	N	4	150
95	R	9	La Bota	Webb	4/7/15	0	0	-	4	150
96	RS	10	San Ygnacio	Zapata	2/26/15	0	0	-	4	150
97	BH	10	San Ygnacio	Zapata	11/14/14	0	0	-	4	150
98	BW	10	San Ygnacio	Zapata	11/13/14	0	0	-	4	150
99	R	10	San Ygnacio	Zapata	2/18/15	0	0	-	4	150
100	R	10	San Ygnacio	Zapata	2/18/15	0	0	-	4	150
101	RS	10	San Ygnacio	Zapata	2/18/15	0	0	-	4	150
102	MC	10	San Ygnacio	Zapata	11/15/14	0	0	-	4	150
103	RS	10	San Ygnacio	Zapata	2/18/15	0	0	-	4	150
104	MC	10	San Ygnacio	Zapata	11/15/14	0	0	-	4	150
105	BH	10	San Ygnacio	Zapata	11/13/14	0	0	-	4	150
106	BW	10	San Ygnacio	Zapata	2/26/15	0	0	-	4	150
107	BH	11	Salenino	Starr	11/19/14	0	0	-	4	150
108	BW	11	Salenino	Starr	11/19/14	0	0	-	4	150
115	Pool	-	Devils	Val Verde	9/15/15	0	0	-	4	150
116	Pool	-	Devils	Val Verde	9/15/15	0	0	-	4	150
117	MC	-	Devils	Val Verde	9/15/15	0	0	-	4	150
118	Pool	-	Devils	Val Verde	9/16/15	0	0	-	4	150
119	MC	-	Devils	Val Verde	9/16/15	0	0	-	4	150
120	RS	-	Devils	Val Verde	9/17/15	0	0	-	4	150
121	BW	-	Devils	Val Verde	9/17/15	0	0	-	4	100
122	R	-	Devils	Val Verde	9/17/15	34	8.50	N	4	150
123	MC	-	Devils	Val Verde	9/17/15	0	0	-	4	150
124	BH	-	Devils	Val Verde	9/18/15	0	0	-	4	150
125	BW	-	Devils	Val Verde	9/18/15	1	0.25	N	4	150
126	RS	ICP	Pecos	Terrell	3/17/2016	0	0	-	4	150
127	R	ICP	Pecos	Terrell	3/17/2016	0	0	-	4	150
128	P	ICP	Pecos	Terrell	3/17/2016	0	0	-	4	150
129	BH	ICP	Pecos	Terrell	3/17/2016	0	0	-	4	150
130	R	ICP	Pecos	Terrell	3/17/2016	0	0	-	4	150
131	R	PDL	Pecos	Val Verde	3/18/2016	0	0	-	4	150
132	RS	PDL	Pecos	Val Verde	3/18/2016	0	0	-	4	150
133	RS	PDL	Pecos	Val Verde	3/18/2016	0	0	-	4	150
134	RS	PDL	Pecos	Val Verde	3/19/2016	1	0.25	N	4	150
135	R	PDL	Pecos	Val Verde	3/19/2016	0	0	-	4	150
136	BH	PDL	Pecos	Val Verde	3/19/2016	0	0	-	4	150

Table 1. Continued.

Site	Habitat	Reach	Locality	County	Date of collection	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
137	RS	WER	Pecos	Val Verde	3/20/2016	0	0	-	4	150
138	RS	WER	Pecos	Val Verde	3/21/2016	0	0	-	4	150
139	RS	WER	Pecos	Val Verde	3/20/2016	0	0	-	4	150
140	RS	WER	Pecos	Val Verde	3/21/2016	0	0	-	4	150

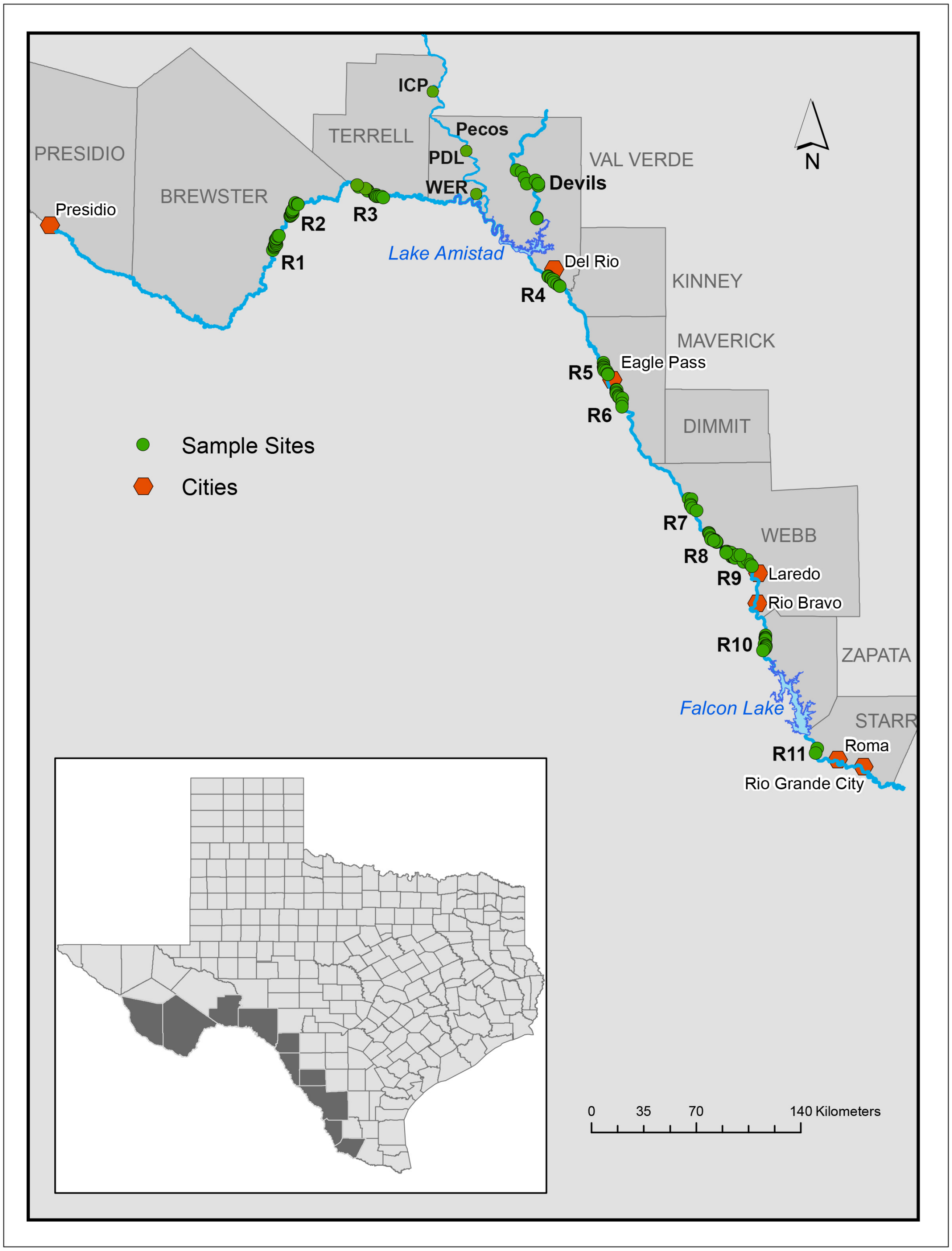

Figure 1. Map of study area. Shaded circles denote sampling locations and letters with numerals indicate sampling reaches.

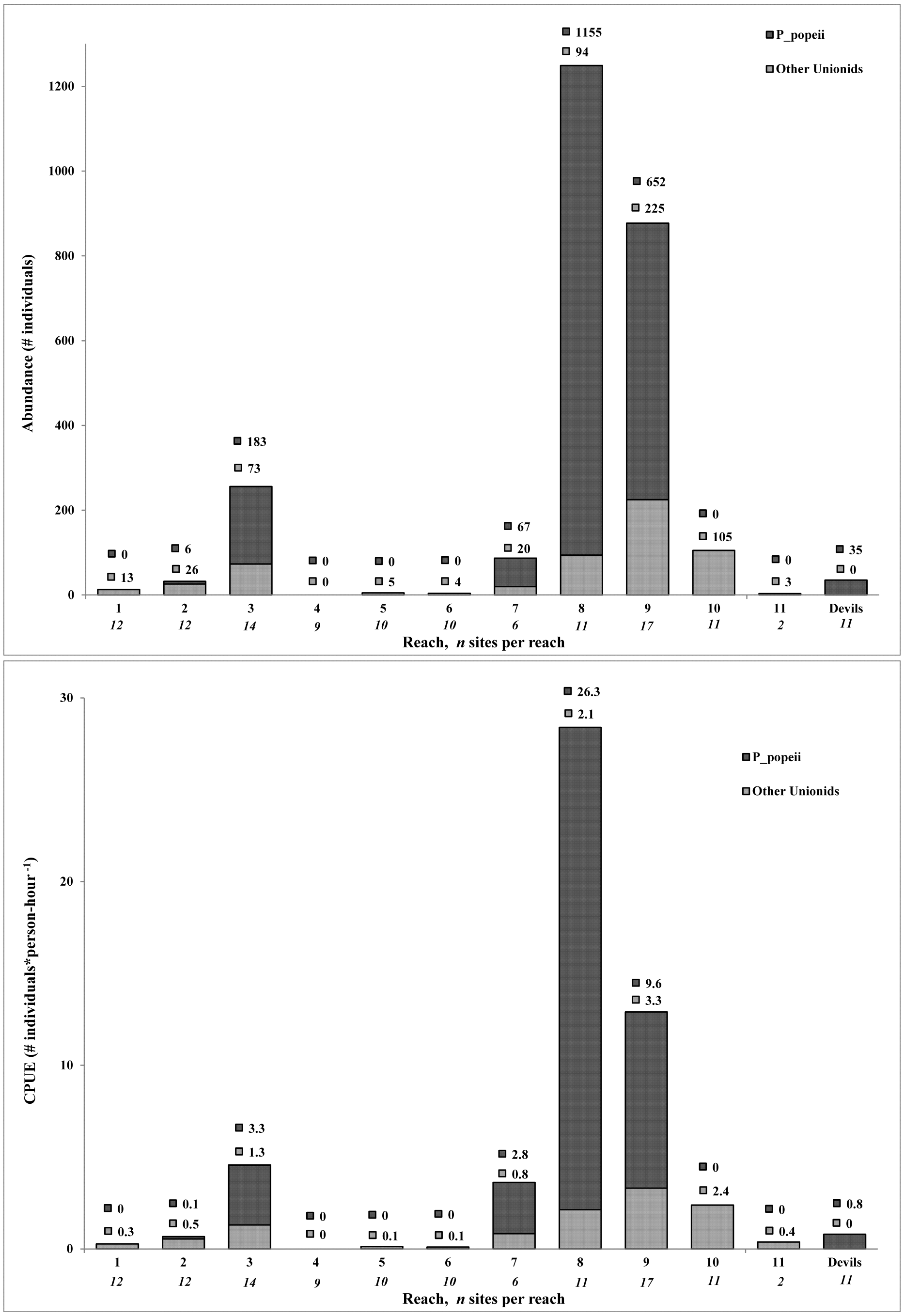

Figure 2. Total abundance (top) and relative abundance (bottom) by reach for all other mussels, "Other Unionids," and *Popenaias popeii* (Texas hornshell), "*P_popeii*," (see Table 1 for codes). Labels above bars denote total (top) or relative (bottom) number of live individuals collected. CPUE = mussel abundance per site divided by 4 person-hours, as effort was standardized at all sites. The CPUE numbers in this figure represent the totality of abundance divided by the totality of effort expended in each reach. The number of sites per reach determines the amount of effort expended per reach. The Pecos River population is not included in either graph because sample size (number of individuals and number of sites) is too small to draw any conclusions.

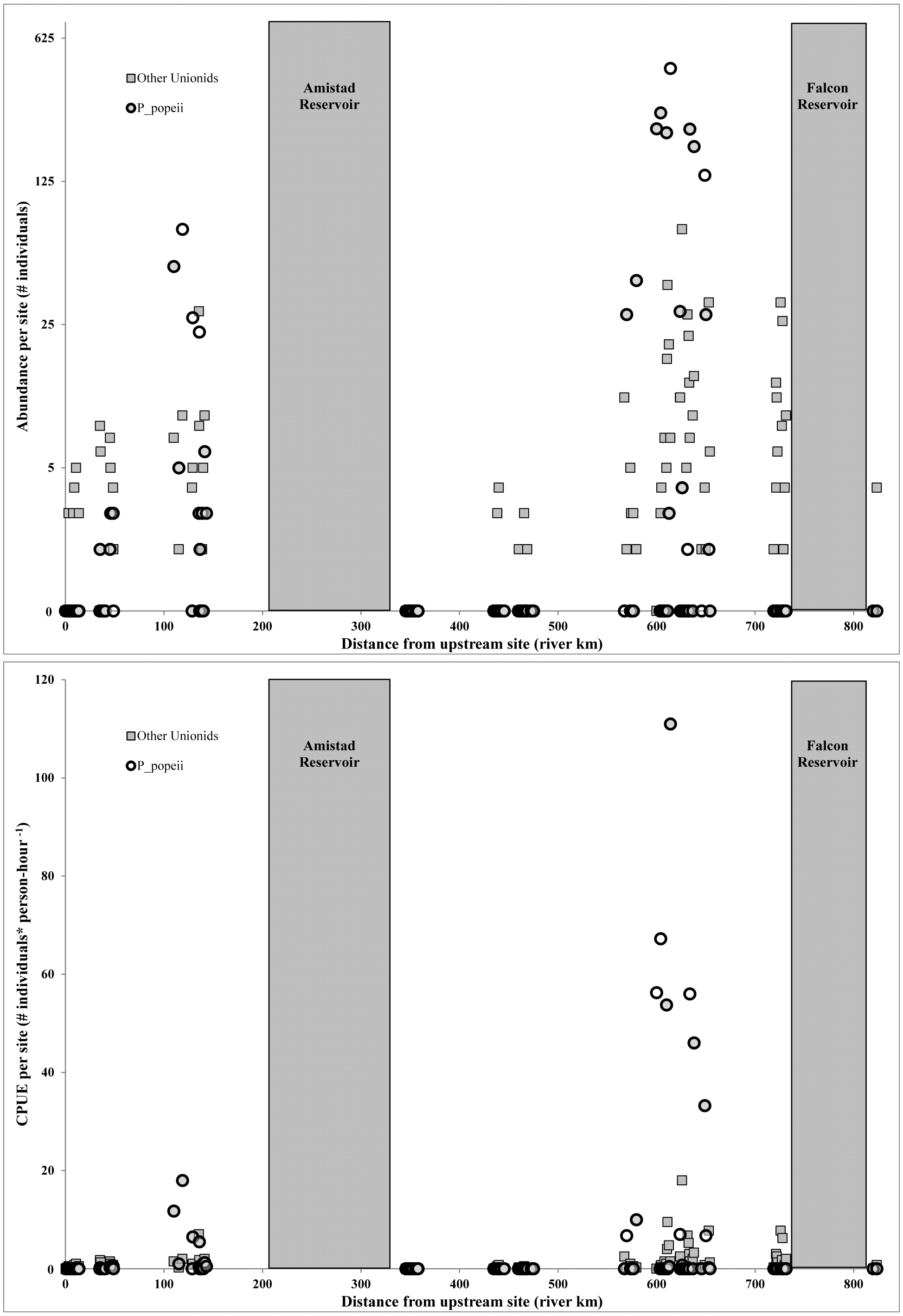

Figure 3. Longitudinal distribution of raw abundance (top) and relative abundance data (bottom) for all other mussel species, "Other Unionids," and *Popenaias popeii* (Texas hornshell), from La Linda to Saleniño, TX. The vertical axis in the top graph is transformed to logarithmic scale (base 5) to display low abundances more clearly. Each point represents species at one sample site. CPUE = total number of either TX hornshell or all other mussels encountered at each site divided by the number of person hours (4) searched at each site.

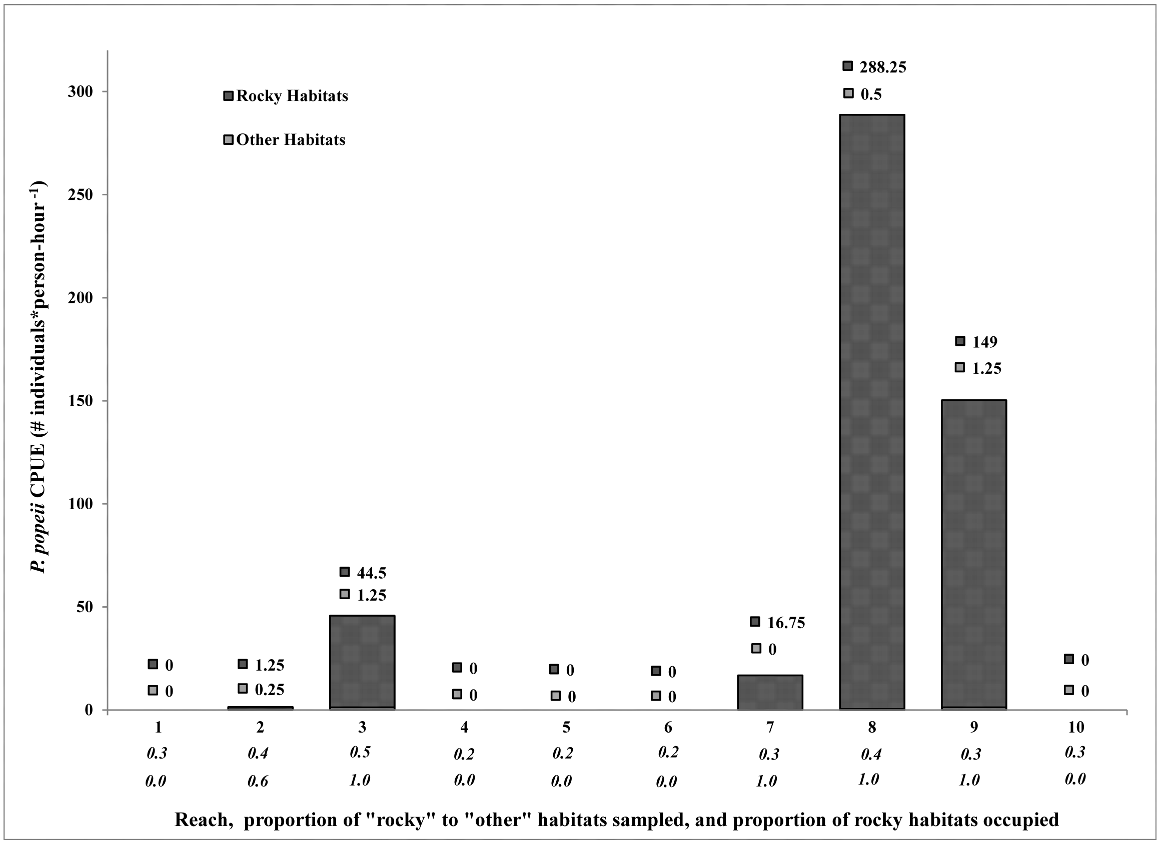

Figure 4. Relative abundance of Texas hornshell at “Rocky” habitats and all other habitat types. Values above bars denote CPUE (i.e., relative abundance) per habitat grouping. The proportion of rocky habitat to other habitat types sampled per reach and presence/absence of Texas hornshell at rocky-type habitats in each reach are listed below the x-axis. CPUE = Texas hornshell abundance per “rocky” or “other” habitat type and represents the totality of abundance divided by the totality of effort expend for each grouping. Note that Reach 11 and the Devils and Pecos Rivers have not been included because sample sizes (i.e., number of sites surveyed) for each are too small to draw meaningful conclusions regarding *P. popeii* habitat associations.

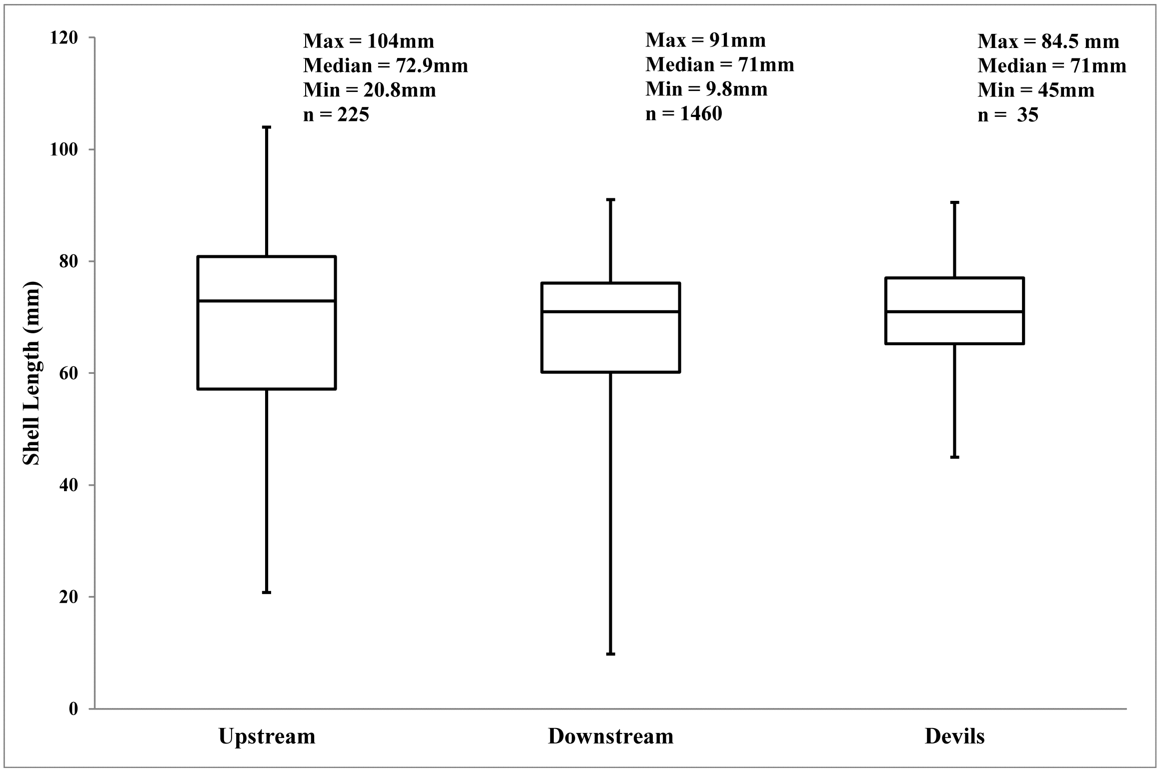

Figure 5. Box and whisker plot of shell length data for *Popenaias popeii* (Texas hornshell) populations from the Rio Grande and Devils River. Labeling on the x-axis denotes the following: "Upstream," represents the population located upstream of Lake Amistad between Black Gap WMA and John's Marina, "Downstream," represents the population from Apache Ranch to Laredo, TX, and "Devils," describes the population between Baker's Crossing at the Highway 163 bridge and the Big Satan unit of the Devils River State Natural Area. The Pecos River population is not included because sample size (number of individuals and number of sites) is too small to draw any conclusions.

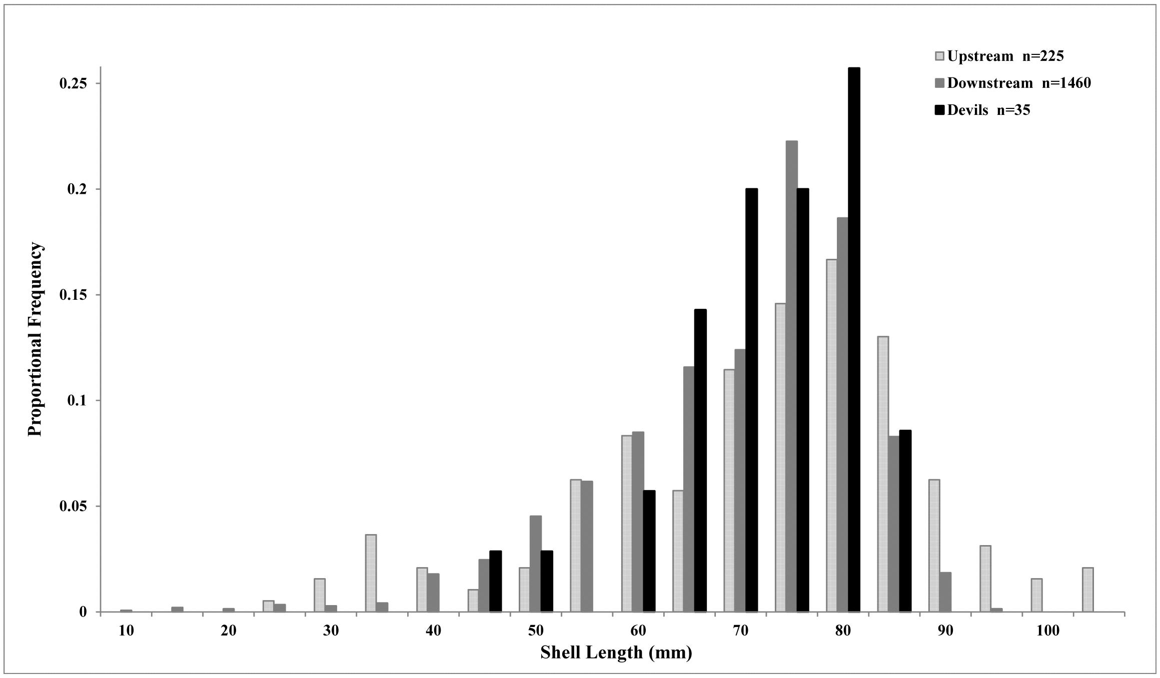

Figure 6. Proportional frequency of shell lengths for *Popenaias popeii* (Texas hornshell) from the Rio Grande and Devils River. The "Upstream" population is located in the Rio Grande upstream of Lake Amistad between Black Gap WMA and John's Marina, the "Downstream" population is located in the Rio Grande and ranges from Apache Ranch to Laredo, TX, and the "Devils River" population is located between Baker's Crossing at the Highway 163 bridge and the Big Satan unit of the Devils River State Natural Area. Shell lengths are binned into 5 mm groups. The Pecos River population is not included in because sample size (number of individuals and number of sites) is too small to draw any conclusions.

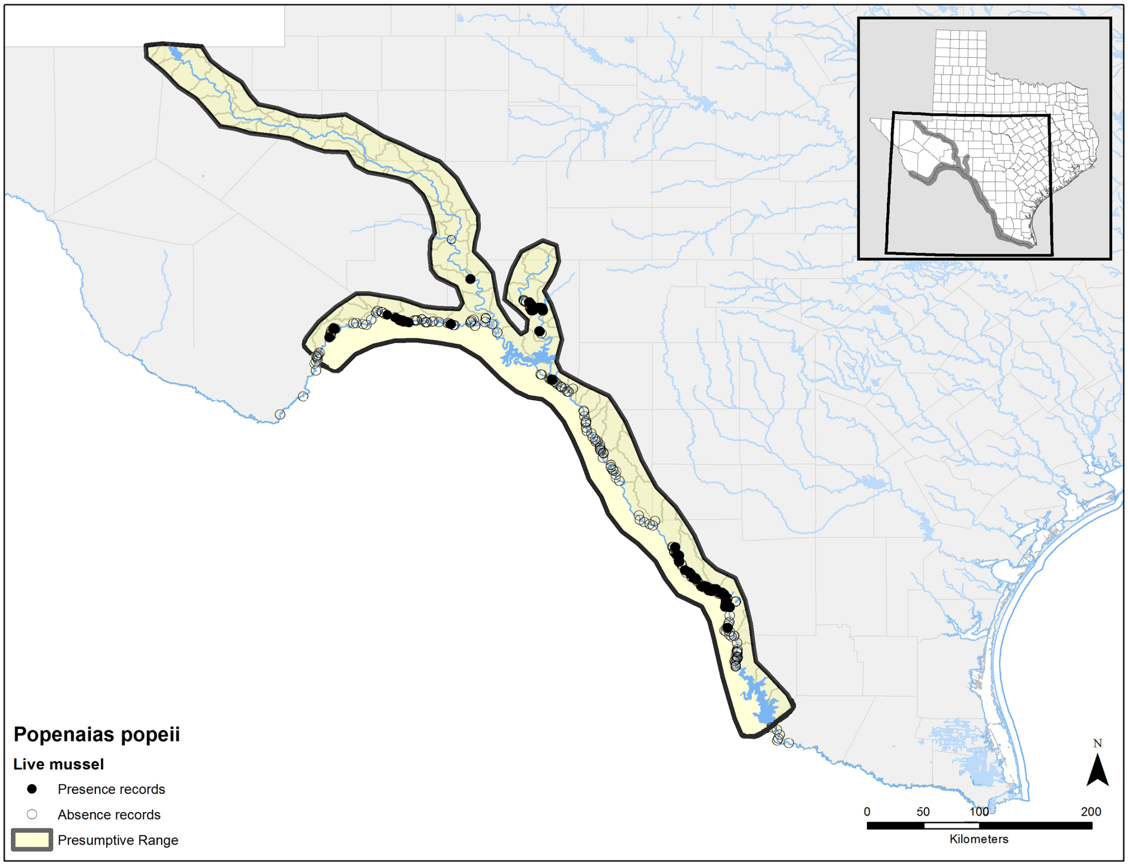

Figure 7. Map of survey locations in the Rio Grande. Shaded circles denote presence and unshaded circles indicate absence for *Popenaias popeii* (Texas hornshell). Survey sites shown are from 2000 to present and are taken from the present study plus those obtained from academic, state, and federal agencies.

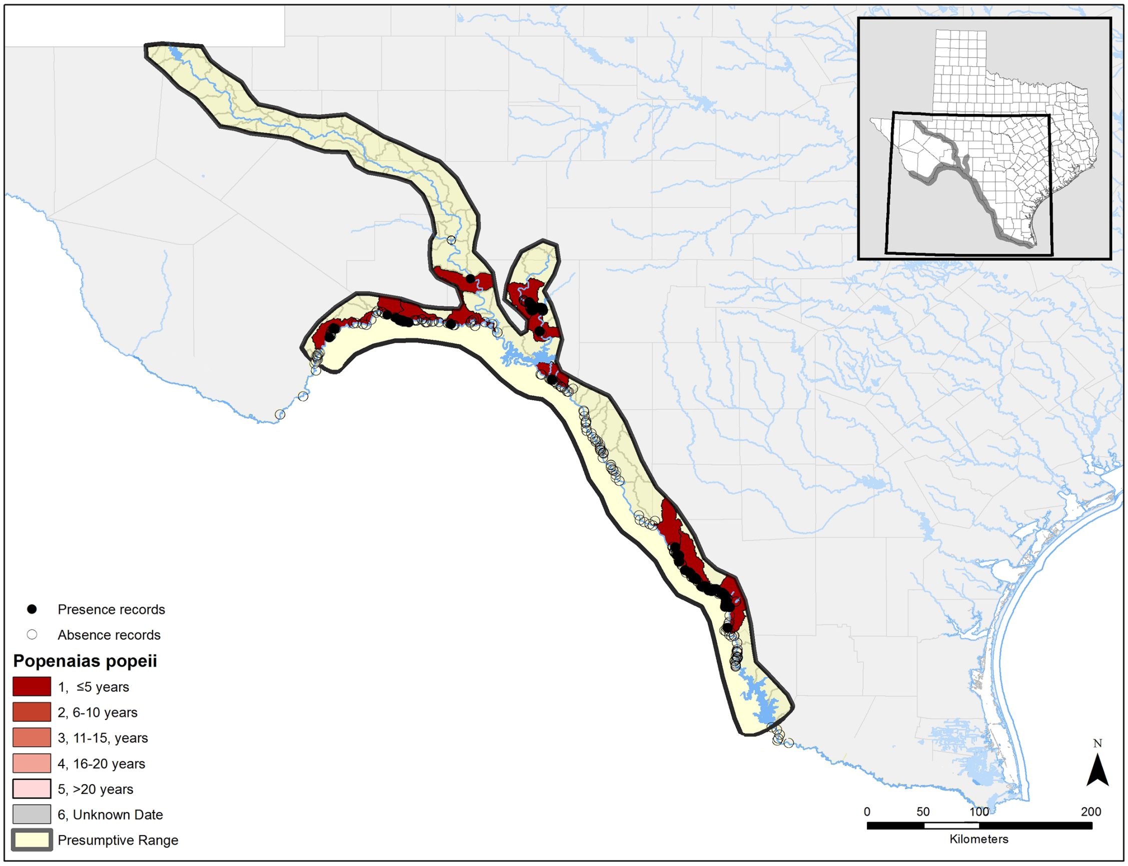

Figure 8. Conservation assessment map for *Popenaias popeii* (Texas hornshell). Shaded circles denote presence and unshaded circles indicate absence for *P. popeii*. Survey sites shown are from 2000 to present and are taken from the present study plus those obtained from academic, state, and federal agencies. HUCs are colored based on date of sampling.

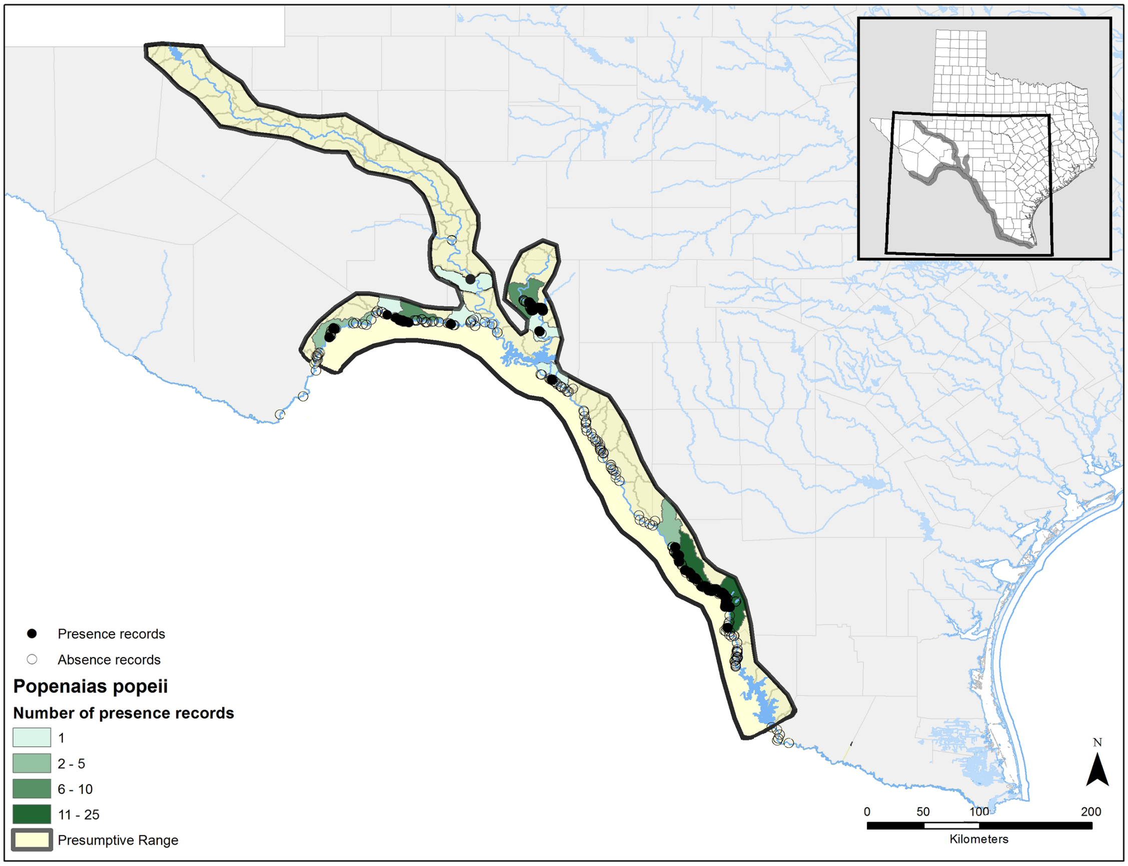

Figure 9. Map of prevalence for *Popenaias popeii* (Texas hornshell) in the Rio Grande. Shaded circles denote presence and unshaded circles indicate absence for *P. popeii*. Survey sites shown are from 2000 to present and are taken from the present study plus those obtained from academic, state, and federal agencies. HUCs are colored based on the number of times *P. popeii* was detected in a given HUC.

Survey Results for Popenaias popeii (Texas hornshell) in the Devils and Lower Pecos Rivers, Texas.

Section Summary

The goal of this study was to determine the status, distribution, and mesohabitat associations for *Popenaias popeii* (Texas Hornshell), a candidate for protection under the Endangered Species Act, in the Lower Pecos and Devils Rivers of the Rio Grande drainage in Val Verde, Terrell, and Crockett Counties, Texas. We used recent and historical data to inform a sampling program within this section of the range of *P. popeii.* In total, we surveyed 43 sites from the Independence Creek confluence with the Pecos River to the influence of Amistad Reservoir, and found 3 live *P. popeii* at 2 of 43 (4.6%) sites surveyed, with three live individuals found immediately downstream of Pandale, TX in rock wall habitats. In the Devils River, we surveyed 39 sites from approximately 3 river kilometers above Baker's Crossing (HWY 163) to Satan Canyon where the influence of Amistad Reservoir begins and found 127 live *P. popeii* at 15 of 39 (38%) sites surveyed. The majority of individuals from the Devils River were found in riffle habitats in the central portion of the study area near Dolan Falls. Within the Devils River, size frequency distributions, using shell length as a proxy for age, suggest that some level of recruitment is occurring, and observations of reproductive activity (i.e. brooding females) are congruent with observations from the population of Texas Hornshell in the Black River, New Mexico.

Introduction

The objectives of this study were to assess the distribution, abundance, and habitat use for *P. popeii* in the Lower Pecos and Devils Rivers of west Texas as a continuation of surveys conducted within the Rio Grande from the Big Bend National Scenic Riverway to Roma, TX. Prior data suggested that a population existed in these tributaries, but significant knowledge gaps remained including presence or absence of live individuals within the Pecos River. We developed a survey program to inform the habitat use and distribution of populations within the Devils River, and to detect the presence of live individuals that may persist in the Pecos River, which had not been surveyed comprehensively to date.

In tributaries of the Rio Grande, Texas hornshell is known to have existed in the Devils River from the confluence with the Rio Grande upstream to Miller Canyon, Val Verde County at present (Strecker 1931, data herein). Other historical records of *P. popeii* from the Devils River were collected by William Lloyd: USNM_118394 (in Stearns 1891) without locality info. Bereza and Fuller collected specimens in 1976 (ANSP_34891); listed as from Comstock, but this locality information is suspect. Other records without dates or locality information from the Devils river include the following: C.R. Orcutt: USNM_252546, J.D. Mitchell: USNM_464728, and a type specimen collected by Captain Pope: USNM_25735. Since then shell material has been collected upstream of Dolan Springs (Howells 2001) and a small number of live individuals have been found between Baker's Crossing to the Devils River SNA (South unit; below Dolan Falls) (Burlakova and Karatayev 2014; C.R. Robertson, personal communication), though most of these were from within the TNC Dolan Falls Preserve.

For reaches of the Pecos that flow through Texas, the last collections of live *P. popeii* were near Pandale, Val Verde County, in 1973 (A.L. Metcalf 1974, USNM 709228). Since then weathered shell material for *P. popeii* has been collected near Barstow, TX, Ward County (J.D. Mitchell, ~1890, USNM 464732), though Karatayev et al. (2012), Burlakova and Karatayev (2014) and Karatayev et al. (2015) incorrectly reported these individuals as live at time of collection. Downstream of this location, shell material for *P. popeii* has been found from the Pecos River at Iraan, TX (fragment of a valve; Burlakova and Karatayev 2014), near Pandale, TX (2 shells – relatively-long dead; Howells 2000), at the Old Ingram Dam Pump site located ~ 59 km downstream of Pandale (2 shells – relatively-long dead; Howells 2000), upstream of Painted Canyon located ~ 63 km downstream of Pandale (one valve and shell – relatively-long dead; Howells 2000), and from an ~ 8 km stretch upstream from the confluence with the Rio Grande (unspecified number– subfossil to long-dead; Burlakova and Karatayev 2014).

Until recently Texas hornshell was considered extremely rare. Singley (1893) recorded *P. popeii* from very few locations (in the Devils and Pecos Rivers) and commented that this species was rare. Neck (1982) suggested considering this species for listing by the USFWS. Williams et al. (1993) listed the species as threatened and more recently elevated it to endangered (Williams et al. in review). NatureServe ranks *P. popeii* as critically imperiled across its range and this species is currently listed as a candidate for protection under the U.S. Endangered Species Act (USFWS 2001). Surveys by Miller et al. (unpublished data), Karatayev et al. (2012), and Burlakova and Karatayev (2014) have reported live individuals or recently dead specimens for this species from the Devils River (Val Verde Co.), Rio Grande near John's Marina (Terrell Co.), Del Rio, TX (Val Verde Co.), and Laredo, TX (Webb Co.). These surveys were not initially designed to detect species with low abundance, assess evidence of recruitment, or provide population estimates. Thus, the conservation status of this species throughout its historic range is still uncertain.

Popenaias popeii have been reported to reside in rock crevices, travertine shelves, and under large boulders, where small-grained material, such as clay, silt, or sand gathers (references in Carman 2007; Howells 2010). Karatayev et al. (2012) and Burlakova and Karatayev (2013) performing surveys in portions of the upper and middle Rio Grande reported similar observations, however, their findings were also anecdotal as they primarily focused on habitats that were known or suspected to harbor *P. popeii* populations (i.e., rock slabs and boulders). Other habitats that may be suitable for mussels (e.g., banks or backwater areas) were not surveyed. Thus, habitat associations for this species remain untested and for juveniles, undescribed.

Methods

Study Area

The World Wildlife Fund currently ranks the Rio Grande as the most imperiled river in the United States due to water over-extraction and over-appropriation by human populations along the river (Wong et al. 2007). The Devils River is a pristine tributary to the Rio Grande originating in Sutton County, TX, and flows intermittently southward into Val Verde County, TX, where it becomes perennial. Flow is unregulated and provided from groundwater seepage and springs. The river lies within the Edwards Plateau region and drains an approximate area of 10,000 km^2, which is sparsely populated (Cantu and Winemiller 1997). The Pecos River is the largest tributary to the Rio Grande from the North and originates in New Mexico, draining approximately 115,000 km^2. This river is highly saline in Texas due to saline aquifer input as well as anthropogenic impacts such as groundwater extraction and irrigation; and has experienced a dramatic shift in fish fauna as well as harmful algal blooms from golden alga (*Prymnesium parvum*) since the 1980s (Southard 2010).

Sampling Methods

Site selection on the Devils and Pecos was accomplished *a priori* by one of two methods. In all cases habitat types were identified and categorized using aerial imagery. Then depending on access, sites were chosen randomly within 2km up and downstream from an access location, or the river was broken into 1km segments (reaches) and each habitat type was selected at random from those possible in each segment, then sampled during a downstream paddling trip. In both cases due to logistical constraints, we focused on locating live individuals. Riffles were targeted in the Devils River after determining that habitat type to be the most frequently occupied during early sampling trips. In the Pecos, we targeted habitats most similar to those occupied in the Rio Grande due to similarity, presence of shell material at those sites, and lack of riffle habitats as found in the Devils River.

Qualitative surveys using the timed search method were performed in each selected mesohabitat type. The timed search method was chosen because it provides a more effective means of detecting rare species than quantitative sampling methodologies (Vaughn et al. 1997). At each site (i.e., mesohabitat type), we confined the search boundaries to the specific habitat type, ensuring that the search area did not exceed 150 m^2. Each site was surveyed tactilely and visually for a total of 4 person-hours (p-h). However, because we are interested in the amount of effort needed to detect *P. popeii* (which will be important for designing long-term monitoring programs), we divided the total search time into 4, 1 p-h intervals. At the end of each search interval, surveyors combined all live specimens into a mesh bag, which was kept submerged in water until completion of the survey. During each interval, surveyors were spread out in the search area and every effort was made to search all available microhabitats. Following completion of the survey, all live mussels from each time period were identified to species, counted, measured, checked for gravidity, and then returned back to the river into the appropriate habitat.

Data Analysis

Scatter plots were used to visually explore the longitudinal distribution and abundance of *P. popeii* in each river (Figures 1 and 2). Bar graphs were used to display habitat associations for live and *in situ* shells of *P. popeii* (Figures 3 and 4). Boxplots and length-frequency histograms were developed for *P. popeii* to assess demographic patterns and population structuring within each river, where sufficient data existed (Figures 5 - 7). Generally, multimodal size class distribution may indicate recruitment, whereas truncated distributions (absence of a particular age class, large, or small individuals) may indicate a lack of recent recruitment or a localized extinction event. In the present study, we defined groups by available mesohabitat type (i.e., riffle, midchannel, pool, backwater, and bank).

Results/Discussion

Devils River

A total of 152 person hours were spent surveying 39 sites spanning 62 River Kilometers in the Devils River (Figure 8). A total of 127 *P. popeii* individuals were found from 15 of the 39 sites (Table 1). Relative abundance in the Devils River was 2.1 ± 2.7 mussels per person-hour (mean ± SD) at sites where *P. popeii* was present. No other native mussel species were encountered in the study area. Based on our findings Texas hornshell ranges throughout the Devils River, but is most abundant within and near the TNC Dolan Falls Preserve.

The size frequency distribution of *P. popeii* in the Devils River indicates that some level of recruitment is occurring as the shape of the histogram approximates an inverted teardrop, though the population does appear to have a relatively high number of mid-sized individuals suggesting either a large recruitment event recently, or a past event that removed larger individuals from our sample sites (Figure 5). In contrast to the Rio Grande, individuals in the Devils River appear to reach a smaller maximum size which may be due to reduced growth rate as determined by lower temperatures and nutrient levels in this tributary. Median shell length for this population was 55 mm and minimum and maximum shell lengths were 16 mm and 84.5mm, respectively (Figure 6). We observed reproductive activity (gills containing maturing eggs or glochidia) in September of 2015 and May and June of 2016, which corroborate prior observations from the Black River, New Mexico of the reproductive season for this species (Smith et al. 2003).

Results from our data suggest that habitat preferences for *P. popeii* are riffle habitats in the Devils River (Figure 3) contrary to previous findings in the Rio Grande where most individuals are found in bedrock crevices or under boulders. These habitats are present in the Devils River, but are frequently covered in silt, which may limit their suitability.

Lower Pecos River

A total of 172 person-hours were spent surveying 43 sites in the Lower Pecos River of Texas, downstream from Independence Creek (Figure 9). A total of 3 live individuals of *P. popeii* were found from 2 of those 43 sites, all in reach ”B” near the Pandale Crossing (Table 2, Figure 2, and Figure 9). No *P. popeii* shells were found in Reach “A” near Independence Creek, but were present in all other reaches downstream (Figure 2). Shells were found *in situ* and reflect habitat use in the past that is similar to trends present in the lower canyons of the Big Bend Wild and Scenic Riverway (See Rio Grande Texas Hornshell Report) where *P. popeii* predominantly inhabits crevices in rock walls and sloughed pieces of rock walls, or boulder fields. Only three live individuals were encountered in the Lower Pecos River, with shell lengths of 56, 64, and 95mm (Figure 7). No inferences regarding population demographics can be inferred from this sample size. None of the live individuals were exhibiting reproductive behavior at the time of collection, and the lack of small individuals suggests recruitment is not occurring currently. No other native mussels were encountered during sampling on the Lower Pecos River.

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Table 1. Devils River study sites ordered from furthest upstream to the confluence of the Rio Grande. Sub-adults defined as individuals less than 35 mm in length.

Site	Reach	Habitat	County	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
1	A	Riffle	Val Verde	0	0	-	4	150
2	A	Riffle	Val Verde	3	0.75	Y	4	150
3	A	Backwater	Val Verde	0	0	-	4	150
4	A	Riffle	Val Verde	0	0	-	4	150
5	A	Pool	Val Verde	0	0	-	4	150
6	A	Riffle	Val Verde	1	0.25	N	4	150
7	A	Riffle	Val Verde	0	0	-	4	150
8	A	Riffle	Val Verde	0	0	-	4	150
9	A	Pool	Val Verde	0	0	-	4	150
10	B	Mid-Channel	Val Verde	0	0	-	4	150
11	B	Pool	Val Verde	0	0	-	4	150
12	B	Riffle	Val Verde	1	0.25	N	4	150
13	B	Bank	Val Verde	0	0	-	4	150
14	B	Bank	Val Verde	10	2.5	Y	4	150
15	B	Mid-Channel	Val Verde	0	0	-	4	150
16	B	Mid-Channel	Val Verde	0	0	-	4	150
17	C	Riffle	Val Verde	30	7.5	Y	4	150
18	C	Riffle	Val Verde	14	3.5	Y	4	150
19	C	Backwater	Val Verde	0	0	-	4	150
20	C	Rock slab	Val Verde	0	0	-	4	150
21	C	Mid-Channel	Val Verde	0	0	-	4	150
22	C	Backwater	Val Verde	0	0	-	4	150
23	C	Riffle	Val Verde	34	8.5	N	4	150
24	C	Riffle	Val Verde	1	0.25	N	4	150
25	C	Riffle	Val Verde	16	4	N	4	150
26	C	Riffle	Val Verde	8	2	N	4	150
27	C	Mid-Channel	Val Verde	0	0	-	4	150
28	C	Bank	Val Verde	0	0	-	4	150
29	C	Riffle	Val Verde	0	0	-	4	150
30	D	Rock slab	Val Verde	1	0.25	N	4	150
31	D	Bank	Val Verde	0	0	-	4	150
32	D	Riffle	Val Verde	0	0	-	4	150
33	D	Riffle	Val Verde	4	1	N	4	150
34	D	Backwater	Val Verde	0	0	-	4	150
35	E	Bank	Val Verde	0	0	-	4	150
36	E	Backwater	Val Verde	1	0.25	N	4	150
37	E	Riffle	Val Verde	2	0.5	N	4	150
38	E	Riffle	Val Verde	1	0.25	N	4	150
39	E	Riffle	Val Verde	0	0	-	4	150

Table 2. Lower Pecos River basin study sites ordered from furthest upstream to the confluence of the Rio Grande. Sub-adults defined as individuals less than 35 mm in length.

Site	Reach	Habitat	County	Number of live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
1	A	Riffle	Terrell/Crockett	0	0	-	4	150
2	A	Rock slab	Terrell/Crockett	0	0	-	4	150
3	A	Bank	Terrell/Crockett	0	0	-	4	150
4	A	Riffle	Terrell/Crockett	0	0	-	4	150
5	A	Pool	Terrell/Crockett	0	0	-	4	150
6	B	Riffle	Val Verde	0	0	-	4	150
7	B	Rock slab	Val Verde	0	0	-	4	150
8	B	Boulder field	Val Verde	0	0	-	4	150
9	B	Bank	Val Verde	0	0	-	4	150
10	B	Riffle	Val Verde	0	0	-	4	150
11	B	Rock slab	Val Verde	1	0.25	N	4	150
12	B	Rock wall	Val Verde	0	0	-	4	150
13	B	Boulder field	Val Verde	0	0	-	4	150
14	B	Boulder field	Val Verde	0	0	-	4	150
15	B	Rock wall	Val Verde	0	0	-	4	150
16	B	Riffle	Val Verde	0	0	-	4	150
17	B	Backwater	Val Verde	0	0	-	4	150
18	B	Rock wall	Val Verde	0	0	-	4	150
19	C	Rock wall	Val Verde	2	0.5	N	4	150
20	C	Boulder field	Val Verde	0	0	-	4	150
21	C	Boulder field	Val Verde	0	0	-	4	150
22	C	Rock wall	Val Verde	0	0	-	4	150
23	C	Boulder field	Val Verde	0	0	-	4	150
24	C	Backwater	Val Verde	0	0	-	4	150
25	C	Boulder field	Val Verde	0	0	-	4	150
26	C	Riffle	Val Verde	0	0	-	4	150
27	C	Rock wall	Val Verde	0	0	-	4	150
28	D	Rock wall	Val Verde	0	0	-	4	150
29	D	Rock wall	Val Verde	0	0	-	4	150
30	D	Boulder field	Val Verde	0	0	-	4	150
31	D	Boulder field	Val Verde	0	0	-	4	150
32	D	Backwater	Val Verde	0	0	-	4	150
33	D	Riffle	Val Verde	0	0	-	4	150
34	E	Boulder field	Val Verde	0	0	-	4	150
35	E	Rock wall	Val Verde	0	0	-	4	150
36	E	Riffle	Val Verde	0	0	-	4	150
37	E	Boulder field	Val Verde	0	0	-	4	150
38	E	Backwater	Val Verde	0	0	-	4	150
39	E	Rock wall	Val Verde	0	0	-	4	150
40	F	Boulder field	Val Verde	0	0	-	4	150
41	F	Rock slab	Val Verde	0	0	-	4	150
42	F	Rock slab	Val Verde	0	0	-	4	150
43	F	Rock slab	Val Verde	0	0	-	4	150

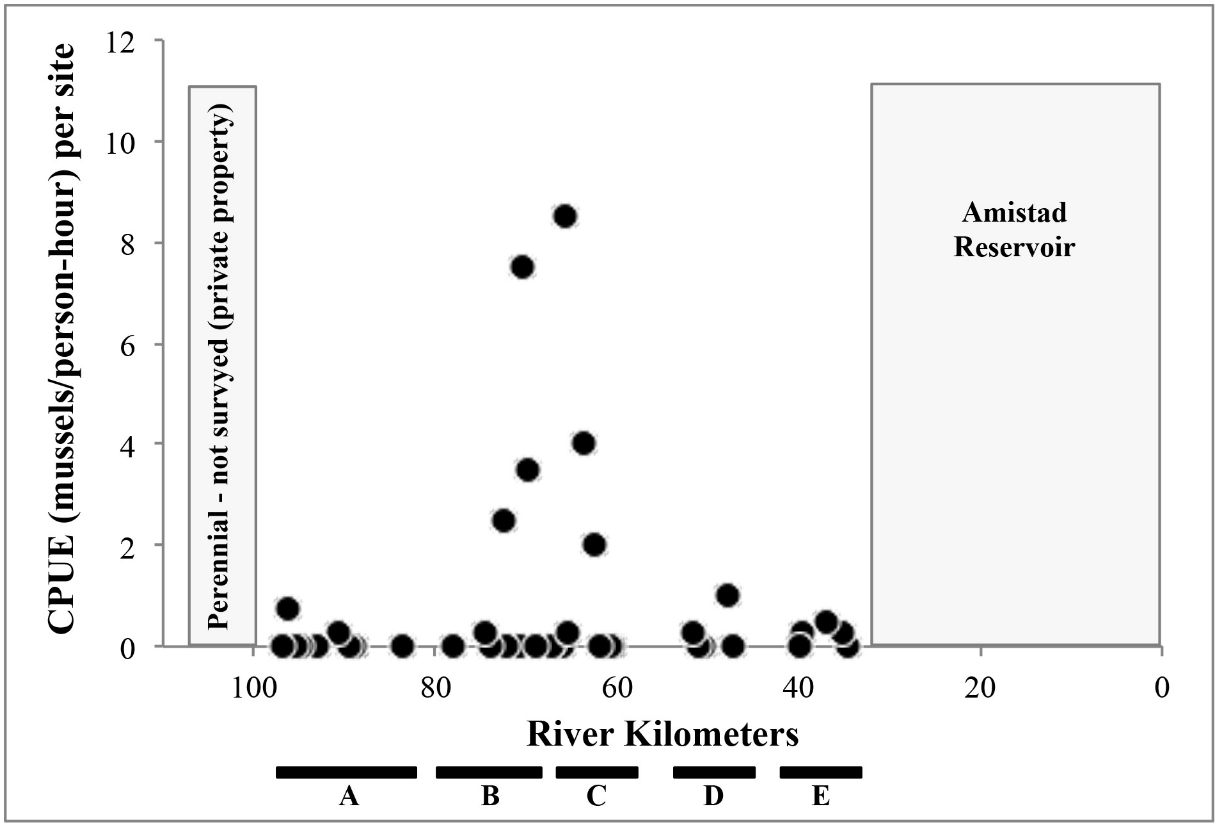

Figure 1. Longitudinal distribution of relative abundance data of *Popenaias popeii* (Texas hornshell) on the Devils River. CPUE = total number of TX hornshell found at each site divided by the number of person hours (4) searched at each site. River Kilometers are measured upstream from the confluence with the Rio Grande, now inundated by Amistad Reservoir. Reaches are labeled under the X- axis.

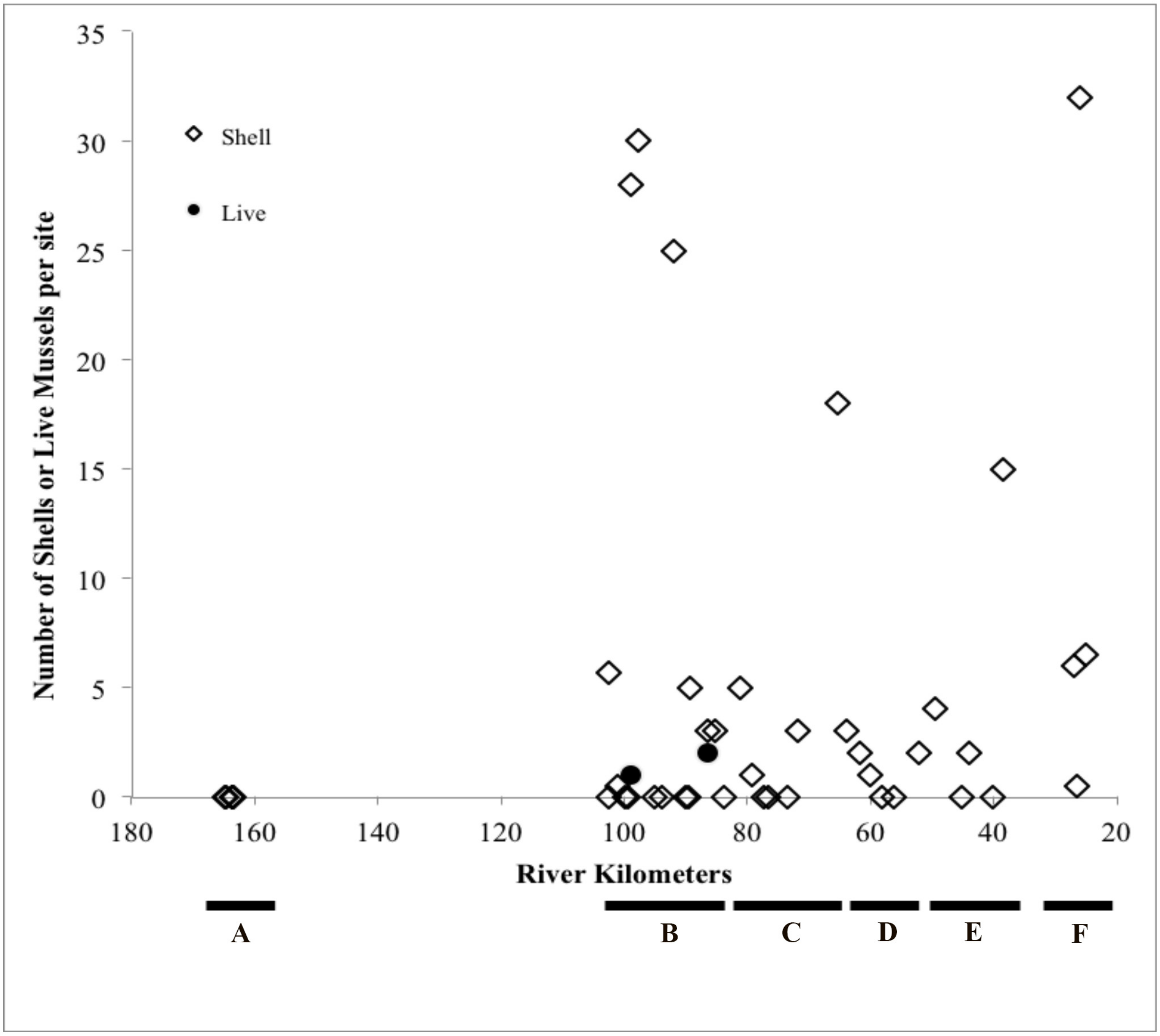

Figure 2. Longitudinal distribution of raw abundance of shell and live individuals of *Popenaias popeii* (Texas hornshell) on the Pecos River. River Kilometers are measured upstream from the confluence with the Rio Grande. Symbols indicating zero shells found at a site also represent zero live mussels at that location. Reaches are labeled under the X-axis.

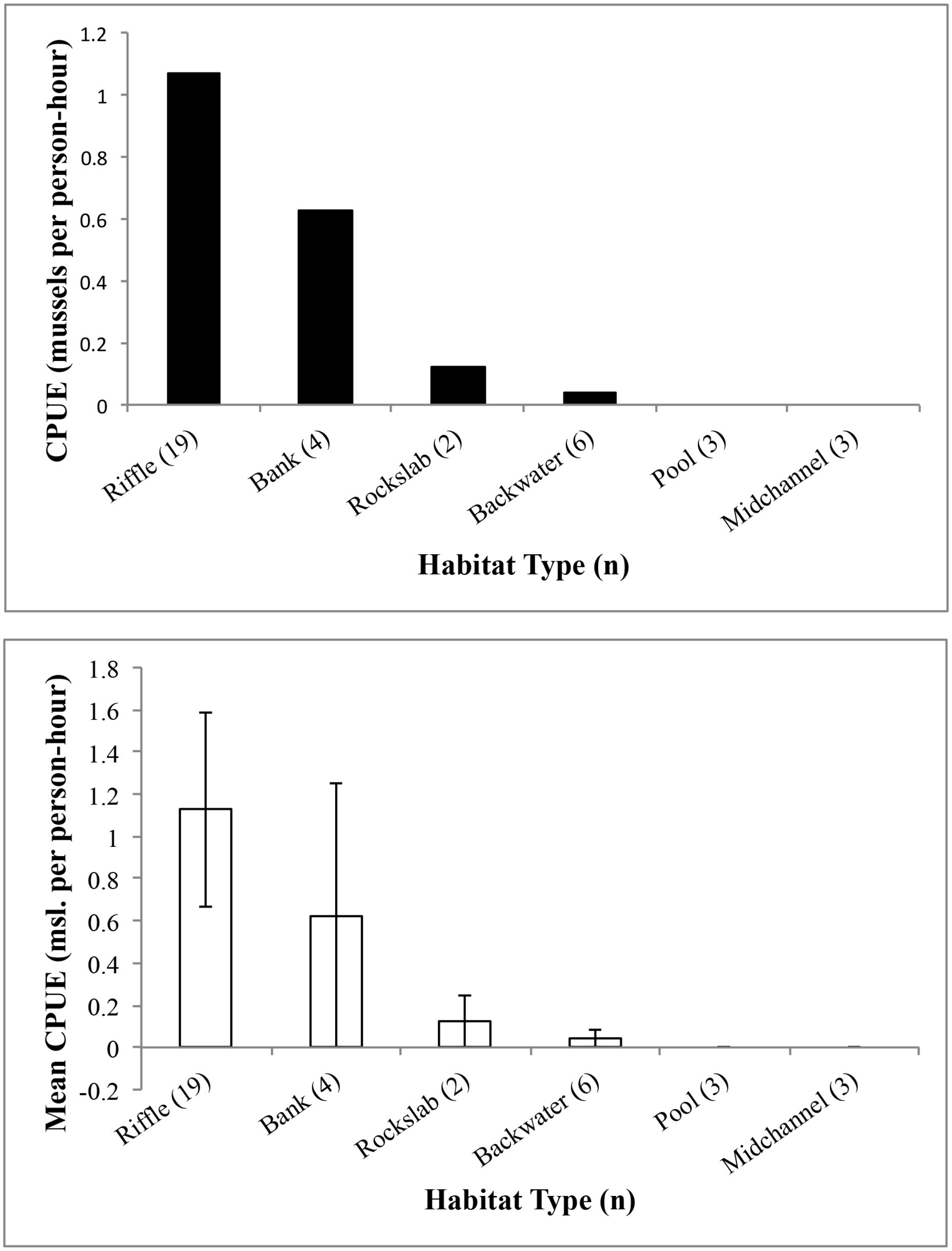

Figure 3. Relative abundance (top) and mean relative abundance (bottom) of *Popenaias popeii* (Texas hornshell) across all habitats sampled in the Devils River. CPUE = represents the totality of Texas hornshell abundance across all sites sampled for a given habitat type divided by the totality of effort expended across those sites. Mean CPUE (± 1 SE) = represents the mean relative abundance across a given habitat type. The number of each habitat type sampled follows the label. "Rock" habitat includes rock slabs and boulder fields.

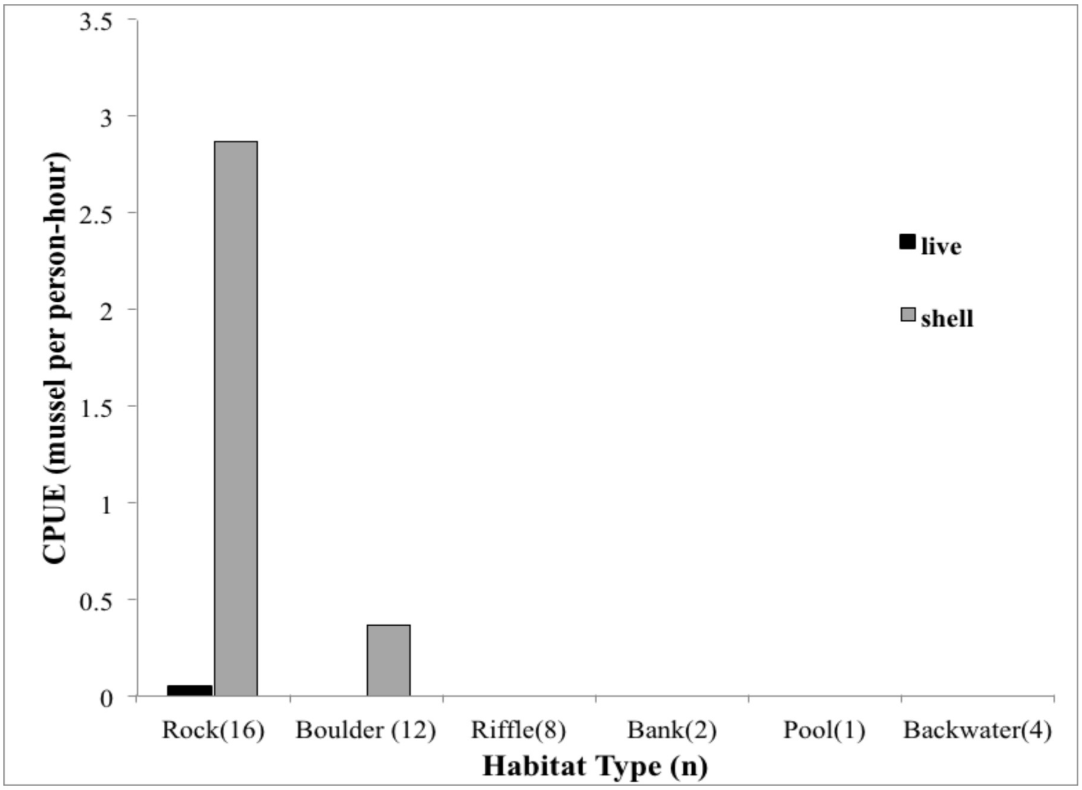

Figure 4. Relative abundance (mussels or shells per person-hour of effort) of *Popenaias popeii* (Texas hornshell) in each habitat type surveyed in the Pecos River. CPUE = represents the totality of Texas hornshell abundance across all sites sampled for a given habitat type divided by the totality of effort expended across those sites. The number of each habitat type sampled follows the label. “Rock” habitat includes rock walls and rock slabs from Table 2.

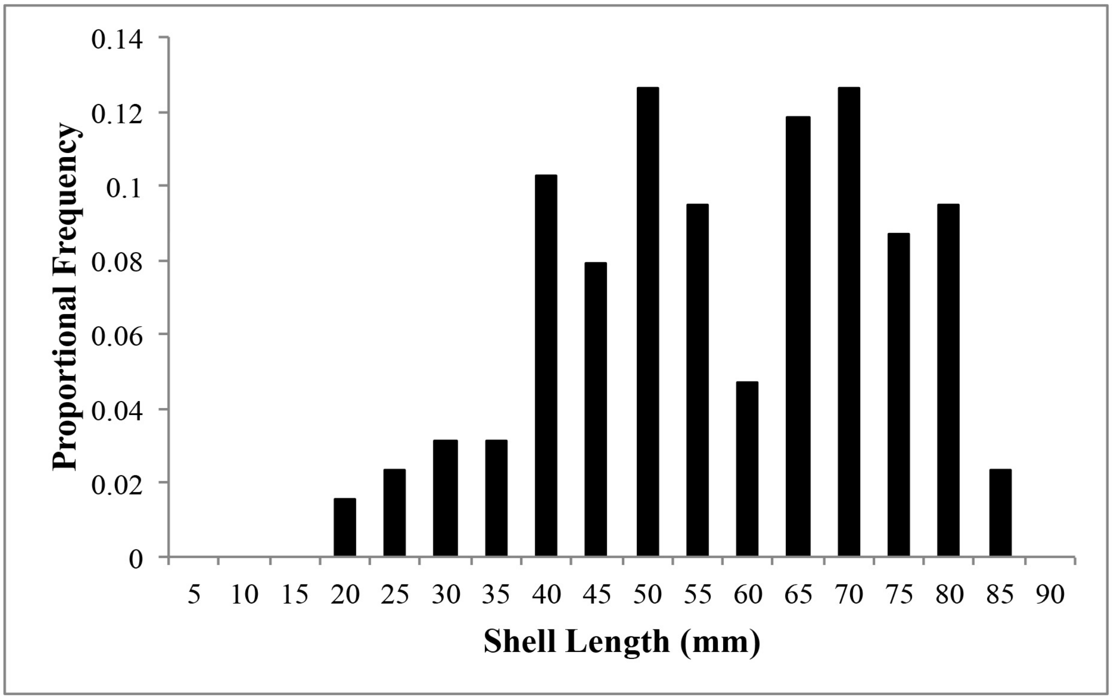

Figure 5. Histogram of *Popenaias popeii* (Texas hornshell) shell length data (n = 127) from the Devils River.

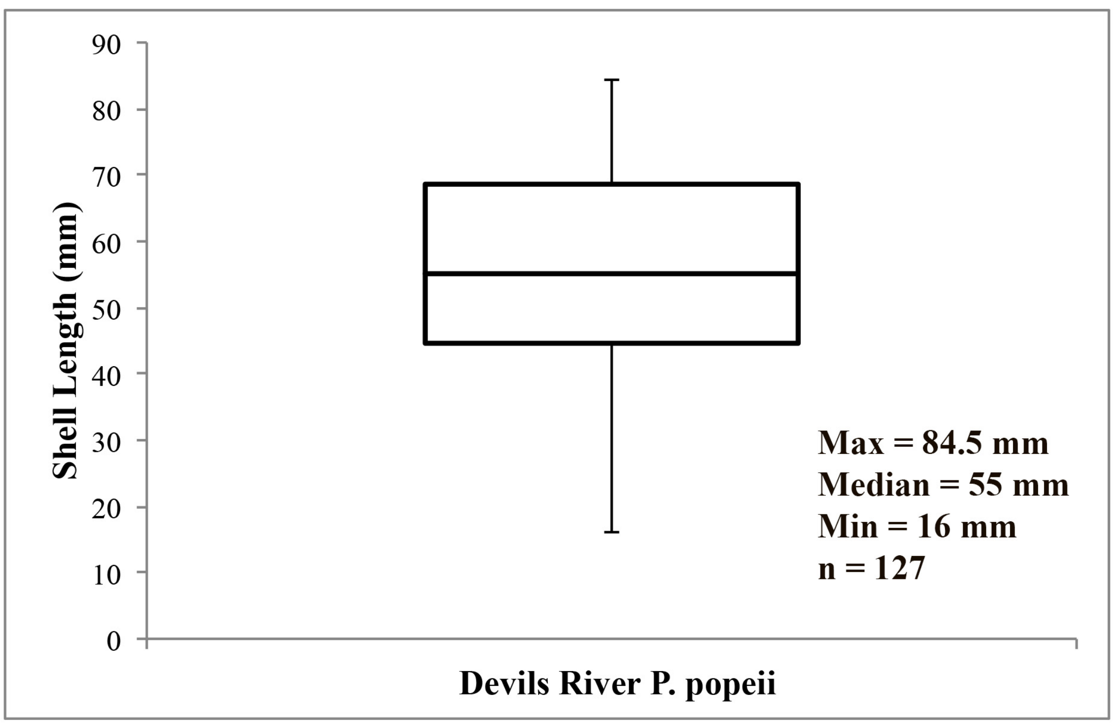

Figure 6. Box and Whisker plot of *Popenaias popeii* (Texas hornshell) shell length data (n = 127) from the Devils River. Insufficient sample size from the Pecos River prevent use of those data in this figure.

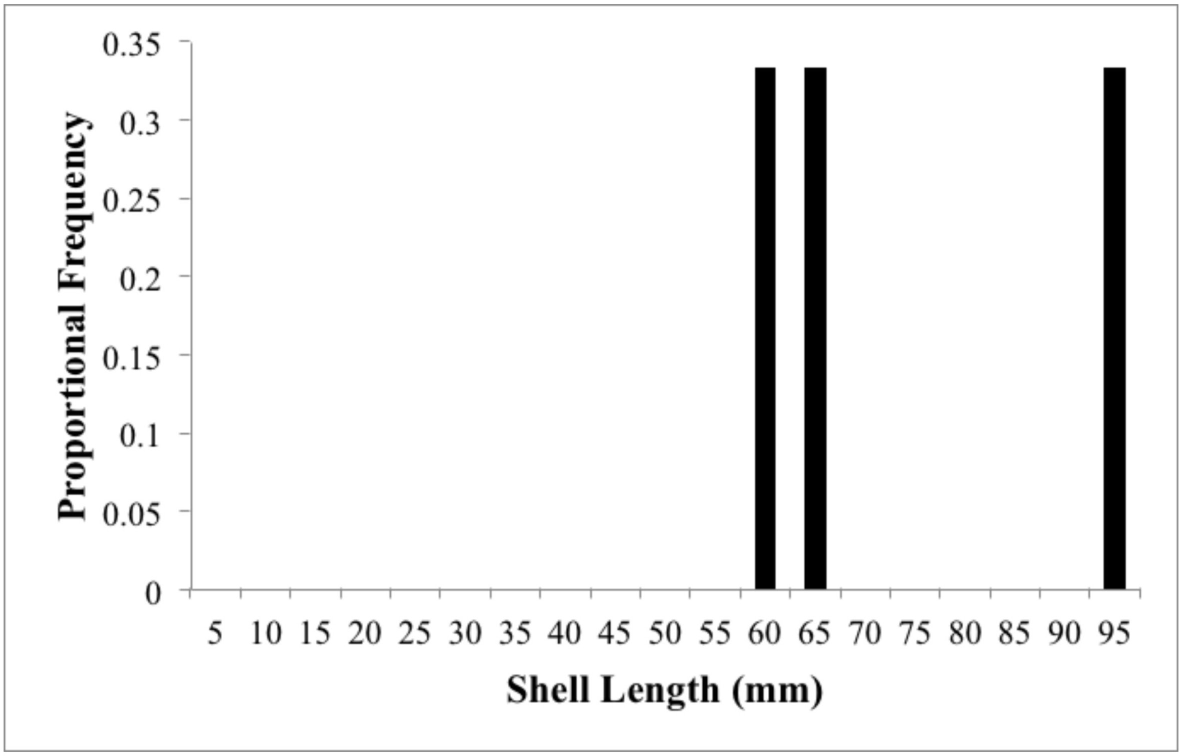

Figure 7. Histogram of live *Popenaias popeii* (Texas hornshell) shell length data (n = 3) from the lower Pecos River.

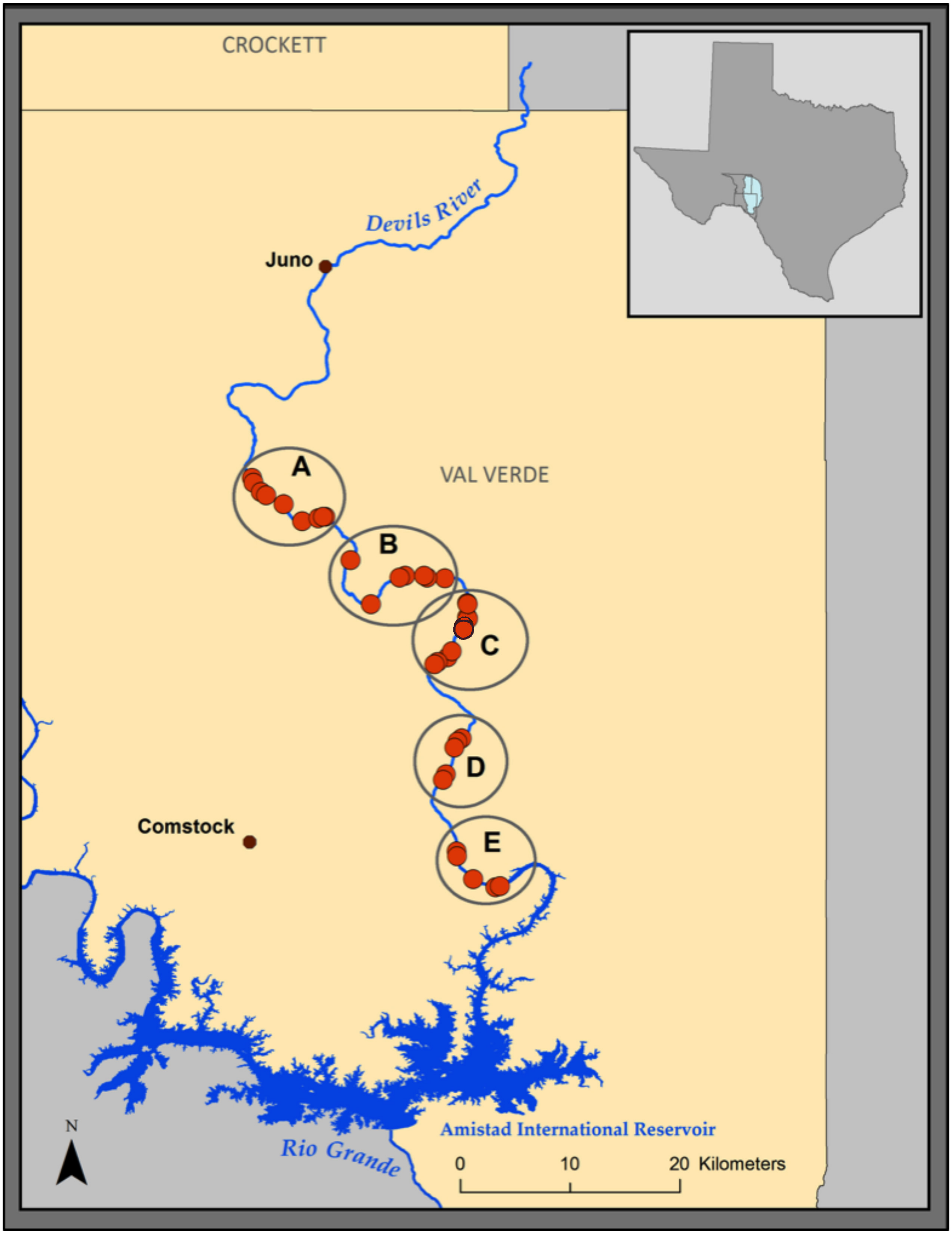

Figure 8. Devils River study area. Reaches correspond to table 1.

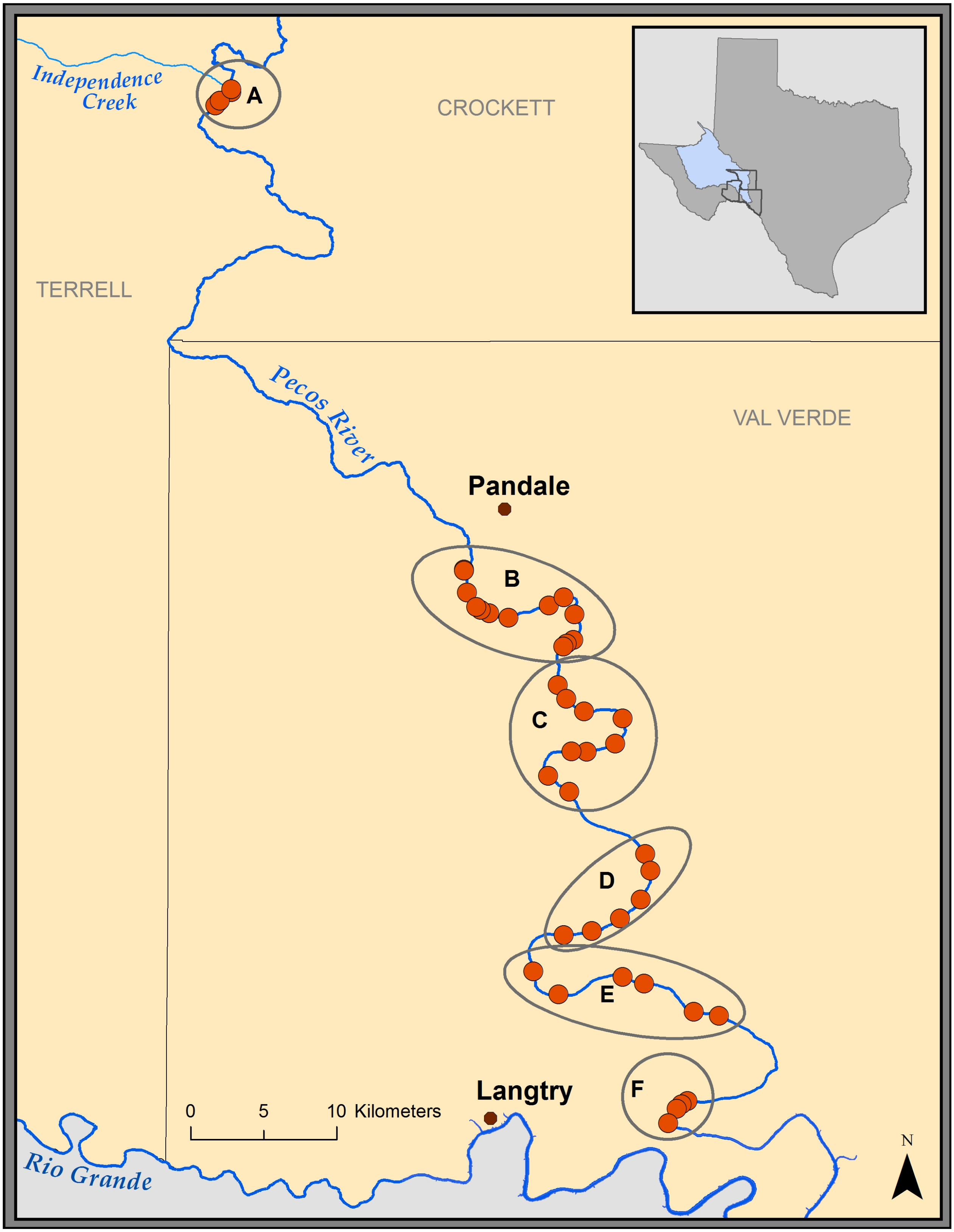

Figure 9. Pecos River study area. Reaches correspond to table 2.

Distribution and Habitat Use for *Truncilla cognata* (Mexican fawnsfoot)

Section Summary

The goal of this study was to determine the status, distribution, and mesohabitat associations for *Truncilla cognata* (Mexican fawnsfoot), a species currently under review for protection under the Endangered Species Act, in the Rio Grande drainage. We used recent and historical data to inform a sampling program within the range of *T. cognata.* In total, we surveyed 196 sites in the Rio Grande, Devils River, and Pecos River, and found 213 live *T. cognata* from 30 of 196 (15.3%) sites. All live individuals were found in the lower reach of the Rio Grande. The majority of live individuals were found in riffle habitat. Size frequency distributions, using shell length as a proxy for age, suggest that some level of recruitment is occurring in the Rio Grande. We surveyed 39 sites in the Devils River and 43 sites in the Pecos River, and found no live individuals or shell material of *T. cognata*. Our results indicate that *T. cognata* occurs at low abundance in the lower reach of the Rio Grande (Maverick, Webb, and Zapata counties) and is likely extirpated above Lake Amistad.

Introduction

Truncilla cognata, (Mexican fawnsfoot), is known historically from the Rio Grande and the Rio Salado, Nuevo Leon, Mexico (Lea, 1860; Johnson, 1999). The holotype specimen was collected by Isaac Lea from the Rio Salado in Nuevo Leon, Mexico in 1860 (Lea 1860). *Truncilla cognata* is considered a valid and distinct species, though its taxonomy has not been validated with genetic analysis (Burlakova and Karatayev, 2010; Howells 2010).

In the Rio Grande basin, historic records of *T. cognata* have come from the mainstem of the Rio Grande and its major tributaries. The presence of *T. cognata* has been sporadically reported in Texas, and Taylor (1966) concluded that this species was extremely rare in the Rio Grande. The first observation of live *T. cognata* in Texas was by Metcalf in the Rio Grande near Del Rio, Texas in 1972 (Howells et al. 1997; Howells 2001, 2010). However, no live or dead specimens were found from the Rio Grande system until 2003, when a live individual was found in the Rio Grande near Laredo, Webb County, Texas (Howells et al. 2003; Howells 2007). Additional live individuals were found within the same stretch of river in 2008 (n = 5) and in 2011 (n = 12) (Burlakova and Karatayev 2008; Karatayev et al. 2012). To date, live *T. cognata* have not been found above Lake Amistad despite occurring there in the past (Howells 2001). Due to declines in its distribution, *T. cognata* is listed as state-threatened by Texas Parks and Wildlife Department (TPWD 2010) and is being considered for listing under the U.S. Endangered Species Act (USFWS 2011).

Currently, little is known about the life history or reproductive requirements of *T. cognata* (Howells 2010). Like other freshwater mussel species, it is an obligate ectoparasite on one or more host-fish species. Congeners are long-term brooders with glochidia observed between August and May and use freshwater drum, *Aplodinotus grunniens* as a host (Haag, 2012). It is likely that a similar reproductive life history and host-fish use is present in *T. cognata*. Based on recent observations from field surveys throughout *T. cognata*'s range, sub-adults and adults appear to occur most often in mixed sand and gravel as well as soft and unconsolidated sediments (e.g. sand, sand, and clay) in shallow protected near shore areas adjacent to riffles and backwater habitats (Randklev et al. unpublished data).

The objectives of this study were to assess the distribution, abundance, and habitat use for *T. cognata* in the Rio Grande drainage. The resulting survey information was then used to develop Conservation Status Assessment Maps for this species within the Rio Grande and major tributaries.

Methods

Study Area

The Rio Grande originates in Colorado and is considered the 4th largest river in the United States, with an approximate length of 3,050 km and draining a total of 870,236 km^2 (Kammerer 1990). The river flows from San Juan County, Colorado, through New Mexico and into Texas where it forms the shared border between Texas and Mexico before emptying into to the Gulf of Mexico near Brownsville, Texas (Benke and Cushing 2011). Throughout its length the Rio Grande flows through arid and semiarid desert scrubland and grassland habitats (Dahm et al. 2005). Flow in the Rio Grande in Texas is regulated by two large reservoirs (Falcon and Amistad reservoirs) and a number of small low-head dams. The World Wildlife Fund currently ranks the Rio Grande as the most imperiled river in the United States due to water over-extraction and over-appropriation by human populations along the river (Wong et al. 2007).

The Devils River is a pristine tributary to the Rio Grande originating in Sutton County, Texas, and flows intermittently southward into Val Verde County, Texas, where it becomes perennial. Flow is unregulated and provided from groundwater seepage and springs. The river lies within the Edwards Plateau region and drains an approximate area of 10,000 km^2 before emptying in Amistad Reservoir (Cantu and Winemiller 1997). The basin is sparsely populated.

The Pecos River is the largest tributary to the Rio Grande and originates in Mora County, New Mexico, draining approximately 115,000 km^2 before emptying in the Rio Grande above Amistad Reservoir. The Pecos River in southeastern New Mexico and Texas becomes highly saline due to saline aquifer input as well as anthropogenic impacts such as groundwater extraction and irrigation. The river has experienced a dramatic shift in fish fauna as well as harmful algal blooms from the golden alga (*Prymnesium parvum*) since the 1980s (Southard 2010).

Sampling Methods

Survey sites within the Rio Grande were selected following methods outlined by Albanese et al. (2007). Specifically using 10-digit HUC watersheds, potential survey sites were selected from the Texas potion of the Rio Grande. We identified the following HUCs based on occurrence data from previous sampling efforts: 1) HUC watersheds where live individuals had been reported; 2) HUC watersheds that had been surveyed, but no live individuals were found; and 3) HUC watersheds that had not been surveyed. To select survey sites, we first identified a subset of the categorized HUCs that could be safely accessed by a motorized boat, and then separated the river into 10 km reaches within each accessible HUC. Within each reach, survey sites were randomly selected based on mesohabitat types, which include banks, backwater, midchannel, riffles, rock slabs, canyon walls (only for reaches located in the upper Rio Grande and lower Pecos River), pools (only for reaches in the Devils River), and boulder fields (only for reaches in the Pecos River). At least two sites per available habitat type were selected for each reach.

Site selection on the Devils and Pecos rivers was accomplished via aerial imagery. Mesohabitat types were identified and categorized in the entire reach of the Devils River and the lower reach of the Pecos River. The rivers were broken into 1 km reaches, and survey sites were randomly selected in the same fashion as that on the Rio Grande.

Qualitative surveys using the timed search method were performed at each site (i.e., mesohabitat type). The timed search method was chosen because it provides a more effective means of detecting rare species than quantitative sampling methodologies (Vaughn et al. 1997). At each site, we confined the search boundaries within the randomly selected mesohabitat and standardized the search area to 150 m^2. Each site was surveyed tactilely and visually for a total of 4 person-hours (p-h). However, because we were interested in the amount of effort needed to detect *T. cognata* (which will be important for designing long-term monitoring programs), we divided the total search time into 4, 1 p-h intervals. At the end of each search interval, surveyors combined all live specimens into a mesh bag, which was kept submerged in water until completion of the survey. During each interval, surveyors were spread out in the search area and every effort was made to search all available microhabitats. Following completion of the survey, all live mussels from each time period were identified to species, counted, measured, checked for gravidity, and then returned back to the river into the appropriate habitat.

Data Analysis

Scatter plots of abundance (CPUE: number of individuals/total person-hours) vs. river kilometer (RKM) were used to examine the effect of stream position on total mussel and *T. cognata* abundance in each river. Boxplots and length-frequency histograms were developed for *T. cognata* to assess demographic patterns and population structure. Generally, multimodal size class distribution may indicate recruitment, whereas truncated distributions (absence of a particular age class, large, or small individuals) may indicate a lack of recent recruitment or a localized extinction event. Bar graphs were also used to visually represent presence of *T. cognata* by mesohabitat type (i.e., riffle, mid-channel, pool, bank, and backwater).

Results/Discussion

In total, 456 person-hours (p-h) were spent surveying mussels at 114 sites in the Rio Grande (Figure 1). Overall, we found 213 live individuals of *T. cognata*, which were found at 30 of 114 (26.3%) sites. Catch-per-unit-effort (CPUE) ranged from 0 to 17.25 mussels/p-h and averaged 0.5 ± 0.2 mussels/p-h (± SE) for *T. cognata* (Table 1), while CPUE averaged 5.8 ± 1.5 mussels/p-h for all mussels (Figure 2). Relative abundance of *T. cognata* was 8.1% of all mussels collected within the Rio Grande. The highest abundance (1.9 ± 0.9 mussels/p-h) of *T. cognata* by habitat type was observed in riffle habitat (Figure 3). The highest abundance (1.7 ± 1.0 mussels/p-h) of *T. cognata* by reach was observed in Reach 9 (Table 1 and Figure 1). Live individuals of *T. cognata* were found primarily in the lower reach of the Rio Grande in Webb and Zapata counties upstream of Falcon Lake (Reaches 7, 8, 9, and 10). Three live individuals were found in Reaches 5 and 6 in Maverick County. Previous studies reported that live individuals were only found in Webb County (Howells 2006, 2009; Burlakova and Karatayev 2010). To our knowledge, only shell material has been documented over the last 30 years from Val Verde, Maverick, and Zapata counties (Metcalf 1982; Johnson 1999; Karatayev et al. 2012). Thus, individuals from Reaches 5, 6, and 10 represent one of the few recent observations of live individuals in Maverick and Zapata counties. Median shell length for *T. cognata* in the Rio Grande was 19.9 mm and minimum and maximum shell lengths were 9.9 mm and 55.2 mm, respectively (Figure 4). Shell length distributions were right skewed with mussels predominately belonging to median size classes, although the presence of smaller size-classes indicates recruitment in recent years (Figure 5).

In total, 156 person-hours were spent surveying mussels at 39 sites in the Devils River (Figure 6). No live individuals or shell material of *T. cognata* were found. In the lower Pecos River, a total of 172 person-hours were spent surveying mussels at 43 sites between the confluence of Independence Creek and the confluence of the Rio Grande (Figure 7). No live individuals or shell material of *T. cognata* were found. To our knowledge, no recent or historical records of *T. cognata* occurrence exist from the Devils River. In the Pecos River, *T. cognata* was only documented from the lower Pecos River before the impoundment of Lake Amistad (Metcalf 1982; Karatayev et al. 2012).

In summary, our results indicate that *T. cognata* occurs at low abundances in the lower reach of the Rio Grande above Falcon Lake in Maverick, Webb, and Zapata counties. If it occurs in the Devils or Pecos rivers, densities are too low to detect using conventional sampling methods. Previous studies reported that live individuals were only found from near Laredo in Webb County (Howells 2006; Karatayev et al. 2012); thus, our results extend the previous occurrence records of live individuals in the Rio Grande.

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Table 1. Locality and collection information for mussel survey sites in the Rio Grande. CPUE = total number of *T. cognata* encountered at each site during qualitative sampling divided by the number of person hours (4) searched at each site. Habitat key: BH = Bank, BW = Backwater, MC = Mid-Channel, R = Riffle, RS = Rock Slab, RW = Rock Wall. Sites are ordered upstream to downstream.

Site	Reach	Habitat	Locality	County	Date of collection	Number of Live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
1	1	MC	La Linda	Brewster	6/8/2015	0	0	-	4	150
2	1	BW	La Linda	Brewster	6/8/2015	0	0	-	4	150
3	1	RW	La Linda	Brewster	6/8/2015	0	0	-	4	150
4	1	BH	La Linda	Brewster	6/8/2015	0	0	-	4	150
5	1	R	La Linda	Brewster	6/8/2015	0	0	-	4	150
6	1	RS	La Linda	Brewster	6/8/2015	0	0	-	4	150
7	1	BW	La Linda	Brewster	6/9/2015	0	0	-	4	150
8	1	BH	La Linda	Brewster	6/9/2015	0	0	-	4	150
9	1	R	La Linda	Brewster	6/9/2015	0	0	-	4	150
10	1	RW	La Linda	Brewster	6/9/2015	0	0	-	4	150
11	1	MC	La Linda	Brewster	6/9/2015	0	0	-	4	150
12	1	RS	La Linda	Brewster	6/9/2015	0	0	-	4	150
13	2	R	Black Gap	Brewster	6/11/2015	0	0	-	4	150
14	2	RW	Black Gap	Brewster	6/11/2015	0	0	-	4	150
15	2	RW	Black Gap	Brewster	6/11/2015	0	0	-	4	150
16	2	BW	Black Gap	Brewster	6/11/2015	0	0	-	4	150
17	2	RS	Black Gap	Brewster	6/11/2015	0	0	-	4	150
18	2	MC	Black Gap	Brewster	6/11/2015	0	0	-	4	150
19	2	R	Black Gap	Brewster	6/11/2015	0	0	-	4	150
20	2	BH	Black Gap	Brewster	6/10/2015	0	0	-	4	150
21	2	BH	Black Gap	Brewster	6/10/2015	0	0	-	4	150
22	2	RW	Black Gap	Brewster	6/10/2015	0	0	-	4	150
23	2	RS	Black Gap	Brewster	6/10/2015	0	0	-	4	150
24	2	BW	Black Gap	Brewster	6/10/2015	0	0	-	4	150
25	3	RW	John's Marina	Terrell	9/8/2015	0	0	-	4	150
26	3	RW	John's Marina	Terrell	5/16/2015	0	0	-	4	150
27	3	RS	John's Marina	Terrell	9/8/2015	0	0	-	4	150
28	3	BH	John's Marina	Terrell	5/16/2015	0	0	-	4	150
29	3	RW	John's Marina	Terrell	5/16/2015	0	0	-	4	150
30	3	BW	John's Marina	Terrell	5/14/2015	0	0	-	4	150
31	3	RW	John's Marina	Terrell	5/14/2015	0	0	-	4	150
32	3	R	John's Marina	Terrell	5/14/2015	0	0	-	4	150
33	3	BH	John's Marina	Terrell	5/15/2015	0	0	-	4	150
34	3	BW	John's Marina	Terrell	5/15/2015	0	0	-	4	150
35	3	R	John's Marina	Terrell	5/14/2015	0	0	-	4	150
36	3	BH	John's Marina	Terrell	5/14/2015	0	0	-	4	150
37	3	RW	John's Marina	Terrell	5/15/2015	0	0	-	4	150
38	3	RS	John's Marina	Terrell	5/15/2015	0	0	-	4	150
39	4	RS	Del Rio	Val Verde	5/13/2015	0	0	-	4	150
40	4	BW	Del Rio	Val Verde	5/12/2015	0	0	-	4	150
41	4	BH	Del Rio	Val Verde	5/13/2015	0	0	-	4	150
42	4	R	Del Rio	Val Verde	5/12/2015	0	0	-	4	150

Table 1. Continued.

Site	Reach	Habitat	Locality	County	Date of collection	Number of Live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
43	4	BW	Del Rio	Val Verde	5/12/2015	0	0	-	4	150
44	4	R	Del Rio	Val Verde	5/12/2015	0	0	-	4	150
45	4	MC	Del Rio	Val Verde	5/12/2015	0	0	-	4	150
46	4	BH	Del Rio	Val Verde	5/12/2015	0	0	-	4	150
47	4	RS	Del Rio	Val Verde	5/12/2015	0	0	-	4	150
48	5	RS	Eagle Pass	Maverick	4/10/2015	0	0	-	4	150
49	5	BH	Eagle Pass	Maverick	4/11/2015	0	0	-	4	150
50	5	MC	Eagle Pass	Maverick	4/10/2015	0	0	-	4	150
51	5	R	Eagle Pass	Maverick	4/10/2015	0	0	-	4	150
52	5	BW	Eagle Pass	Maverick	4/11/2015	0	0	-	4	150
53	5	BW	Eagle Pass	Maverick	4/11/2015	1	0.25	N	4	150
54	5	R	Eagle Pass	Maverick	4/10/2015	0	0	-	4	150
55	5	MC	Eagle Pass	Maverick	5/11/2015	0	0	-	4	150
56	5	BH	Eagle Pass	Maverick	5/11/2015	0	0	-	4	150
57	5	RS	Eagle Pass	Maverick	4/10/2015	0	0	-	4	150
58	6	R	El Indio	Maverick	4/9/2015	0	0	-	4	150
59	6	BW	El Indio	Maverick	4/9/2015	0	0	-	4	150
60	6	MC	El Indio	Maverick	4/9/2015	0	0	-	4	150
61	6	BW	El Indio	Maverick	4/9/2015	0	0	-	4	150
62	6	RS	El Indio	Maverick	4/9/2015	0	0	-	4	150
63	6	MC	El Indio	Maverick	4/9/2015	2	0.5	Y	4	150
64	6	BH	El Indio	Maverick	4/9/2015	0	0	-	4	150
65	6	BH	El Indio	Maverick	4/8/2015	0	0	-	4	150
66	6	RS	El Indio	Maverick	4/8/2015	0	0	-	4	150
67	6	R	El Indio	Maverick	4/8/2015	0	0	-	4	150
68	7	R	Apache	Webb	2/24/2015	10	2.5	Y	4	150
69	7	RS	Apache	Webb	2/25/2015	0	0	-	4	150
70	7	R	Apache	Webb	2/24/2015	4	1	Y	4	150
71	7	BW	Apache	Webb	2/25/2015	2	0.5	Y	4	150
72	7	BW	Apache	Webb	2/25/2015	2	0.5	N	4	150
73	7	RS	Apache	Webb	2/24/2015	0	0	-	4	150
74	8	BW	Columbia	Webb	2/20/2015	0	0	-	4	150
75	8	RS	Columbia	Webb	2/19/2015	0	0	-	4	150
76	8	MC	Columbia	Webb	2/20/2015	3	0.75	Y	4	150
77	8	R	Columbia	Webb	2/19/2015	0	0	-	4	150
78	8	BH	Columbia	Webb	2/20/2015	3	0.75	N	4	150
79	8	RS	Columbia	Webb	9/6/2015	0	0	-	4	150
80	8	RS	Columbia	Webb	2/20/2015	1	0.25	N	4	150
81	8	BW	Columbia	Webb	11/20/2014	0	0	-	4	150
82	8	R	Columbia	Webb	2/19/2015	38	9.5	Y	4	150
83	8	RS	Columbia	Webb	9/6/2015	0	0	-	4	150
84	8	BH	Columbia	Webb	11/20/2014	8	2	N	4	150
85	9	RS	La Bota	Webb	9/5/2015	1	0.25	N	4	150
86	9	RS	La Bota	Webb	2/21/2015	1	0.25	N	4	150
87	9	BH	La Bota	Webb	11/18/2014	5	1.25	Y	4	150
88	9	R	La Bota	Webb	2/22/2015	69	17.25	Y	4	150
89	9	MC	La Bota	Webb	2/21/2015	0	0	-	4	150
90	9	BW	La Bota	Webb	11/16/2014	0	0	-	4	150
91	9	MC	La Bota	Webb	2/22/2015	1	0.25	N	4	150
92	9	R	La Bota	Webb	2/21/2015	27	6.75	Y	4	150

Table 1. Continued.

Site	Reach	Habitat	Locality	County	Date of collection	Number of Live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
93	9	BW	La Bota	Webb	2/22/2015	1	0.25	N	4	150
94	9	BH	La Bota	Webb	11/16/2014	1	0.25	N	4	150
95	9	RS	La Bota	Webb	2/22/2015	1	0.25	N	4	150
96	9	BW	La Bota	Webb	11/18/2014	0	0	-	4	150
97	9	RS	La Bota	Webb	9/5/2015	0	0	-	4	150
98	9	R	La Bota	Webb	4/7/2015	1	0.25	Y	4	150
99	9	RS	La Bota	Webb	4/7/2015	1	0.25	N	4	150
100	9	BW	La Bota	Webb	4/7/2015	1	0.25	N	4	150
101	9	R	La Bota	Webb	4/7/2015	5	1.25	Y	4	150
102	10	RS	San Ygnacio	Zapata	2/26/2015	1	0.25	N	4	150
103	10	BH	San Ygnacio	Zapata	11/14/2014	5	1.25	N	4	140
104	10	BW	San Ygnacio	Zapata	11/13/2014	0	0	-	4	150
105	10	R	San Ygnacio	Zapata	2/18/2015	6	1.5	Y	4	150
106	10	R	San Ygnacio	Zapata	2/18/2015	5	1.25	Y	4	150
107	10	RS	San Ygnacio	Zapata	2/18/2015	3	0.75	N	4	150
108	10	MC	San Ygnacio	Zapata	11/15/2014	4	1	Y	4	150
109	10	RS	San Ygnacio	Zapata	2/18/2015	0	0	-	4	150
110	10	MC	San Ygnacio	Zapata	11/15/2014	0	0	-	4	150
111	10	BH	San Ygnacio	Zapata	11/13/2014	0	0	-	4	150
112	10	BW	San Ygnacio	Zapata	2/26/2015	0	0	-	4	150
113	11	BH	Salenino	Starr	11/19/2014	0	0	-	4	150
114	11	BW	Salenino	Starr	11/19/2014	0	0	-	4	150

Table 2. Locality and collection information for mussel survey sites in the Devils River. CPUE = total number of *T. cognata* encountered at each site during qualitative sampling divided by the number of person hours (4) searched at each site. Habitat key: BH = Bank, BW = Backwater, MC = Mid-Channel, R = Riffle, RS = Rock Slab, P = Pool. Sites are ordered upstream to downstream.

Site	Reach	Habitat	County	Date of collection	Number of Live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
1	A	R	Val Verde	5/11/2013	0	0	-	4	150
2	A	R	Val Verde	5/11/2016	0	0	-	4	150
3	A	BW	Val Verde	5/11/2016	0	0	-	4	150
4	A	R	Val Verde	5/11/2016	0	0	-	4	150
5	A	P	Val Verde	9/15/2015	0	0	-	4	150
6	A	R	Val Verde	5/12/2016	0	0	-	4	150
7	A	R	Val Verde	5/12/2016	0	0	-	4	150
8	A	R	Val Verde	5/12/2016	0	0	-	4	150
9	A	P	Val Verde	9/15/2015	0	0	-	4	150
10	B	MC	Val Verde	9/15/2015	0	0	-	4	150
11	B	P	Val Verde	9/16/2015	0	0	-	4	150
12	B	R	Val Verde	4/28/2016	0	0	-	4	150
13	B	BH	Val Verde	4/28/2016	0	0	-	4	150
14	B	BH	Val Verde	4/28/2016	0	0	-	4	150
15	B	MC	Val Verde	4/28/2016	0	0	-	4	150
16	B	MC	Val Verde	9/16/2015	0	0	-	4	150
17	B	R	Val Verde	6/13/2016	0	0	-	4	150
18	B	R	Val Verde	6/13/2016	0	0	-	4	150
19	B	BW	Val Verde	6/13/2016	0	0	-	4	150
20	C	RS	Val Verde	9/17/2015	0	0	-	4	150
21	C	MC	Val Verde	9/17/2015	0	0	-	4	150
22	C	BW	Val Verde	9/17/2015	0	0	-	4	150
23	C	R	Val Verde	9/17/2015	0	0	-	4	150
24	C	R	Val Verde	6/13/2016	0	0	-	4	150
25	C	R	Val Verde	6/13/2016	0	0	-	4	150
26	C	R	Val Verde	4/27/2016	0	0	-	4	150
27	C	MC	Val Verde	4/26/2016	0	0	-	4	150
28	C	BH	Val Verde	4/27/2016	0	0	-	4	150
29	C	R	Val Verde	4/27/2016	0	0	-	4	150
30	D	RS	Val Verde	4/26/2016	0	0	-	4	150
31	D	BH	Val Verde	4/26/2016	0	0	-	4	150
32	D	R	Val Verde	4/26/2016	0	0	-	4	150
33	D	R	Val Verde	4/26/2016	0	0	-	4	150
34	D	BW	Val Verde	4/26/2016	0	0	-	4	150
35	E	BH	Val Verde	9/18/2015	0	0	-	4	150
36	E	BW	Val Verde	9/18/2015	0	0	-	4	150
37	E	R	Val Verde	5/10/2016	0	0	-	4	150
38	E	R	Val Verde	5/10/2016	0	0	-	4	150
39	E	R	Val Verde	5/10/2016	0	0	-	4	150

Table 3. Locality and collection information for mussel survey sites in the lower Pecos River. CPUE = total number of *T. cognata* encountered at each site during qualitative sampling divided by the number of person hours (4) searched at each site. Habitat key: BH = Bank, BF = Boulder Field, BW = Backwater, MC = Mid-Channel, R = Riffle, RS = Rock Slab, RW = Rock Wall, P = Pool. Sites are ordered upstream to downstream.

Site	Reach	Habitat	County	Date of collection	Number of Live	CPUE	Sub Adult	Effort (hrs)	Area (m^2)
1	A	R	Terrell/Crockett	3/17/2016	0	0	-	4	150
2	A	RS	Terrell/Crockett	3/17/2016	0	0	-	4	150
3	A	BH	Terrell/Crockett	3/17/2016	0	0	-	4	150
4	A	R	Terrell/Crockett	3/17/2016	0	0	-	4	150
5	A	P	Terrell/Crockett	3/17/2016	0	0	-	4	150
6	B	R	Val Verde	3/18/2016	0	0	-	4	150
7	B	RS	Val Verde	3/18/2016	0	0	-	4	150
8	B	BF	Val Verde	3/18/2016	0	0	-	4	150
9	B	BH	Val Verde	3/19/2016	0	0	-	4	150
10	B	R	Val Verde	3/19/2016	0	0	-	4	150
11	B	RS	Val Verde	3/19/2016	0	0	-	4	150
12	B	RW	Val Verde	5/20/2016	0	0	-	4	150
13	B	BF	Val Verde	5/20/2016	0	0	-	4	150
14	B	BF	Val Verde	5/20/2016	0	0	-	4	150
15	B	RW	Val Verde	5/20/2016	0	0	-	4	150
16	B	R	Val Verde	5/21/2016	0	0	-	4	150
17	B	BW	Val Verde	5/21/2016	0	0	-	4	150
18	B	RW	Val Verde	5/21/2016	0	0	-	4	150
19	C	RW	Val Verde	5/21/2016	0	0	-	4	150
20	C	BF	Val Verde	5/21/2016	0	0	-	4	150
21	C	BF	Val Verde	5/21/2016	0	0	-	4	150
22	C	RW	Val Verde	5/22/2016	0	0	-	4	150
23	C	BF	Val Verde	5/22/2016	0	0	-	4	150
24	C	BW	Val Verde	5/22/2016	0	0	-	4	150
25	C	BF	Val Verde	5/22/2016	0	0	-	4	150
26	C	R	Val Verde	5/22/2016	0	0	-	4	150
27	C	RW	Val Verde	5/22/2016	0	0	-	4	150
28	D	RW	Val Verde	5/23/2016	0	0	-	4	150
29	D	RW	Val Verde	5/23/2016	0	0	-	4	150
30	D	BF	Val Verde	5/23/2016	0	0	-	4	150
31	D	BF	Val Verde	5/23/2016	0	0	-	4	150
32	D	BW	Val Verde	5/23/2016	0	0	-	4	150
33	D	R	Val Verde	5/23/2016	0	0	-	4	150
34	E	BF	Val Verde	5/23/2016	0	0	-	4	150
35	E	RW	Val Verde	5/24/2016	0	0	-	4	150
36	E	R	Val Verde	5/24/2016	0	0	-	4	150
37	E	BF	Val Verde	5/24/2016	0	0	-	4	150
38	E	BW	Val Verde	5/24/2016	0	0	-	4	150
39	E	RW	Val Verde	5/24/2016	0	0	-	4	150
40	F	BF	Val Verde	3/20/2016	0	0	-	4	150
41	F	RS	Val Verde	3/21/2016	0	0	-	4	150
42	F	RS	Val Verde	3/20/2016	0	0	-	4	150
43	F	RS	Val Verde	3/21/2016	0	0	-	4	150

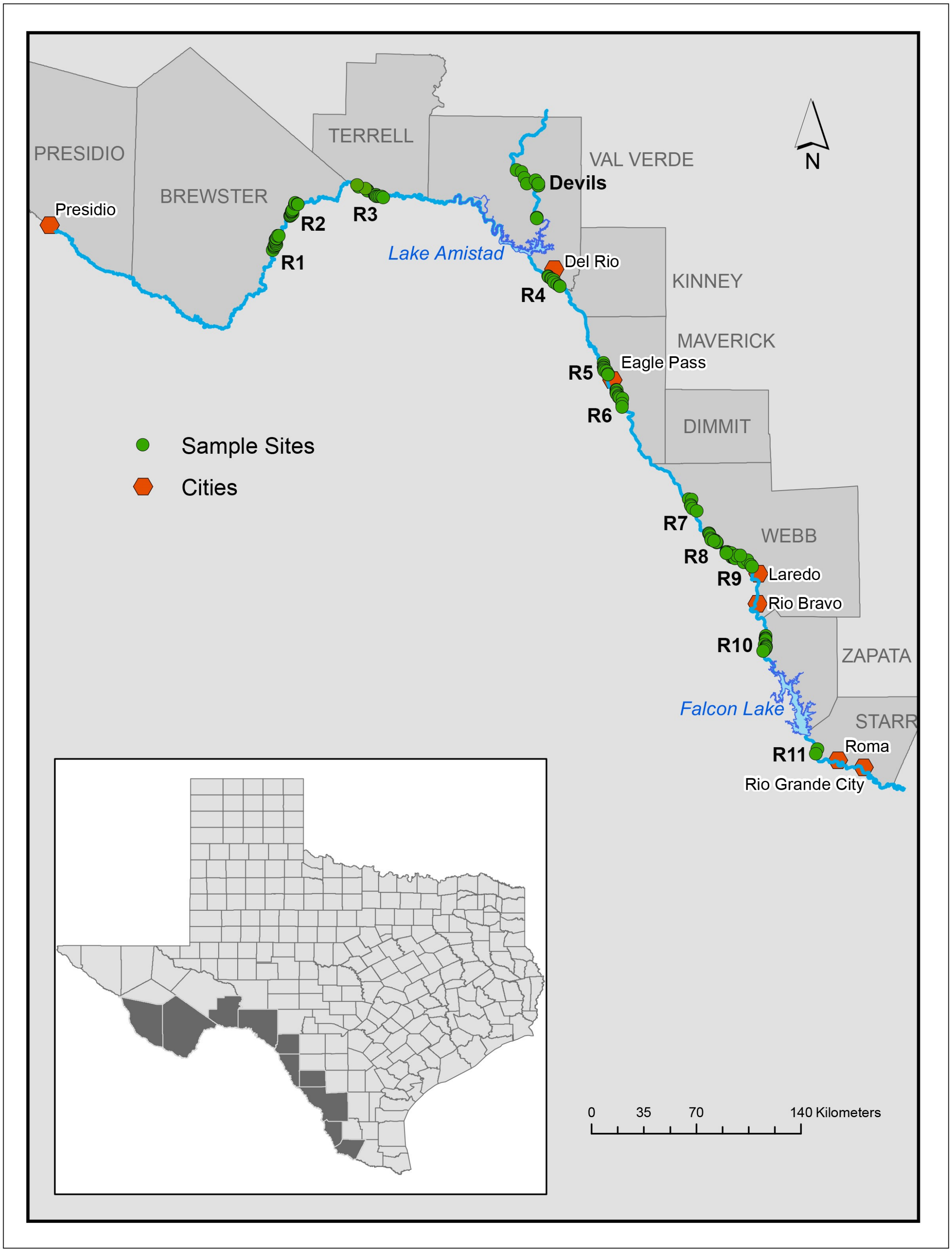

Figure 1. Map of Rio Grande drainage with shaded (green) circles denoting sampling locations. Reaches are indicated by number and correspond to Table 1.

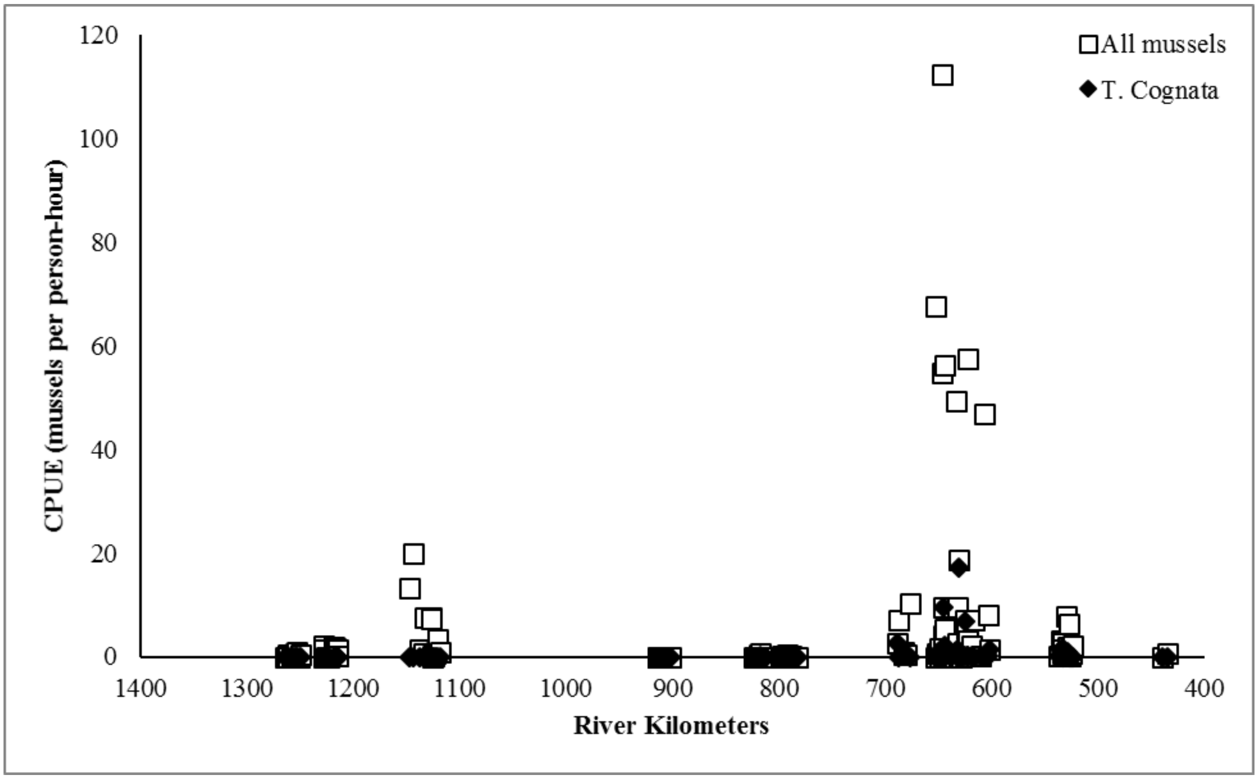

Figure 2. Catch-per-unit effort (CPUE) of *Truncilla cognata* (Mexican fawnsfoot) and all mussel species (All mussels) in the Rio Grande. Each point represents one sample site and its position is determined based on the longitudinal distance upstream from the most downstream site (0 River Kilometers). CPUE = total number of either *T. cognata* or all mussels encountered at each site divided by the number of person hours (4) searched at each site.

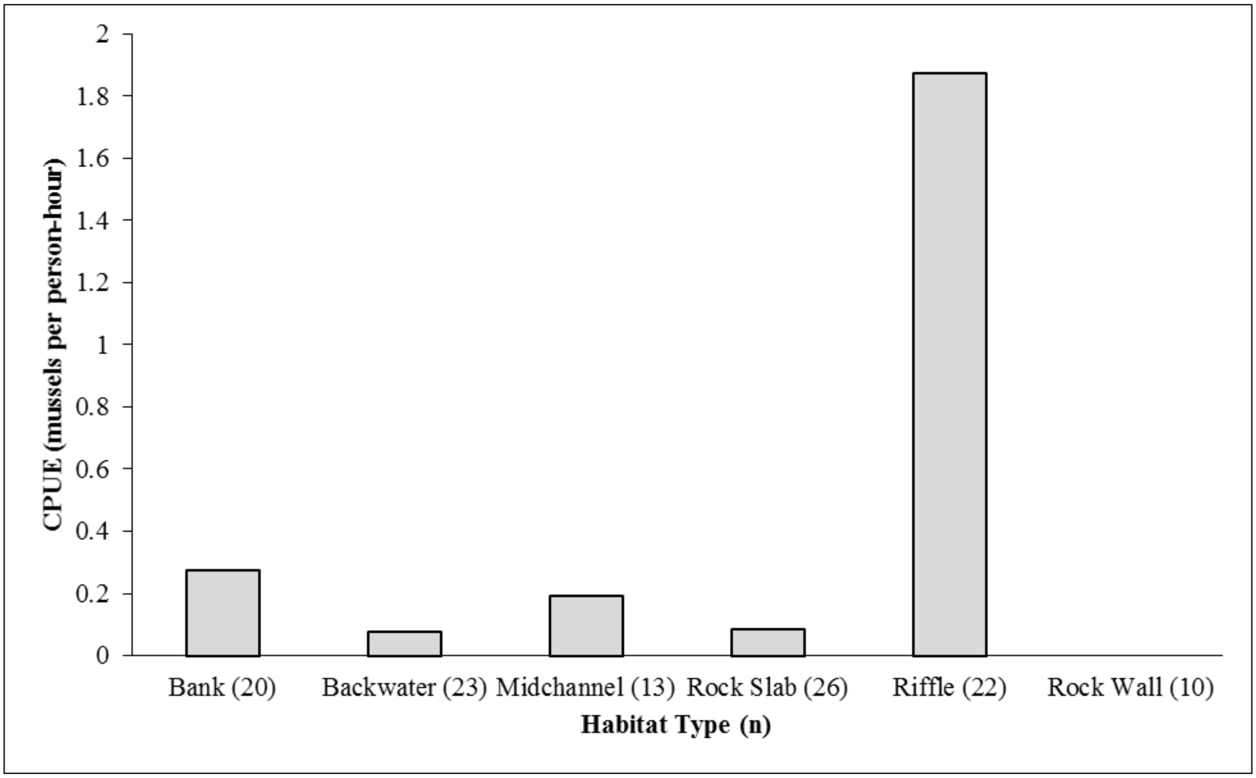

Figure 3. Catch-per-unit-effort (CPUE) of *Truncilla cognata* (Mexican fawnsfoot) by mesohabitat type in the Rio Grande. The total number of sites sampled for each habitat are listed in parenthesis.

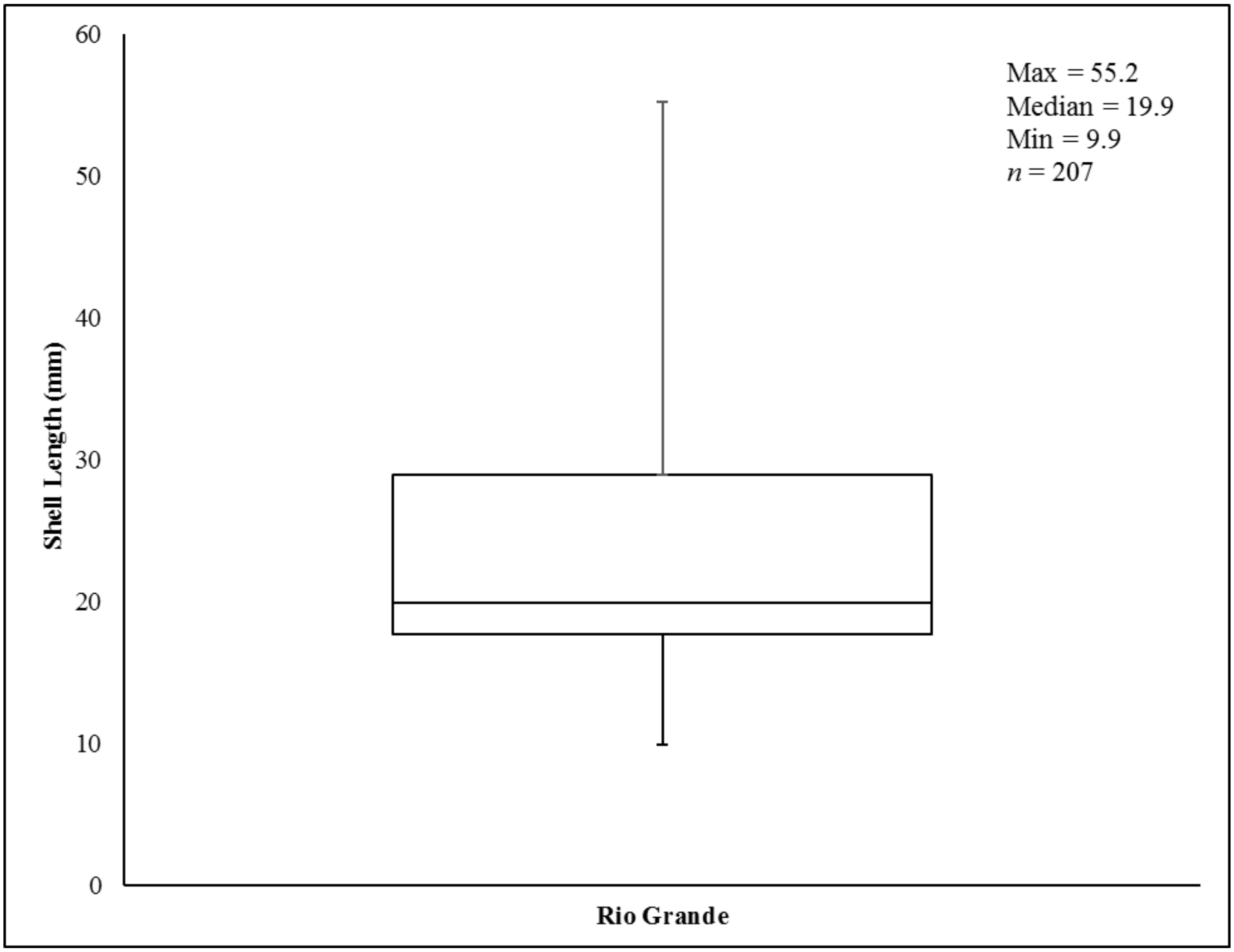

Figure 4. Shell length data of *Truncilla cognata* (Mexican fawnsfoot) populations in the Rio Grande.

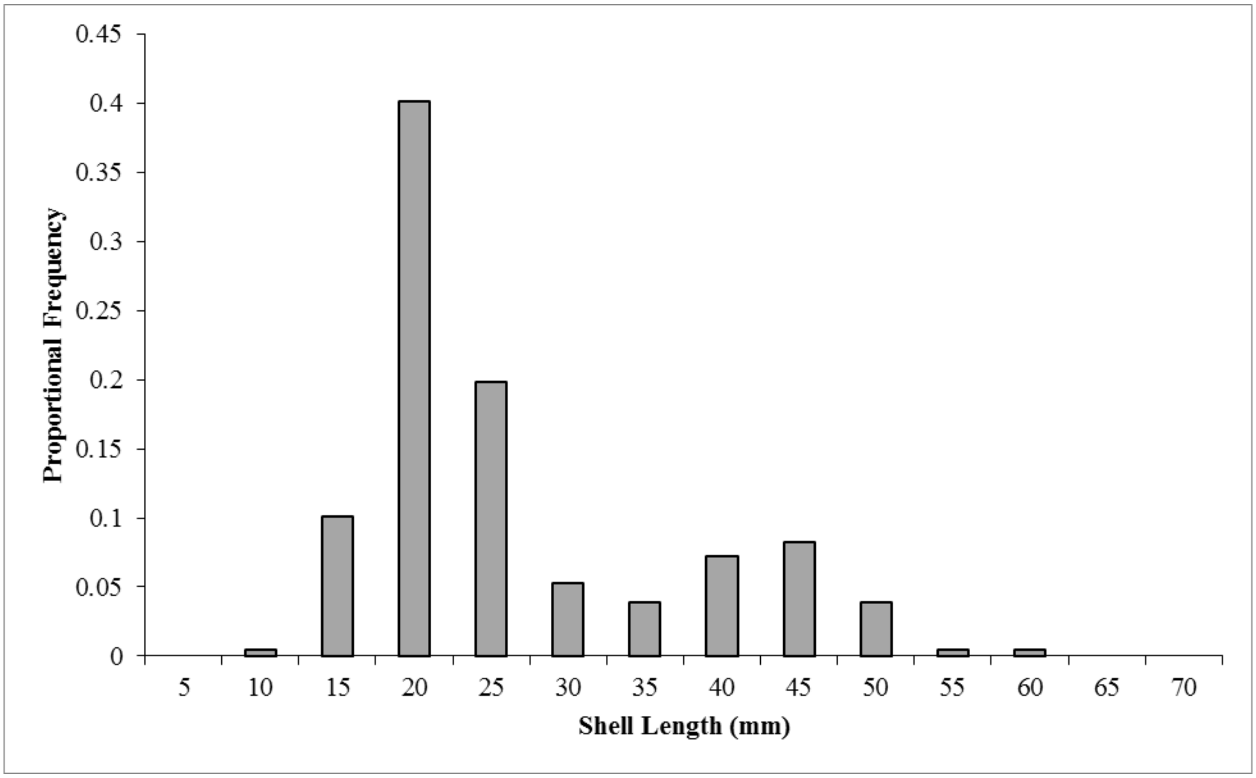

Figure 5. Proportional frequency of shell length of *Truncilla cognata* (Mexican fawnsfoot) in the Rio Grande. Shell lengths are binned into 5 mm groups.

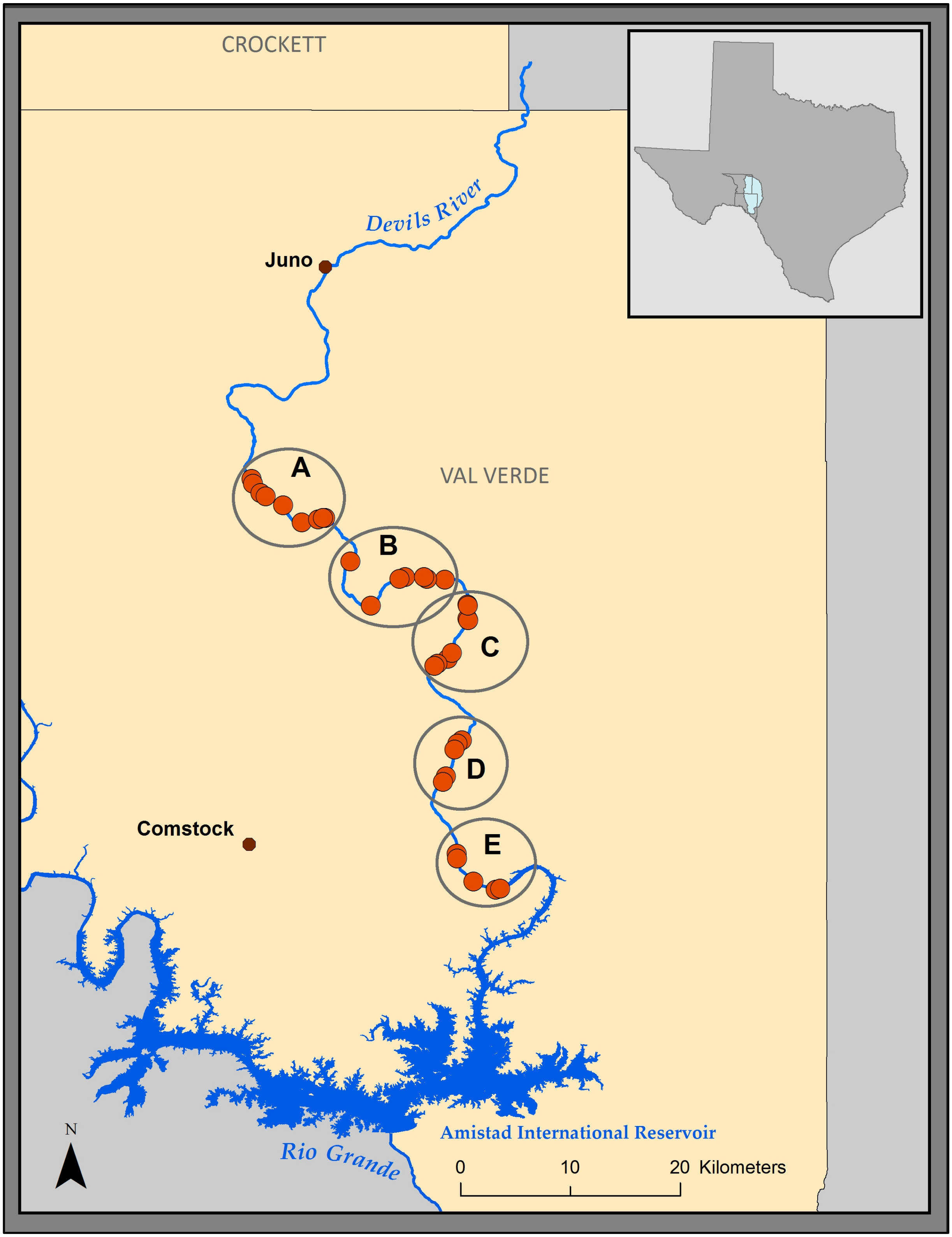

Figure 6. Devils River study area. Reaches correspond to Table 1.

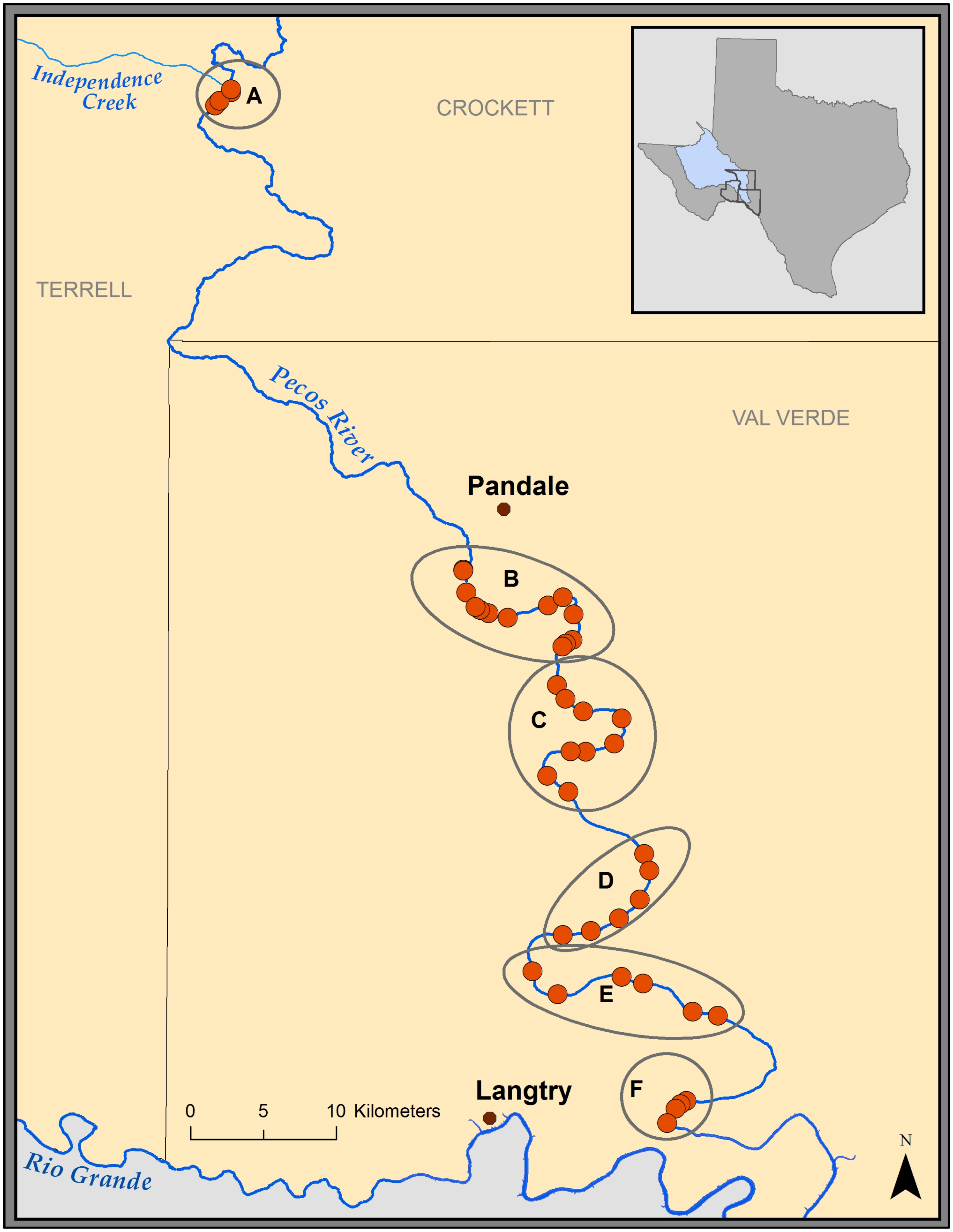

Figure 7. Pecos River study area. Reaches correspond to Table 2.

Occupancy modeling of *Popenaias popeii* (Texas hornshell)

Section Summary

The Rio Grande drainage, in Texas, harbors three unionid mussel species (*Popenaias popeii*, Texas hornshell; *Potamilus metnecktayi*, Salina mucket; and *Truncilla cognata*, Mexican fawnsfoot) proposed for listing under the Endangered Species Act. Due to the remoteness of this river system and historical lack of interest in freshwater mussels, the Rio Grande and tributaries have not been surveyed using a sampling design that accounts for variability in detection, which is problematic because false-negatives can incorrectly suggest extirpation and range reductions. The objective of this chapter was to use an occupancy-modeling approach to identify factors that affect detection and occupancy of *P. popeii* at 153 sites along ~ 800 river kilometers of the Rio Grande and Devils River in Texas. In total, we collected 2,190 live individuals at 44 of the 153 sites surveyed. Detection probabilities varied and were influenced primarily by abundance, whereas occupancy was driven by proximity to urban centers (Middle Rio Grande), cumulative number of springs located upstream of a given sample location (Lower Canyons and Devils River), presence of boulder/bedrock habitat (Middel Rio Grande and Lower Canyons) and siltation (Devils River). Our results provide important information that will likely aide in the conservation and management of mussel species in one of the most endangered river systems in North America and confirms the usefulness of occupancy-modeling to assess the distribution, habitat relationships and anthropogenic stressors on unionid mussels.

Introduction

Popenaias popeii, Texas hornshell, is a freshwater mussel endemic to the Rio Grande River basin in New Mexico, Texas and northern part of Mexico (Karatayev et al. 2012; Strenth 2004). There are only four populations reported in the U.S., of these, three are located in the Rio Grande basin: 1) Lower Canyons of the Rio Grande Wild and Scenic River, TX; 2) Rio Grande near Laredo, TX; and 3) Devils River, a major tributary of the Rio Grande located near Del Rio, TX. The long-term viability of these populations remains in question, as abiotic and biotic factors influencing their persistence have not been well studied. However, it has been hypothesized that impoundments, water pollution, and over-extraction of water may be potential threats (Karatayev et al. 2012). In 2016, USFWS proposed to list *P. popeii* as endangered under the U.S. Endangered Species Act and so information on environmental factors that influence its persistence are needed.

While threats and environmental data on *P. popeii* is limited for populations in the Rio Grande, the population occurring in the Black River near Malaga, NM, a tributary of the Pecos, has been studied for over 20 years. Through mark-recapture studies and other associated lab work it has been demonstrated that *P. popeii* is sensitive to changes in river discharge and water quality (Inoue et al. 2014). These same factors likely affect populations in the Rio Grande proper and Devils River, though they may differ in scale and magnitude, particularly when considering further anthropogenic development, increased water use, and climate change in the Rio Grande basin.

The objectives of this study were to assess occupancy and detection and the environmental factors that affect them of *Popenaias popeii*, Texas hornshell, in the Lower Canyons, middle Rio Grande, and Devils River. This information may be helpful for identifying specific factors that pose the greatest risk to populations of this species in these reaches, which is critical for identifying additional research needs and developing effective mitigation strategies to ensure its persistence and recovery. Our results also provide vital information that may help with developing future monitoring/sampling programs for this species in this basin and elsewhere.

Methods

Study Area

The Rio Grande originates in Colorado and is considered the 4th largest river in the United States, with an approximate length of 3,050 km and draining a totaling 870,236 km^2 (Kammerer 1990). The river flows from San Juan County, Colorado, through New Mexico and into Texas where it forms the shared border between Texas and Mexico before emptying into the Gulf of Mexico near Brownsville, TX (Benke and Cushing 2011). Throughout its length the Rio Grande flows through arid and semiarid desert scrubland and grassland habitats (Dahm et al. 2005). Flow in the Rio Grande is regulated by two large reservoirs (Falcon Reservoir and Lake Amistad) and a number of small low-head dams. The World Wildlife Fund currently ranks the Rio Grande as the most imperiled river in the United States due to water over-extraction and over-appropriation by human populations along the river (Wong et al. 2007).

The Devils River is a pristine tributary to the Rio Grande originating in Sutton County, TX, and flows intermittently southward into Val Verde County, TX, where it becomes perennial. Flow is unregulated and provided from groundwater seepage and springs. The river lies within the Edwards Plateau region and drains an approximate area of 10,000 km^2, which is sparsely populated (Cantu and Winemiller 1997). The present study was located primarily in the Lower Canyons of the Rio Grande Wild and Scenic River (upstream of Lake Amistad), middle portions of the Rio Grande in Texas (between Lake Amistad and Falcon Reservoir, although several sites were surveyed immediately downstream of Lake Falcon, and the entire length of the Devils River.

Sampling

<u>Rio Grande</u>

Survey sites within the Rio Grande were selected following methods outlined by Albanese et al. (2007). Specifically, 10-digit HUC watersheds were used to delineate the entire length of Rio Grande within our study area. Species occurrence data from previous sampling efforts in the Rio Grande were then used to determine the following: 1) HUC watersheds where live individuals for *P. popeii* have been reported; 2) HUC watersheds that have been surveyed, but *P. popeii* was not found; and 3) HUC watershed that have not been surveyed. The resulting map was then used to prioritize survey needs by focusing on areas that have not been surveyed (UNS_HUCs) or in areas where past surveys failed to detect *P. popeii* (ND_HUCs). For a subset of HUCs that met these criteria and could be accessed safely using a motorized boat, we delineated the entire length of the river into 10 km reaches. Within each reach specific sites were selected using a random sampling design with 2 strata: river left or river right (except for midchannel habitats) and 2) mesohabitat: (banks, backwater, midchannel, riffles, rock slabs, and canyon walls (only for reaches located in the upper Rio Grande)). All sites were 150 m^2 in area and were searched for 4 person-hours (p-h) visually and tactilely either by snorkel or SCUBA.

<u>Devils River</u>

Site selection on the Devils was accomplished *a priori* by one of two methods. In all cases habitat types were identified and categorized using aerial imagery. Then depending on access, sites were chosen randomly within 2km up and downstream from an access location, or the river was broken into 1km segments (reaches) and each habitat type was selected at random from those possible in each segment, then sampled during a downstream paddling trip.

Sampling method

We used timed searches in each randomly selected mesohabitat type to locate mussels. The timed search method was chosen because it provides a more effective means of detecting rare species than quantitative sampling methodologies (Vaughn et al. 1997). At each site (i.e., mesohabitat type), we confined the search boundaries to the specific habitat type, ensuring that the search area did not exceed 150 m^2. Each site was surveyed tactilely and visually for a total of 4 p-h. At the end of each search interval, surveyors combined all live specimens into a mesh bag, which was kept submerged in water until completion of the survey. During each interval, surveyors were spread out in the search area and every effort was made to search all available microhabitats. Following completion of the survey, all live mussels from each search period were identified to species, counted, measured and then returned back to the river into the appropriate habitat.

Habitat measurements

Physical characteristics of each site and sampling event were recorded to determine their effect on mussel occupancy (ψ) and detection (p). Substrate composition, current velocity, habitat type, and water depth were visually estimated within each 150 m^2 search area by the same person (CRR) (Table 1). Proximity to nearby reservoirs and major urban areas and cumulative number of springs was determined using ArcGIS 10.5. Surveyor experience and effort were determined using survey information presented in Randklev et al. (2016).

Data analysis

Detection probability and site occupancy was estimated using a single-season occupancy approach described by MacKenzie et al. (2006). Detection probability (p) is the probability of detecting *Popenaias popeii*, Texas hornshell, within a single one-hour search period (p-h), and site occupancy (ψ) is the portion of sites occupied within the overall search area (i.e., Lower Canyons, middle Rio Grande, and Devils River). This method assumes sites are demographically closed, detection and occupancy are independent among sites, and species identifications are correct.

A candidate set of models was built using alternative parameterizations of the environmental covariates described in Table 1. Before fitting these models we used Pearson correlation to screen for autocorrelation and removed covariates having an $r >$ 0.50. Variables representing linear distance from a specific point (e.g., dams, cities) were examined as linear and polynomial terms. Model development consisted of considering various possible combinations of each covariate, though combinations of parameters were screened to ensure they made ecological sense. Parametric bootstrapping (n =10,000) was performed to assess overdispersion ($\hat{c}$). The resulting candidate models were ranked based on Akaike's Information Criterion and adjusted for sample size and overdispersion (AIC_c). AIC_c weights (w), which range from 0 to 1, were calculated and the model with the highest weight was considered the best-approximating model

(Burnham and Anderson 2002). We considered models to be plausible if their $AIC_c \leq 2$. For the best-approximating model odds ratios were calculated to evaluate the effect of a given parameter estimate on detection and occupancy. Occupancy models were developed using the program MARK (White and Burnham 1999).

Results/Discussion

A total of 456 person-hours were spent surveying 114 sites located in the Rio Grande (Figure 1) and 2,063 live individuals of *P. popeii* were found across 29 sites in the Lower Canyons (*n* = 189 individuals) and middle (*n* = 1,874 individuals) Rio Grande. Occurrence and relative abundance in the Lower Canyons was highest in reaches within Terrell County and in the middle Rio Grande within Webb County (Figure 1). Within the Devils River, a total of 152 person-hours were spent surveying 39 sites spanning 62 River Kilometers in the Devils River (Figure 1) and 127 *P. popeii* individuals were found from 15 of the 39 sites surveyed.

Occupancy and detection rates were generated for the Lower Canyons and middle Rio Grande separately due to differences in flow, landuse and habitat/substrate characteristics. Estimated mean occupancy (ψ) of *P. popeii* in the Lower Canyons and middle Rio Grande was 0.37 (85% CI: 0.37-0.37) and 0.20 (0.20-0.20), respectively, which were similar to naïve occupancy estimates (Table 2). Estimated mean detection (*p*) was 0.55 (85% CI: 0.45-0.64) for the Lower Canyons and 0.82 (85% CI: 0.73-0.88) for the middle Rio Grande (Table 2). For the Devils River, estimated mean occupancy was 0.38 (85% CI: 0.38-0.41) and detection was 0.60 (85% CI: 0.48-0.60). These results indicate that occupancy for *P. popeii* in the Lower Canyons and Devils River is almost twice that of the middle Rio Grande, but detection is higher for the middle Rio Grande compared to the other two populations. The likely reason for this is that *P. popeii* in the middle Rio Grande primarily occurs in boulder/bedrock habitats, which are discrete areas that tend to be easily identifiable, but spatially are limited in number compared to other habitat types (hence low occupancy) and so *P. popeii* tends to be locally abundant wherever this habitat occurs (hence high detection). In contrast, within the Lower Canyons boulder/bedrock habitat is less discrete but is spatially more abundant (hence higher occupancy) and so *P. popeii* tends to be less abundant per boulder/bedrock habitat patch (hence lower detection). The Devils River population is similar to the Lower Canyons in that habitat, in this case riffles, is more numerous spatially and so *P. popeii* abundance per habitat patch is relatively low, which explains high accupancy but low detection for this species in this river.

Regarding parameter estimates, the best-approximating model for the lower Canyons indicated that occupancy of *P. popeii* was related to cumulative number of springs and % boulder/canyon wall at a site (Tables 3 & 4). Odds ratios indicate that *P. popeii* occupancy increased by a factor of 12.94 for each cumulative increase in the number of springs located upstream of a given site (Table 4). In addition to proximity to nearby springs, *P. popeii* was 2.56 times more likely to occur for each 1% increase in boulder/canyon wall habitat. This indicates *P. popeii* is habitat specific and corroborates previous findings that it primarily resides in rock crevices, travertine shelves, and under large boulders, where small-grained materials, such as clay, silt, or sand gathers (references in Carman 2007). For detection, time and effort were not important, which indicates that experienced surveyors (in our case a given crew had on average 6.2 years of experience) that are able to identify *P. popeii* habitat are likely to locate live idividuals.

For the middle Rio Grande, the best-approximating model included detection as constant and occupancy varying with distance downstream from major urban centers and % boulder/bedrock (Table 3). Specifically, occupancy of *P. popeii* increased by a factor of 1.07 for each 1 rkm increase in distance from either Del Rio, Eagle Pass, or Laredo, TX, peaking between 150 to 200 rkms (Table 4). This indicates that *P. popeii* is likely sensitive to changes in water quality. Further studies are needed to determine which water quality parameter and/or environmental contaminant is driving this relationship. Occupancy of *P. popeii* was also related to % boulder/bedrock such that occupancy increased by a factor of 1.09 for every 1% increase in boulder/bedrock substrate (Table 4). This result closely mirrors that of our modeling for the Lower Canyon where *P. popeii* appears to be a habitat specialist occurring primarily in rock crevices and under boulders. Similar to the Lower Canyons, detection was not influenced by time or experience, which is probably because surveryors in this study were experienced in surveying for mussels and identifying *P. popeii* habitat.

For the Devils River, the best-approximating model indicated that occupancy and of *P. popeii* was related to cumulative number of springs and % clay/silt at a site (Tables 3 & 4). Odds ratios indicate that *P. popeii* occupancy increased by a factor of 1.40 for each cumulative increase in the number of springs located upstream of a given site (Table 4). In addition to proximity to nearby springs, *P. popeii* was 1.21 times less likely to occur for each 1% increase in silt/clay. In general, areas within the Devils that lack flow have a high percentage of clay/silt, which is a calcium carbonate precipitate. This likely explains why *P. popeii* primarily occurs in riffles or other habitat types with moderate to fast current velocities. For detection, time and effort were not important, which indicates that experienced surveyors (in our case a given crew had on average 6.2 years of experience) that are able to identify *P. popeii* habitat are likely to locate live individuals.

Our results indicate that *P. popeii* in the Rio Grande proper is dependent on spring inflows (Lower Canyons and Devils River), rocky-type habitats (Lower Canyons and Middle Rio Grande) and is sensitive to water quality degradation (middle Rio Grande) and siltation (Devils River). These results also indicate that occupancy of *P. popeii* is likely higher in the Lower Canyons and Devils River compared to the Middle Rio Grande, but is less abundant at any given location within either of those reaches compared to the Middle Rio Grande.

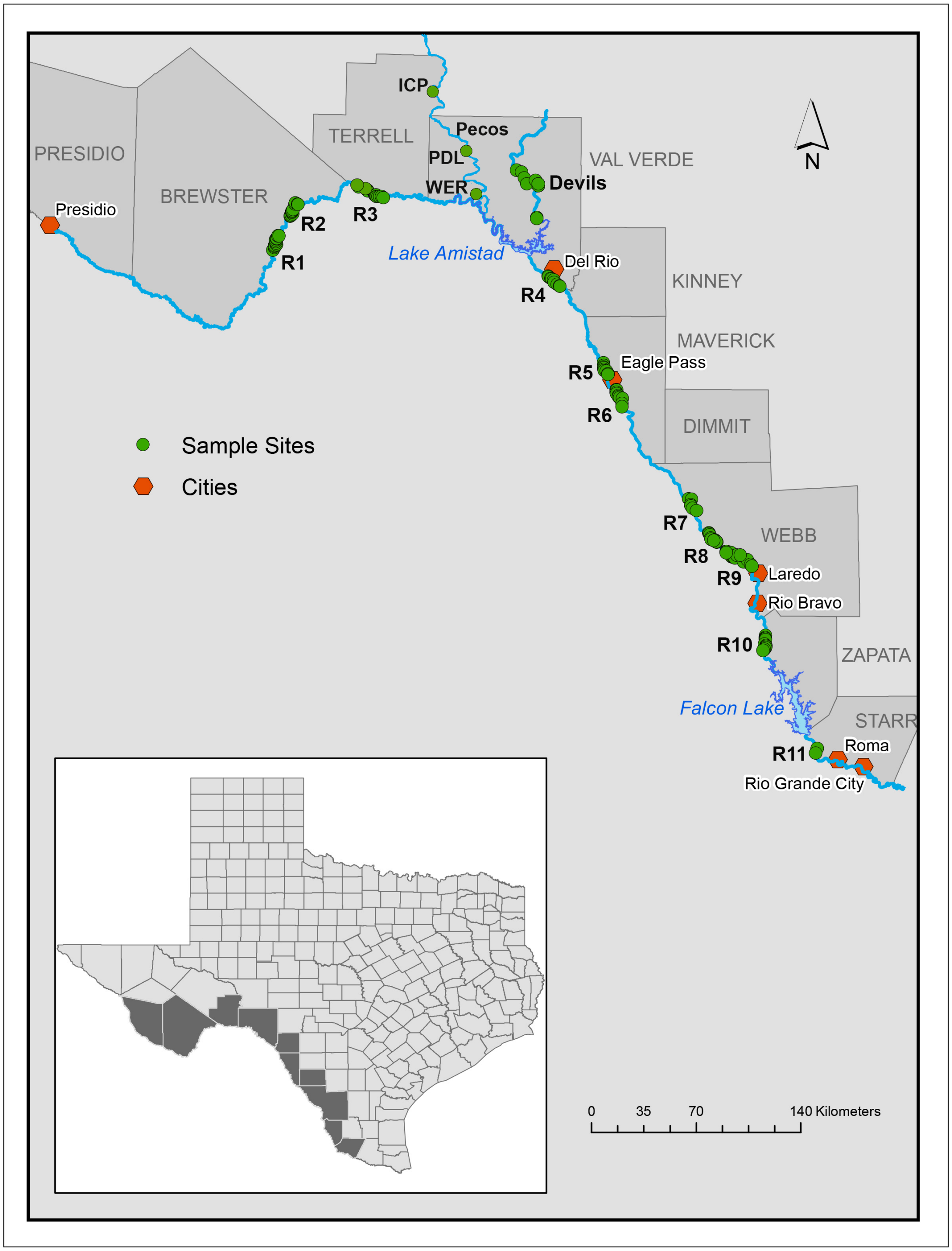

Figure 1. Map of study area. Shaded circles denote sampling locations and letters with numerals indicate sampling reaches.

Table 1. Covariates included in candidate occupancy models for *Popenanias popeii* (Texas hornshell) occupancy (ψ) and detection (*p*) in the Rio Grande, Texas.

Variable	Definition
.	Constant, does not vary
Site specific (*ψ*)	
Dam	Downstream distance (rkm) from Lake Amistad or Falcon Lake depending on sample site location.
City	Downstream distance (rkm) from Del Rio, Eagle Pass, or Laredo depending on sample site location.
Stream position	Location of a given site (rkm) relative to the upstream boundary of a given study area (i.e., Lower Canyons, Middle Rio Grande, Devils River).
Cumulative number of springs	Cumulative number of springs upstream of a given sample site.
Mesohabitat	Bank, Backwater, Riffle, Midchannel, Pool, Rockslab/canyon wall.
Water velocity	Varies by presence of slack water (0), perceivable (1), or swift (2)
Wadeable	% of site that was less than 1.5 m in water depth.
% Clay/silt	% Clay/silt within $150m^2$ search area.
% Sand	% Sand within $150m^2$ search area.
% Gravel	% Gravel within $150m^2$ search area.
% Cobble	% Cobble within $150m^2$ search area.
% Boulder/Bedrock	% Boulder/Bedrock within $150m^2$ search area.
Survey specific (*p*)	
Effort	Total search time (h) per survey period.
Searcher experience	Varies by experience of searcher (y)

Table 2. Estimated mean detection (*p*) and occupancy (ψ) for best-approximating models and naïve occupancy (proportion of sites observed occupied without accounting for incomplete detection) with 85% confidence intervals (CI) of *Popenaias popeii* (Texas hornshell) in the Rio Grande, Texas.

Model	***p***	**85% CI**	***ψ***	**85%CI**	**Naïve ψ**
***Popenaias popeii* (Texas hornshell)**					
Upper Rio Grande – Lower Canyons					
	0.55	0.45 – 0.64	0.37	0.37 – 0.37	0.37
Middle Rio Grande – Lake Amistad to Lake Falcon					
	0.82	0.73 – 0.88	0.20	0.20 – 0.20	0.20
Devils River					
	0.60	0.49 – 0.69	0.38	0.38 – 0.41	0.38

Table 3. Model selection results for examination of factors affecting occupancy (ψ) and detection (*p*) of *Popenaias popeii* (Texas hornshell) in the Rio Grande, Texas.

Study Area Model	**AIC_c**	**ΔAIC_c**	***w_i***	***K***
Popenaias popeii (Texas hornshell)				
Upper Rio Grande – Lower Canyons				
p(.), ψ(%boulder.bedrock + cum.spring)	95.54	0.00	0.46	4
Middle Rio Grande – Lake Amistad to Lake Falcon				
p(.), ψ(%boulder.bedrock + city)	89.57	0.00	0.50	4
p(effort), ψ(%boulder.bedrock + city)	90.38	0.81	0.34	5
Devils River				
p(.), ψ(%clay.silt)	117.65	0.00	0.27	3
p(.), ψ(%clay.silt + cum.spring)	119.36	1.71	0.11	4
p(.), ψ(%clay.silt + stream.position)	119.58	1.93	0.10	4

Table 4. Parameter estimates (SE), lower and upper 85% confidence limits (CL), and odds ratios for models used to map occupancy (ψ) of *Popenaias popeii* (Texas hornshell) in the Rio Grande, Texas.

Parameter	**Estimate (SE)**	**Lower CL**	**Upper CL**	**Odds ratio**
Upper Rio Grande – Lower Canyons				
p				
Intercept	0.19 (0.27)	-0.19	0.59	
ψ				
Intercept	-182.30 (236.32)	-522.49	-157.90	
%boulder.bedrock	0.94 (1.22)	-0.81	2.69	2.56
cum.spring	2.56 (3.33)	-2.23	7.35	12.94
Middle Rio Grande – Lake Amistad to Lake Falcon				
p				
Intercept	1.49 (0.34)	1.01	1.97	
ψ				
Intercept	-13.72 (5.16)	-21.16	-6.29	
%boulder.bedrock	0.09 (0.03)	0.04	0.13	1.09
city	0.07 (0.03)	0.03	0.11	1.07
Devils River				
p				
Intercept	0.37 (0.30)	-0.06	0.80	
ψ				
Intercept	1.51 (1.79)	-1.06	4.09	
%clay.silt	-0.19 (0.09)	-0.31	-0.06	1.21
cum.spring	0.34 (0.41)	-0.24	0.93	1.40

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Task 3: Conservation status assessment maps for nine state-threatened mussel species

Section Summary

We constructed conservation status assessment maps for 9 state-threatened species from central and west Texas using ArcMap 10.0 (ESRI Inc., Redlands, California) using the Conservation Status Map package provided by the Georgia Department of Natural Resources (http://www.georgiawildlife.com/conservation_status_assessment_maps). Conservation status assessment maps are a way to efficiently determine the status of a given species and have been used in conservation assessments by U.S. Fish and Wildlife Service for rare aquatic species. Generally, conservation maps are suitable for coarse-level assessments and are generated using occurrence data mapped at a watershed scale using GIS. The resulting map can then be used to identify range size, survey needs, and high priority areas for conservation. The goal of this task was to illustrate the spatiotemporal distribution of these species at the HUC 10 level. Presence/absence data was obtained from the IRNR – Mussel Database, Texas Parks and Wildlife Department [TPWD], Texas Department of Transportation [TxDOT], Texas Commission on Environmental Quality [TCEQ], Texas Water Development Board [TWDB], and published literature.

Fusconaia mitchelli (false spike)

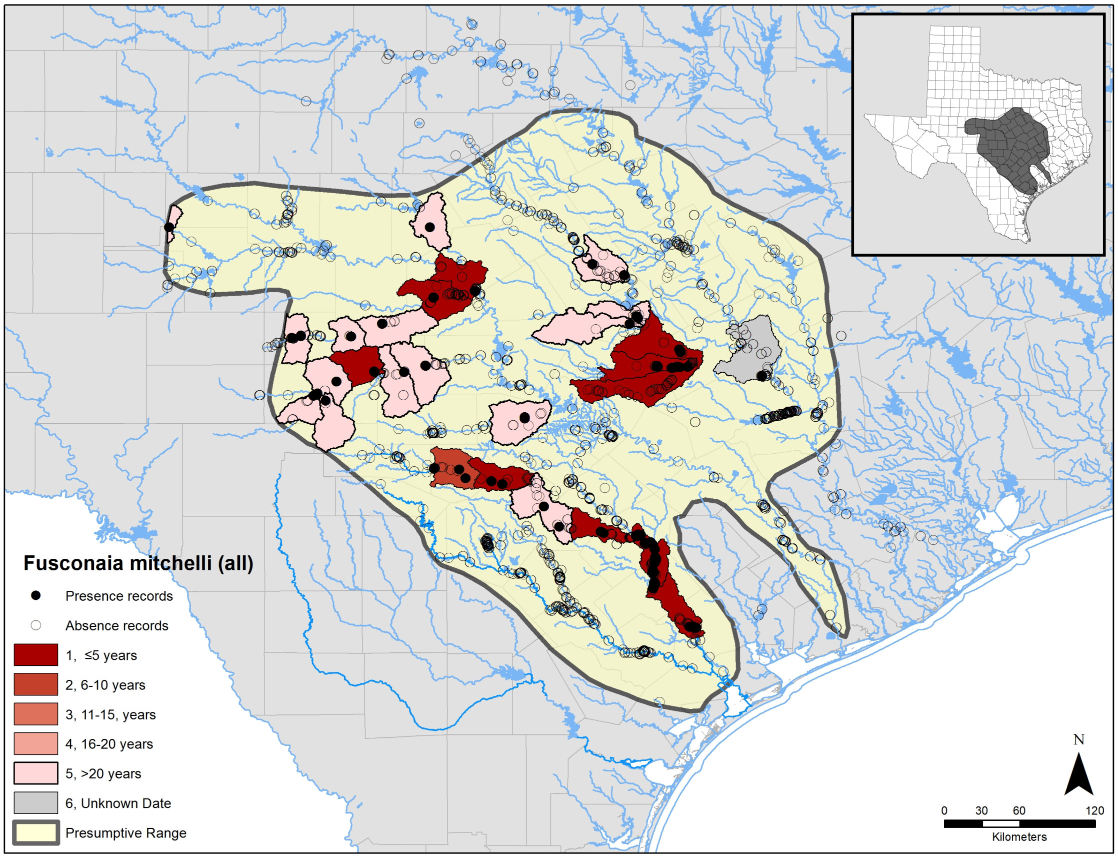

Figure 1. Conservation assessment map for *Fusconaia mitchelli* (false spike) populated with presence/absence records for both shell and live individuals. Shaded circles denote presence and unshaded circles indicate absence for *F. mitchelli*. Survey sites shown are from 1890 to present and are taken from the present study plus those obtained from museums, academic, state, and federal agencies. HUCs are colored based on date of sampling. Solid black line denotes presumptive range.

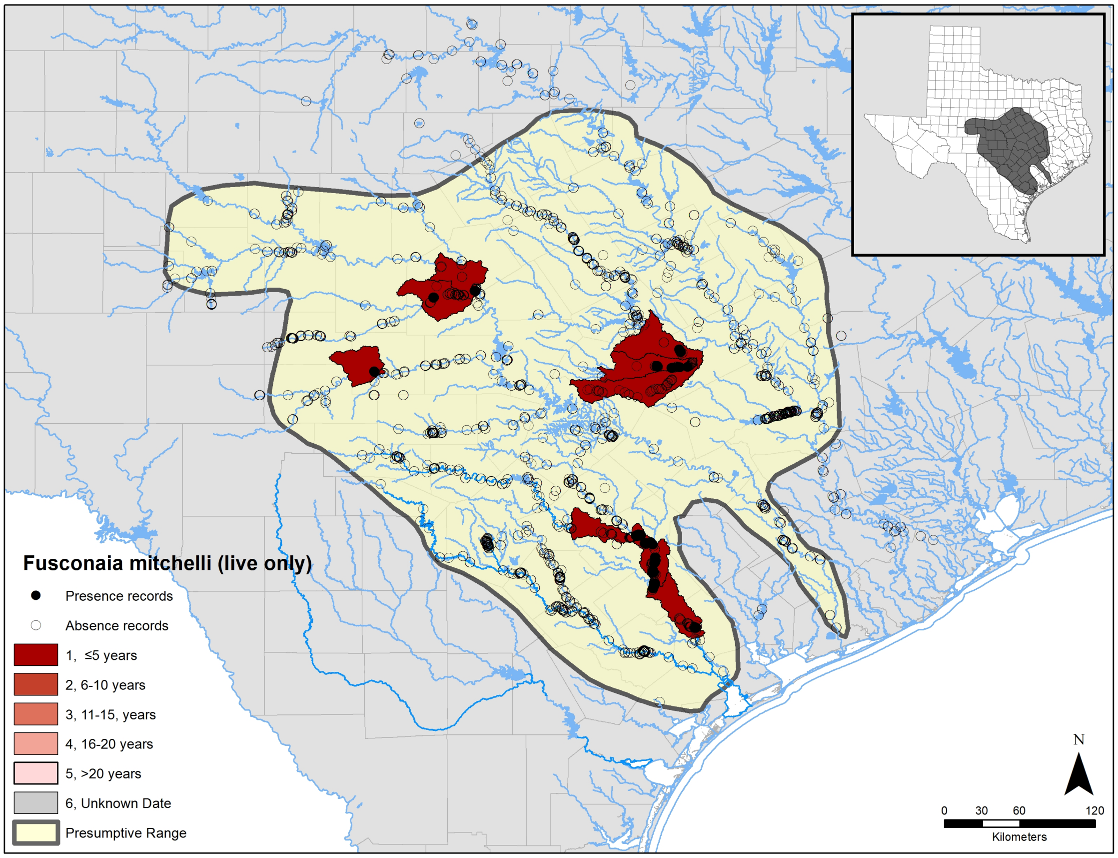

Figure 2. Conservation assessment map for *Fusconaia mitchelli* (false spike) populated with presence/absence records for only live individuals. Shaded circles denote presence and unshaded circles indicate absence for *F. mitchelli*. Survey sites shown of live individuals are from 2012 to present and are taken from the present study plus those obtained from academic, state, and federal agencies. Survey sites shown of absence data are from 1890 to present and are taken from the same sources listed above. HUCs are colored based on date of sampling. Map taken from Randklev et al. (2016).

Lampsilis bracteata (Texas fatmucket)

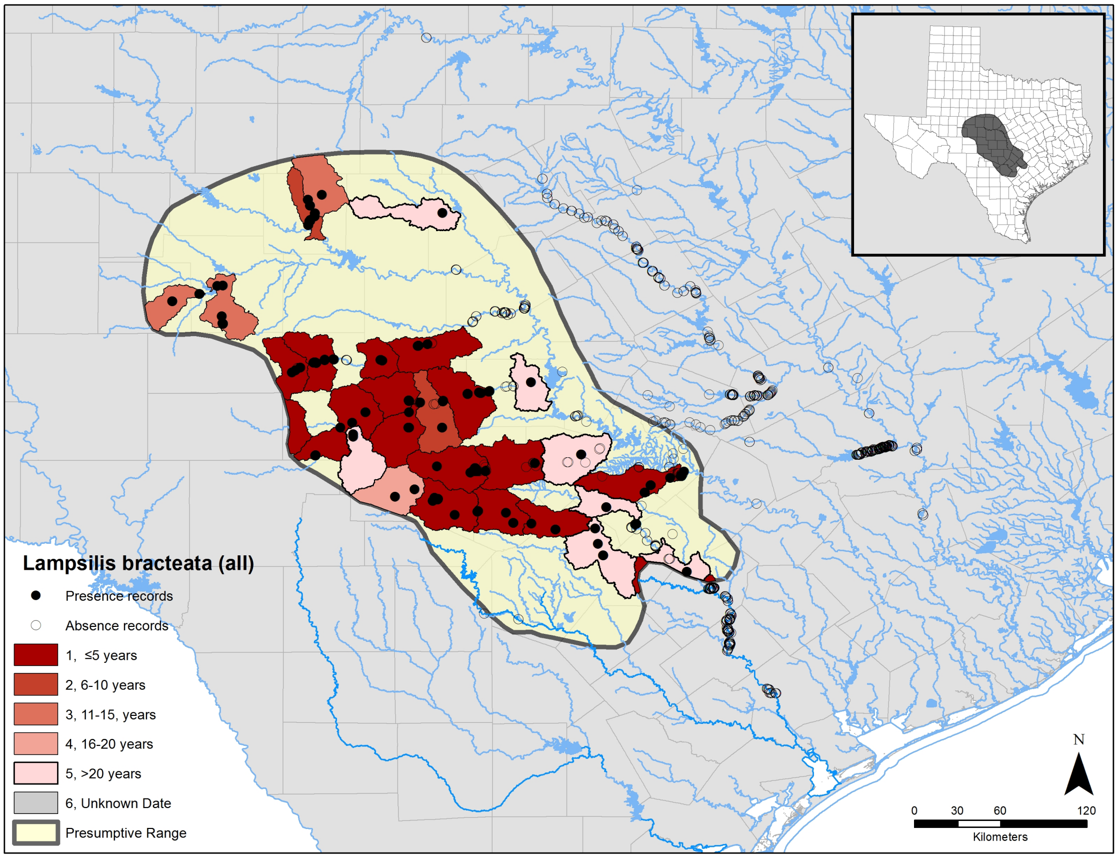

Figure 1. Conservation assessment map for *Lampsilis bracteata* (Texas fatmucket) populated with presence/absence records for both shell and live individuals. Shaded circles denote presence and unshaded circles indicate absence for *L. bracteata*. Survey sites shown are from 1890 to present and are taken from the present study plus those obtained from museums, academic, state, and federal agencies. HUCs are colored based on date of sampling. Solid black line denotes presumptive range.

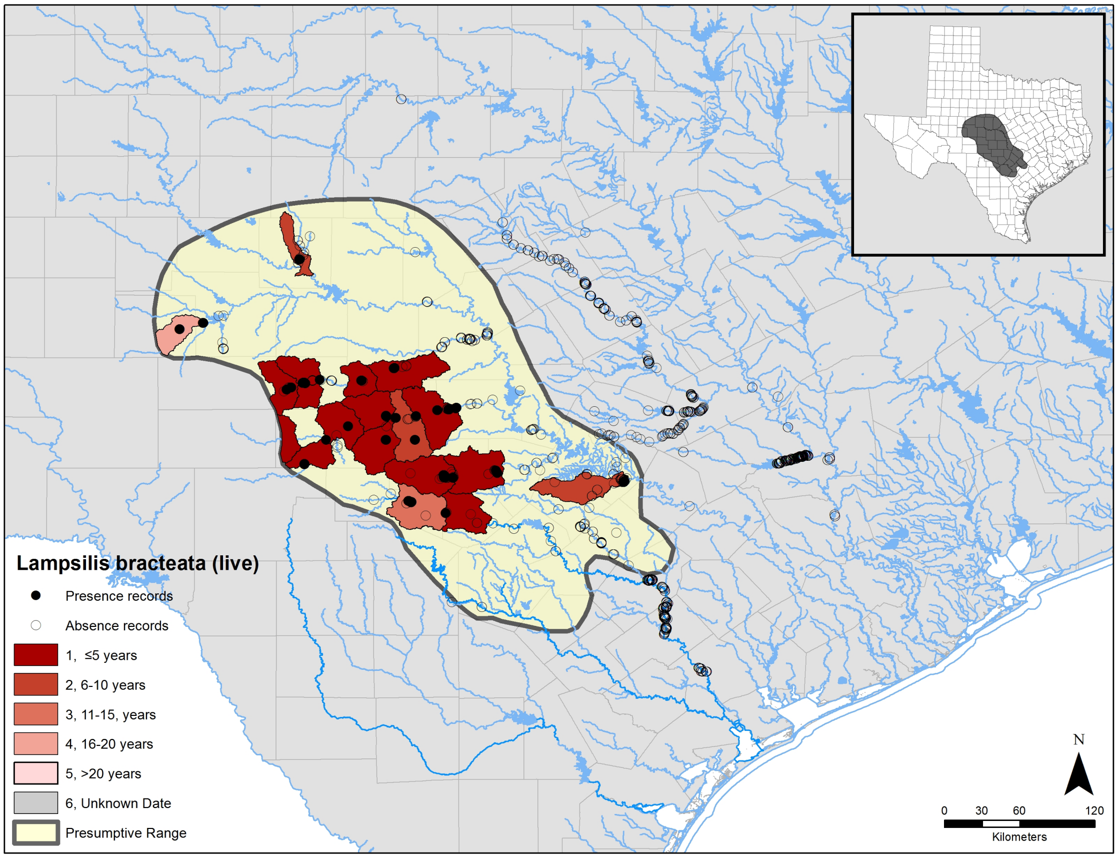

Figure 2. Conservation assessment map for *Lampsilis bracteata* (Texas fatmucket) populated with presence/absence records for only live individuals. Shaded circles denote presence and unshaded circles indicate absence for *L. bracteata*. Survey sites shown of live individuals are from 1993 to present and are taken from the present study plus those obtained from academic, state, and federal agencies. Survey sites shown of absence data are from 1890 to present and are taken from the same sources listed above. HUCs are colored based on date of sampling. Map taken from Randklev et al. (2016).

Potamilus metnecktayi (Salina mucket)

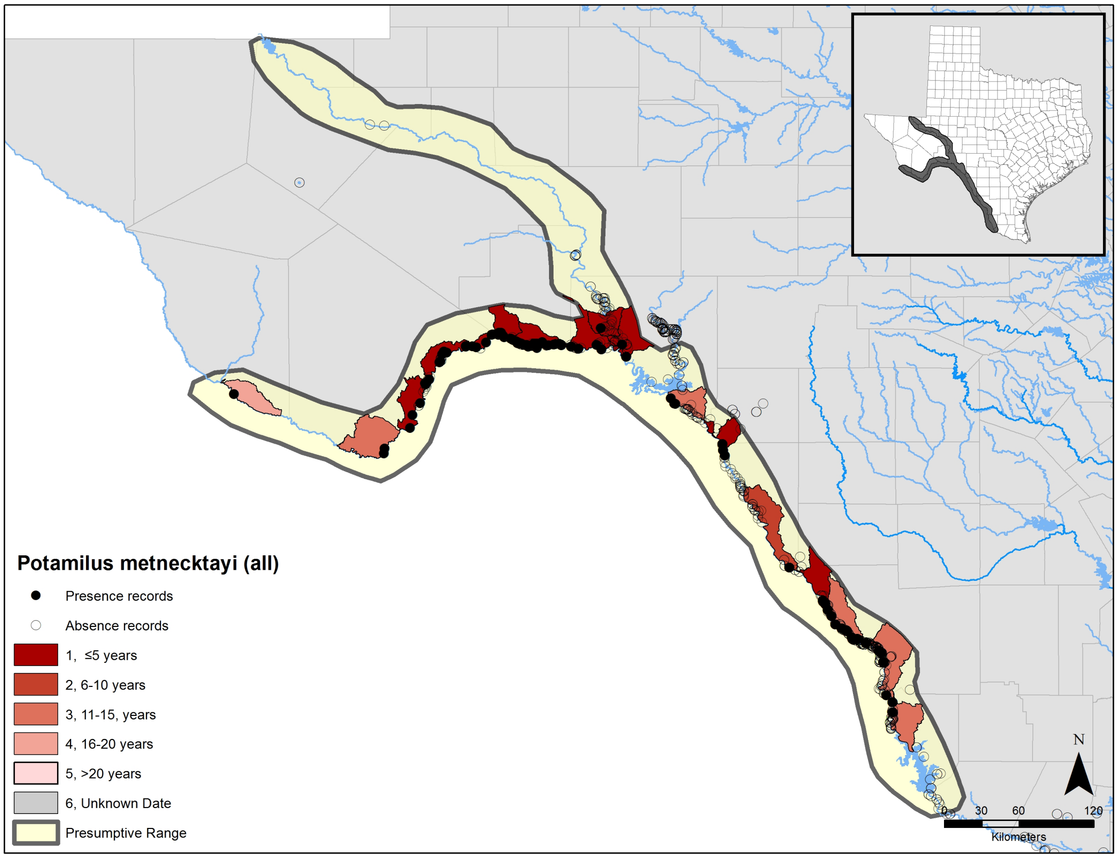

Figure 1. Conservation assessment map for *Potamilus metnecktayi* (Salina mucket) populated with presence/absence records for both shell and live individuals. Shaded circles denote presence and unshaded circles indicate absence for *P. metnecktayi*. Survey sites shown are from 1902 to present and are taken from the present study plus those obtained from museums, academic, state, and federal agencies. HUCs are colored based on date of sampling. Solid black line denotes presumptive range.

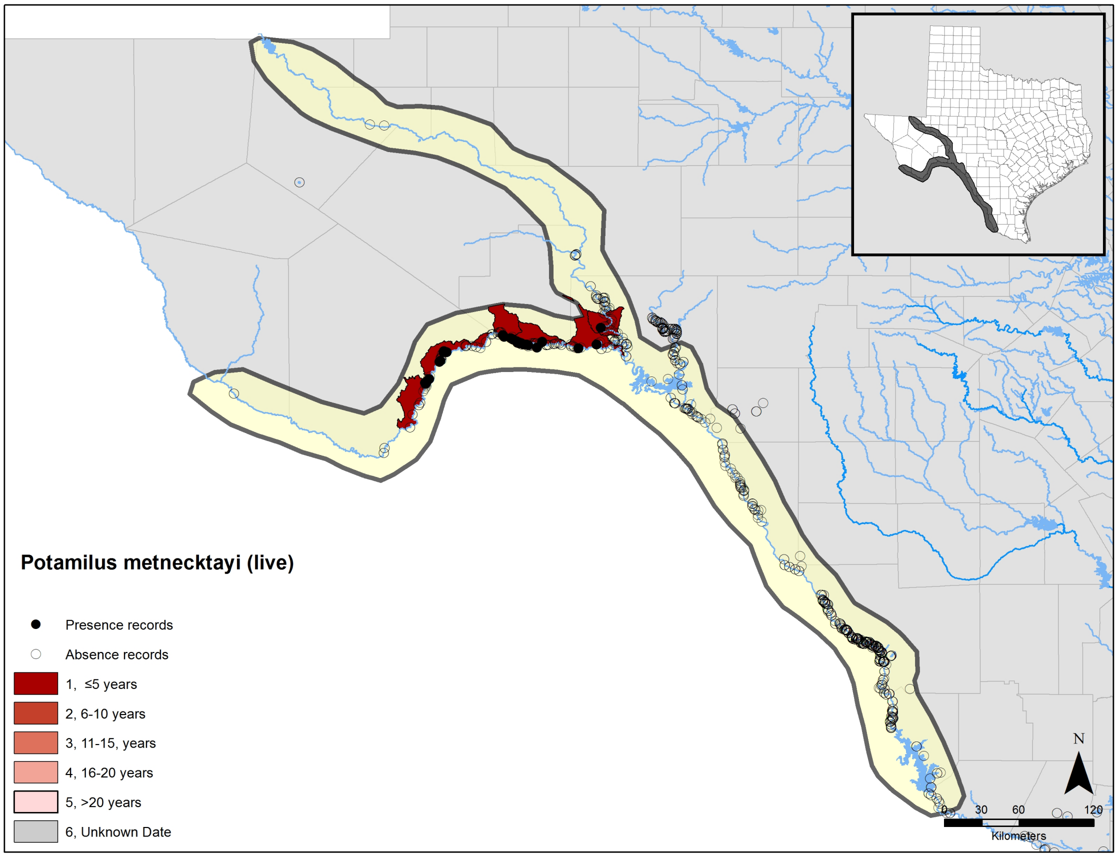

Figure 2. Conservation assessment map for *Potamilus metnecktayi* (Salina mucket) populated with presence/absence records for only live individuals. Shaded circles denote presence and unshaded circles indicate absence for *P. metnecktayi*. Survey sites shown of live individuals are from 2003 to present and are taken from the present study plus those obtained from academic, state, and federal agencies. Survey sites shown of absence data are from 1902 to present and are taken from the same sources listed above. HUCs are colored based on date of sampling. Map taken from Randklev et al. (2016).

Popenaias popeii (Texas hornshell)

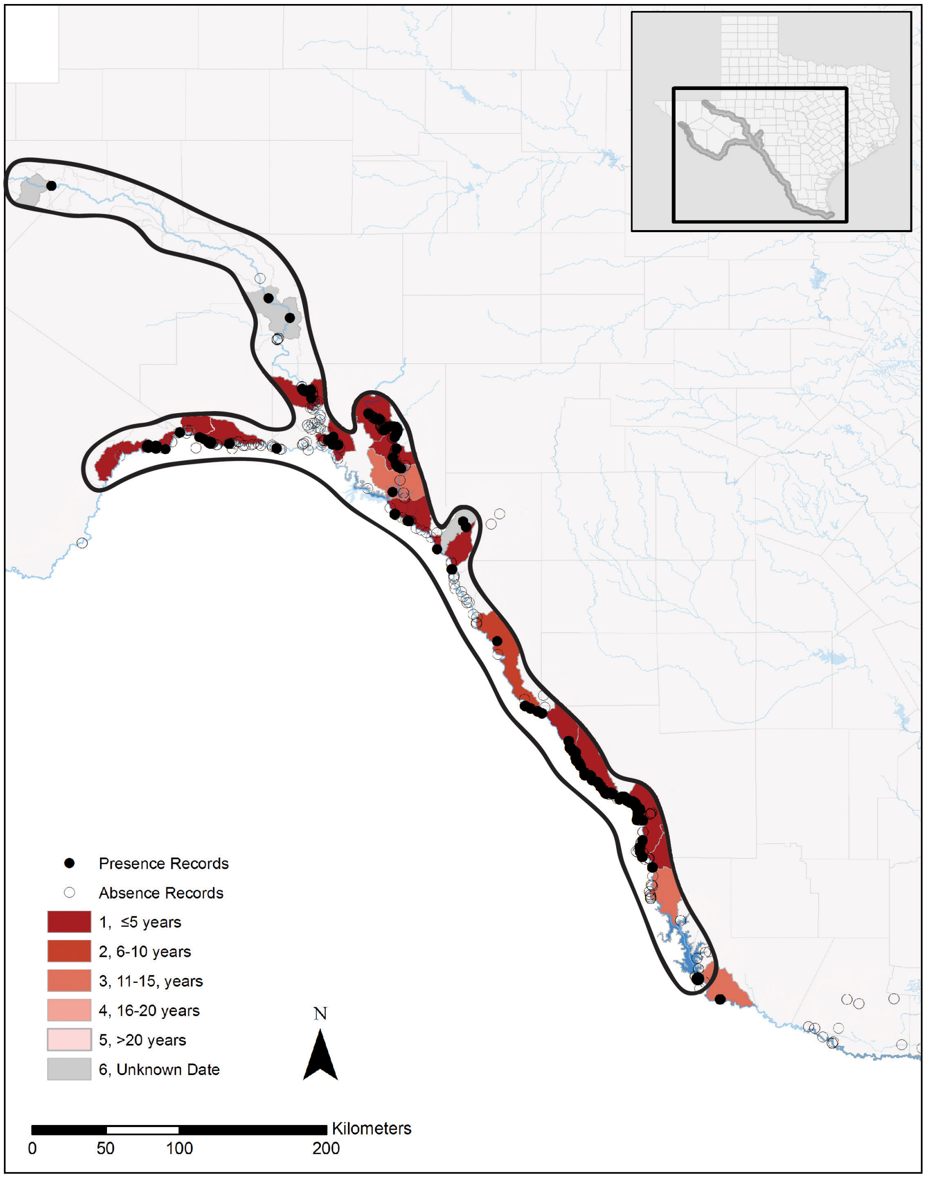

Figure 1. Conservation assessment map for *Popenaias popeii* (Texas hornshell) populated with presence/absence records for both shell and live individuals. Shaded circles denote presence and unshaded circles indicate absence for *P. popeii*. Survey sites shown are from 1905 to present and are taken from the present study plus those obtained from museums, academic, state, and federal agencies. HUCs are colored based on date of sampling. Solid black line denotes presumptive range.

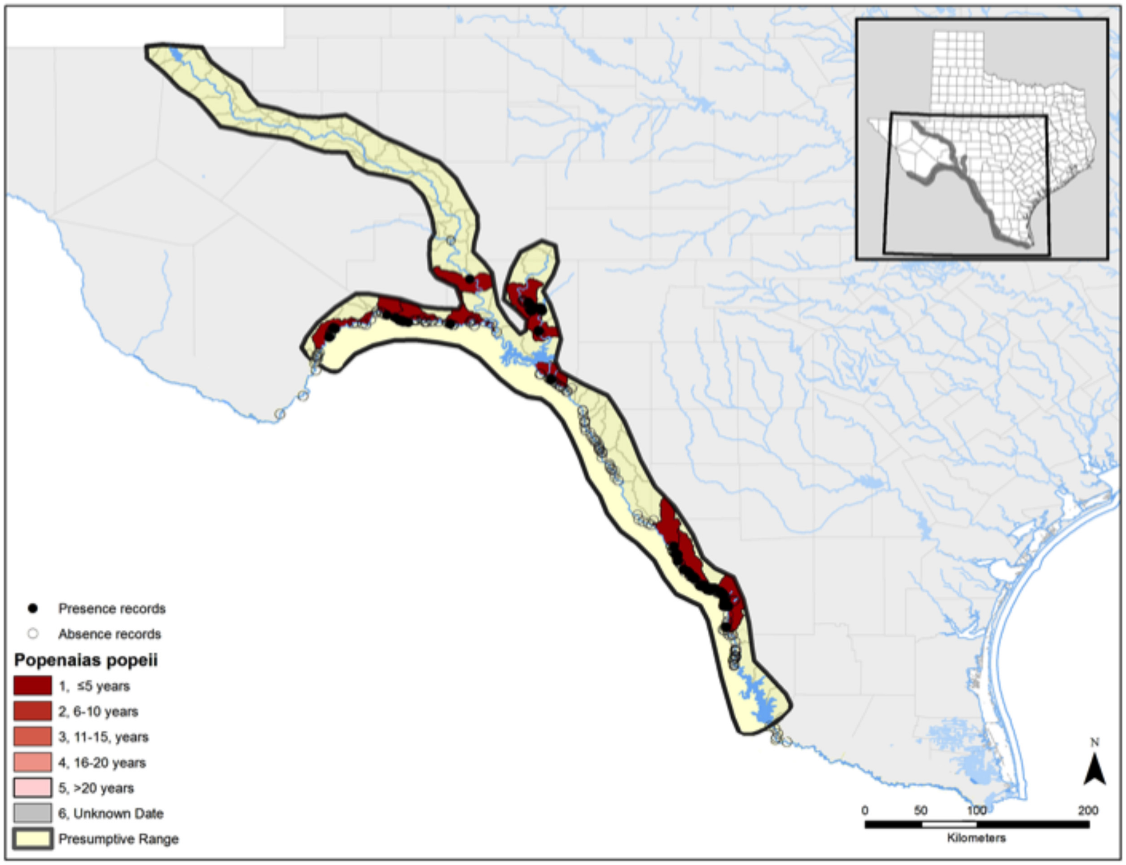

Figure 2. Conservation assessment map for *Popenaias popeii* (Texas hornshell) populated with presence/absence records for only live individuals. Shaded circles denote presence and unshaded circles indicate absence for *P. popeii*. Survey sites shown are from 2000 to present and are taken from the present study plus those obtained from academic, state, and federal agencies. HUCs are colored based on date of sampling. Map taken from Randklev et al. (2016).

Quadrula aurea (golden orb)

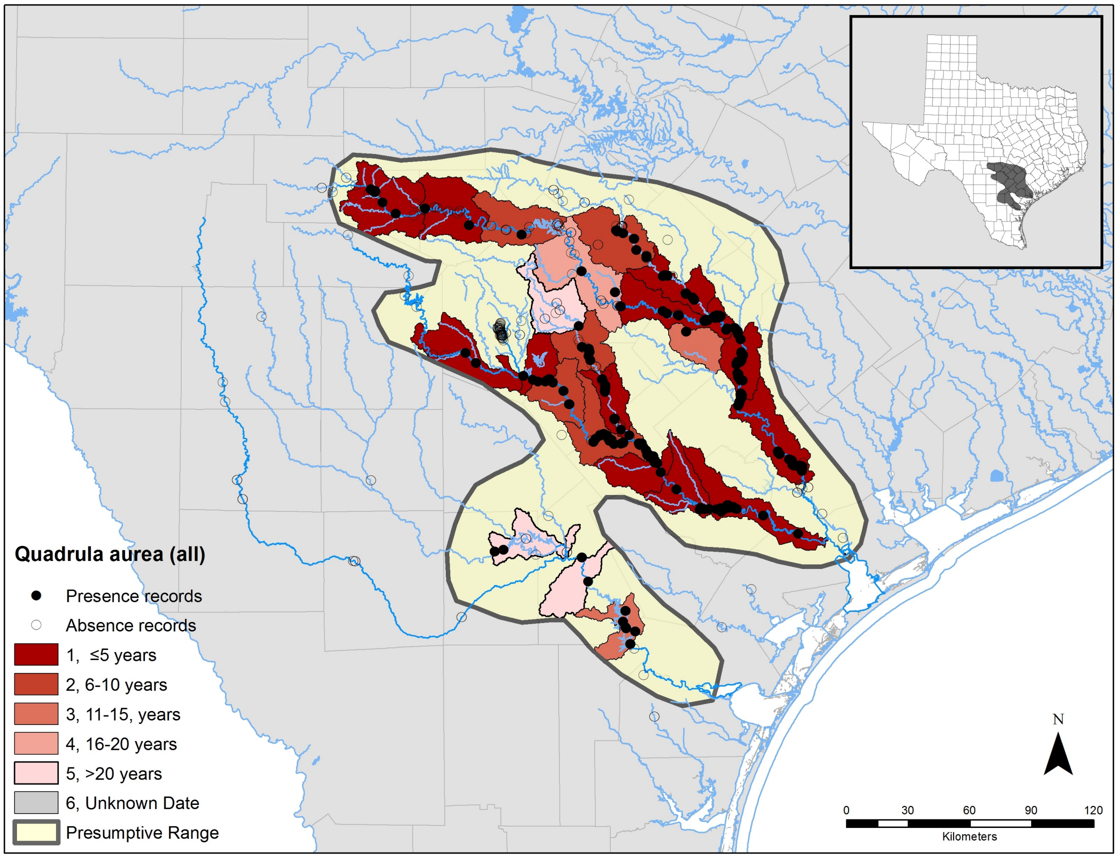

Figure 1. Conservation assessment map for *Quadrula aurea* (golden orb) populated with presence/absence records for both shell and live individuals. Shaded circles denote presence and unshaded circles indicate absence for *Q. aurea*. Survey sites shown are from 1951 to present and are taken from the present study plus those obtained from museums, academic, state, and federal agencies. HUCs are colored based on date of sampling. Solid black line denotes presumptive range.

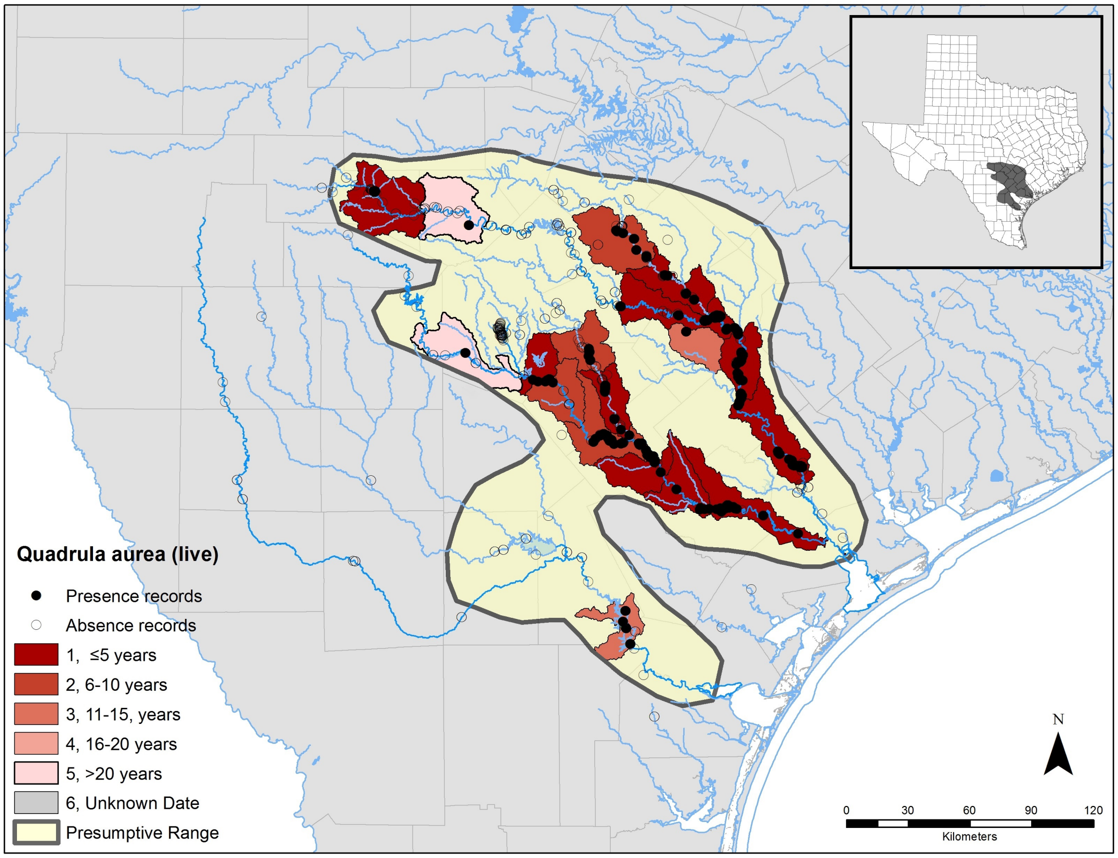

Figure 2. Conservation assessment map for *Quadrula aurea* (golden orb) populated with presence/absence records for only live individuals. Shaded circles denote presence and unshaded circles indicate absence for *Q. aurea*. Survey sites shown of live individuals are from 1973 to present and are taken from the present study plus those obtained from academic, state, and federal agencies. Survey sites shown of absence data are from 1951 to present and are taken from the same sources listed above. HUCs are colored based on date of sampling.

Quadrula houstonensis (smooth pimpleback)

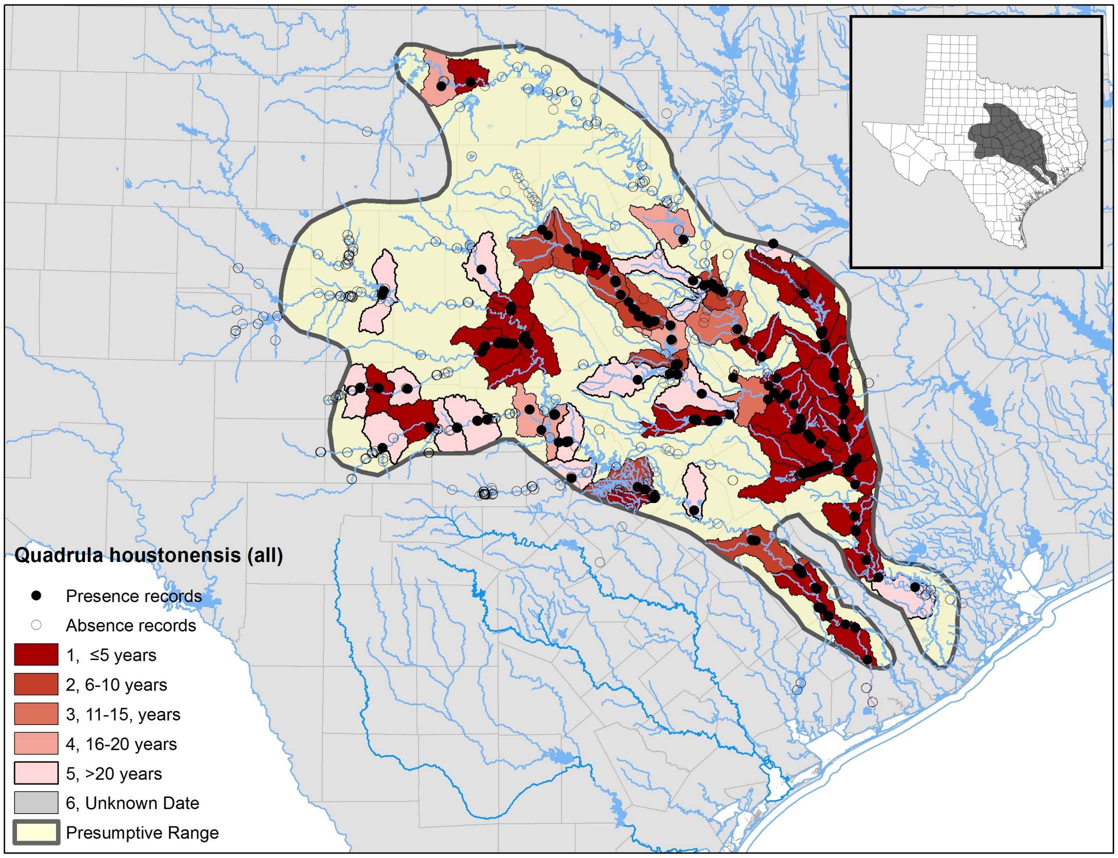

Figure 1. Conservation assessment map for *Quadrula houstonensis* (smooth pimpleback) populated with presence/absence records for both shell and live individuals. Shaded circles denote presence and unshaded circles indicate absence for *Q. houstonensis*. Survey sites shown are from 1900 to present and are taken from the present study plus those obtained from museums, academic, state, and federal agencies. HUCs are colored based on date of sampling. Solid black line denotes presumptive range.

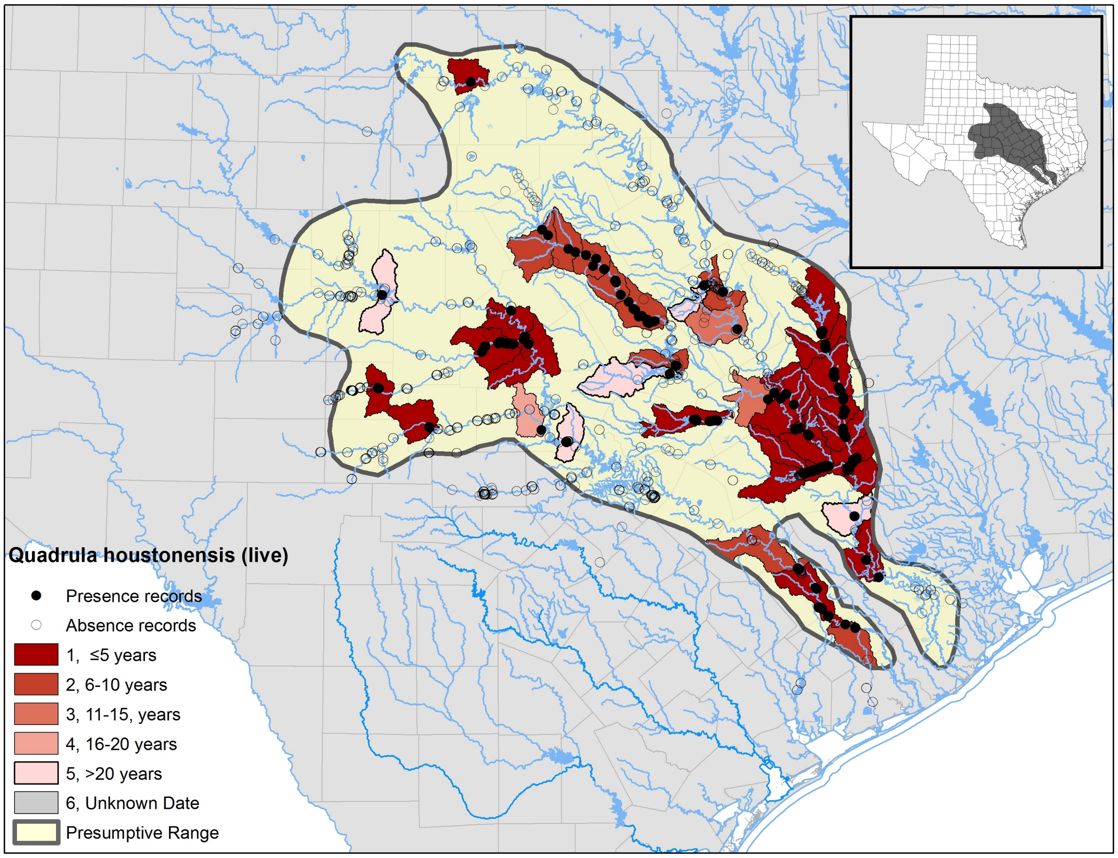

Figure 2. Conservation assessment map for *Quadrula houstonensis* (smooth pimpleback) populated with presence/absence records for only live individuals. Shaded circles denote presence and unshaded circles indicate absence for *Q. houstonensis*. Survey sites shown of live individuals are from 1973 to present and are taken from the present study plus those obtained from academic, state, and federal agencies. Survey sites shown of absence data are from 1900 to present and are taken from the same sources listed above. HUCs are colored based on date of sampling.

Quadrula petrina (Texas pimpleback)

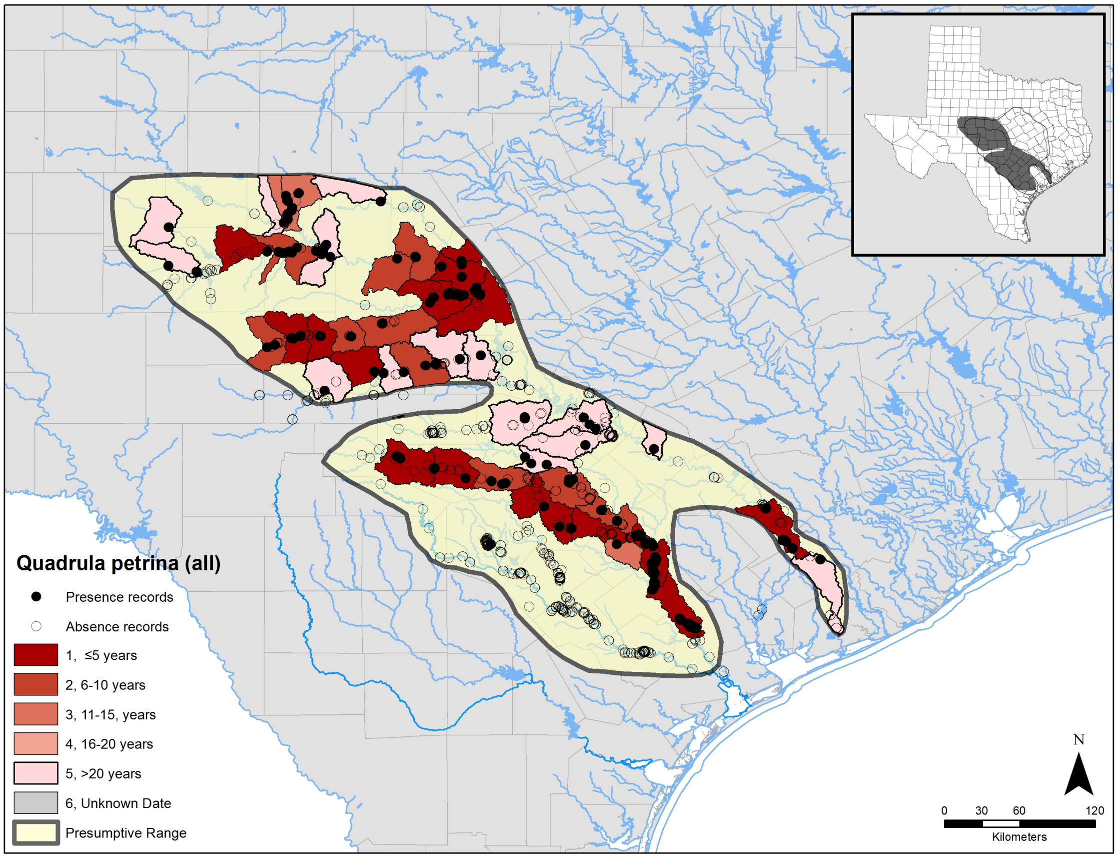

Figure 1. Conservation assessment map for *Quadrula petrina* (Texas pimpleback) populated with presence/absence records for both shell and live individuals. Shaded circles denote presence and unshaded circles indicate absence for *Q. petrina*. Survey sites shown are from 1890 to present and are taken from the present study plus those obtained from museums, academic, state, and federal agencies. HUCs are colored based on date of sampling. Solid black line denotes presumptive range.

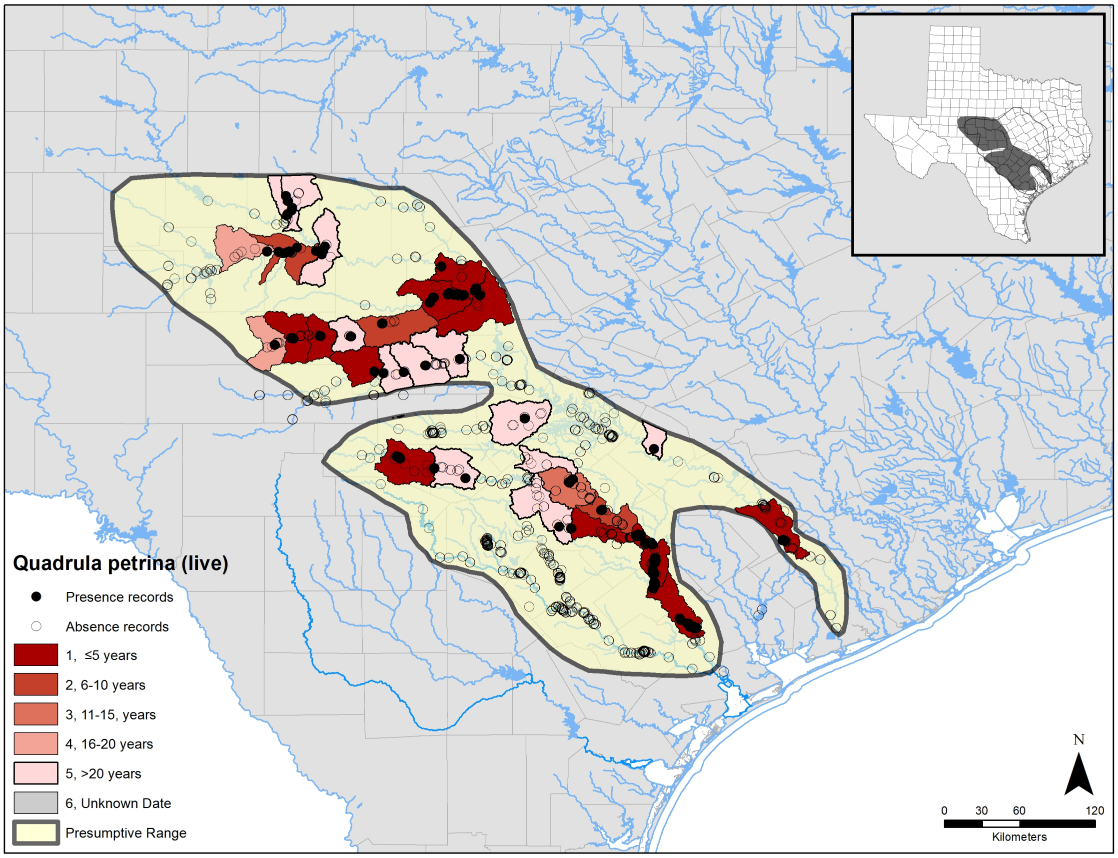

Figure 2. Conservation assessment map for *Quadrula petrina* (Texas pimpleback) populated with presence/absence records for only live individuals. Shaded circles denote presence and unshaded circles indicate absence for *Q. petrina*. Survey sites shown of live individuals are from 1992 to present and are taken from the present study plus those obtained from academic, state, and federal agencies. Survey sites shown of absence data are from 1890 to present and are taken from the same sources listed above. HUCs are colored based on date of sampling.

Truncilla cognata (Mexican fawnsfoot)

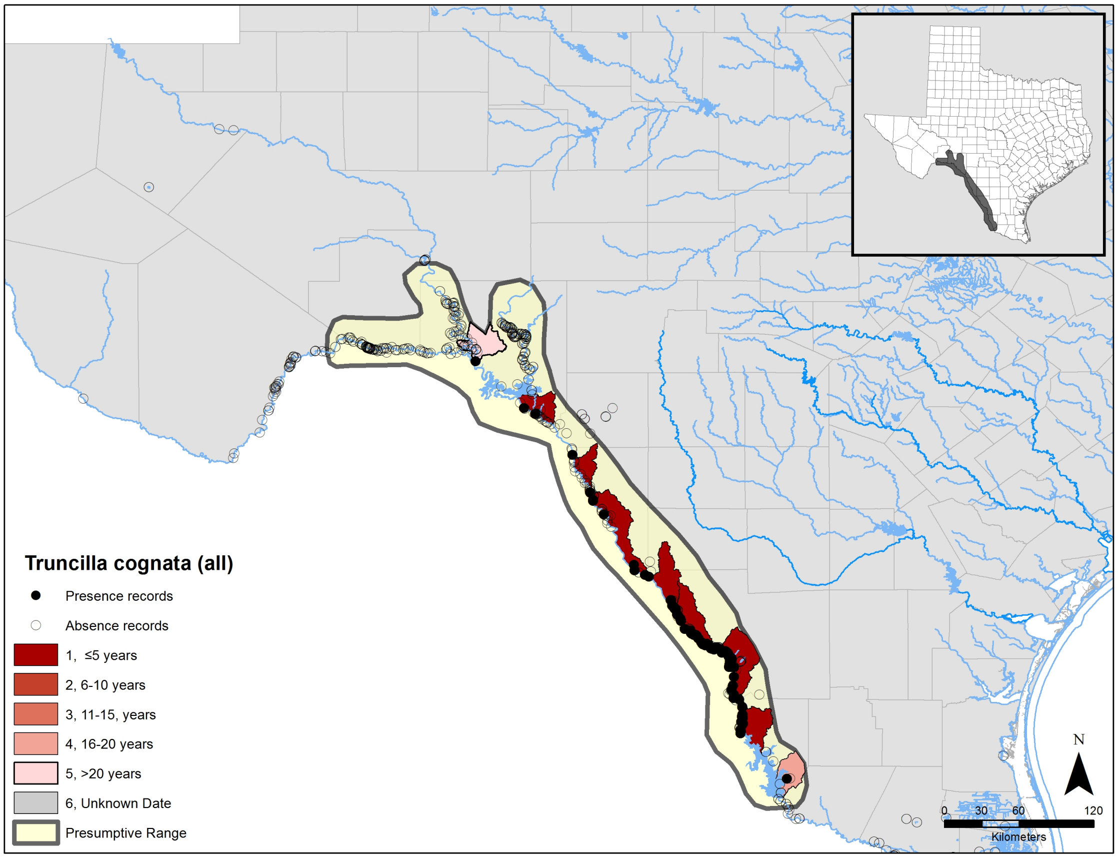

Figure 1. Conservation assessment map for *Truncilla cognata* (Mexican fawnsfoot) populated with presence/absence records for both shell and live individuals. Shaded circles denote presence and unshaded circles indicate absence for *T. cognata*. Survey sites shown are from 1902 to present and are taken from the present study plus those obtained from museums, academic, state, and federal agencies. HUCs are colored based on date of sampling. Solid black line denotes presumptive range.

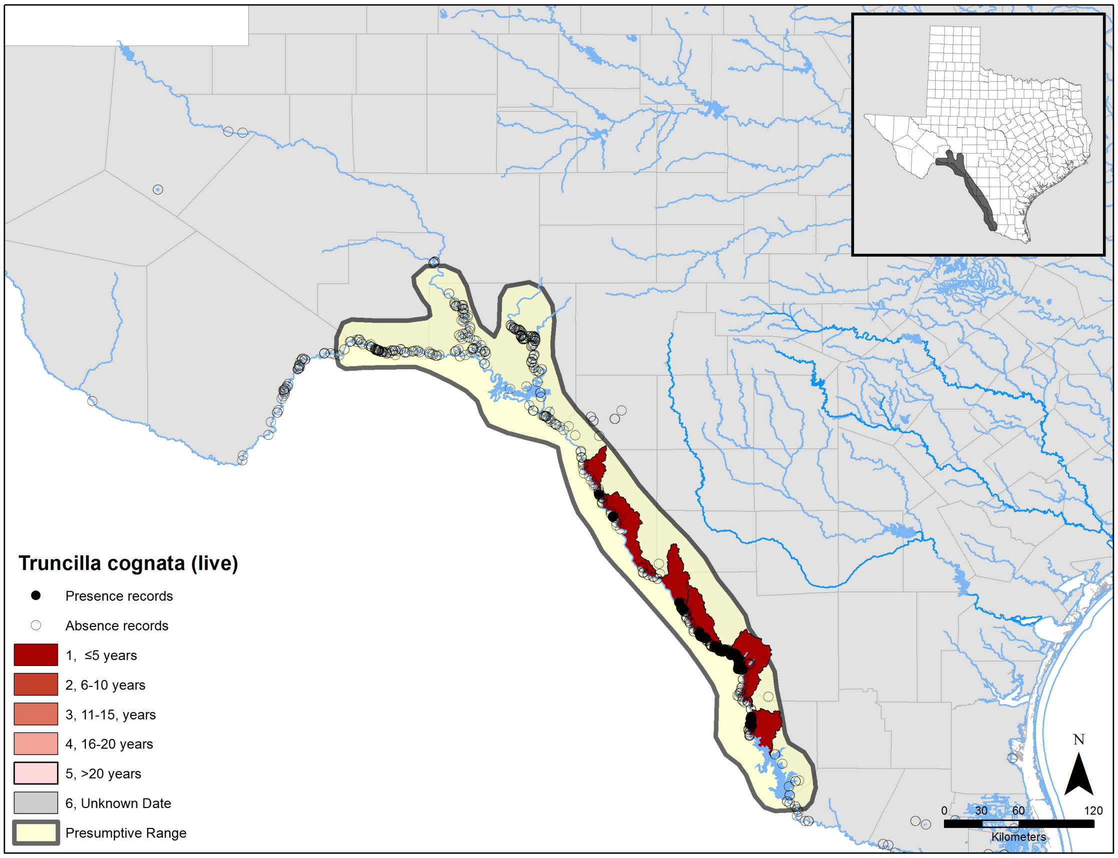

Figure 2. Conservation assessment map for *Truncilla cognata* (Mexican fawnsfoot) populated with presence/absence records for only live individuals. Shaded circles denote presence and unshaded circles indicate absence for *T. cognata*. Survey sites shown of live individuals are from 2003 to present and are taken from the present study plus those obtained from academic, state, and federal agencies. Survey sites shown of absence data are from 1902 to present and are taken from the same sources listed above. HUCs are colored based on date of sampling. Map taken from Randklev et al. (2016).

Truncilla macrodon (Texas fawnsfoot)

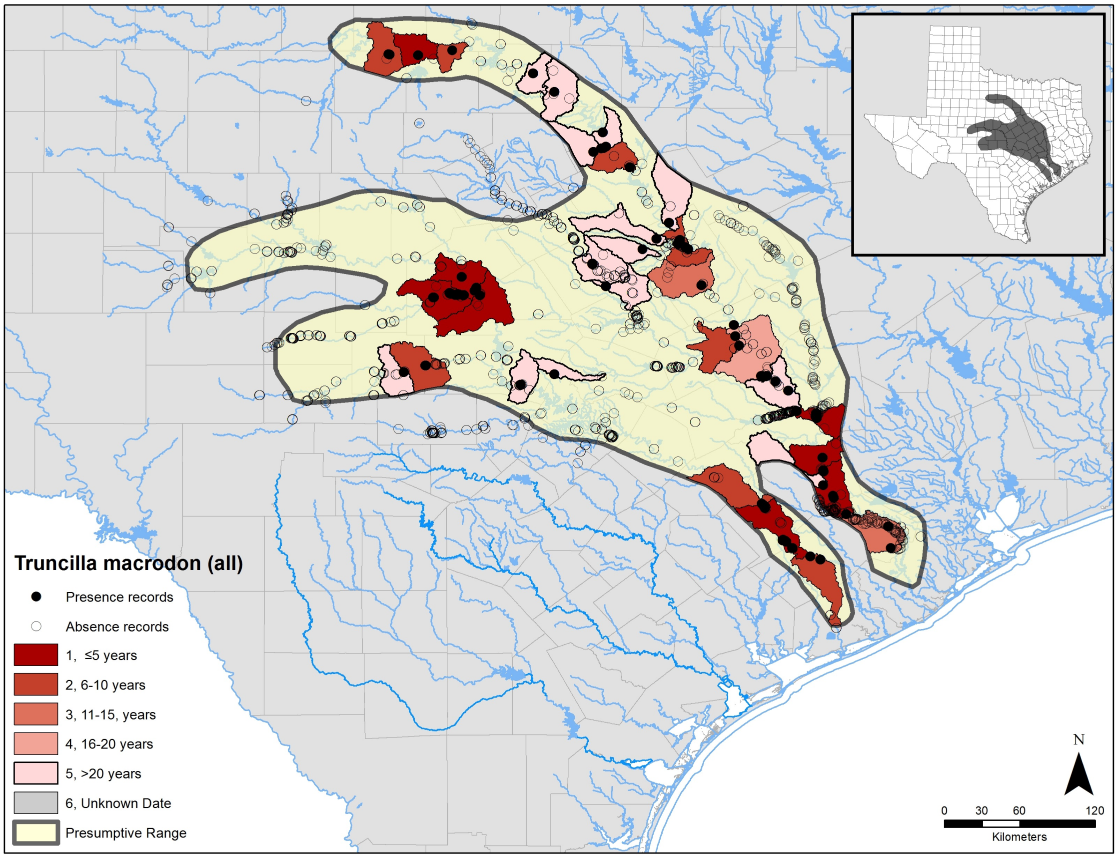

Figure 1. Conservation assessment map for *Truncilla macrodon* (Texas fawnsfoot) populated with presence/absence records for both shell and live individuals. Shaded circles denote presence and unshaded circles indicate absence for *T. macrodon*. Survey sites shown are from 1890 to present and are taken from the present study plus those obtained from museums, academic, state, and federal agencies. HUCs are colored based on date of sampling. Solid black line denotes presumptive range.

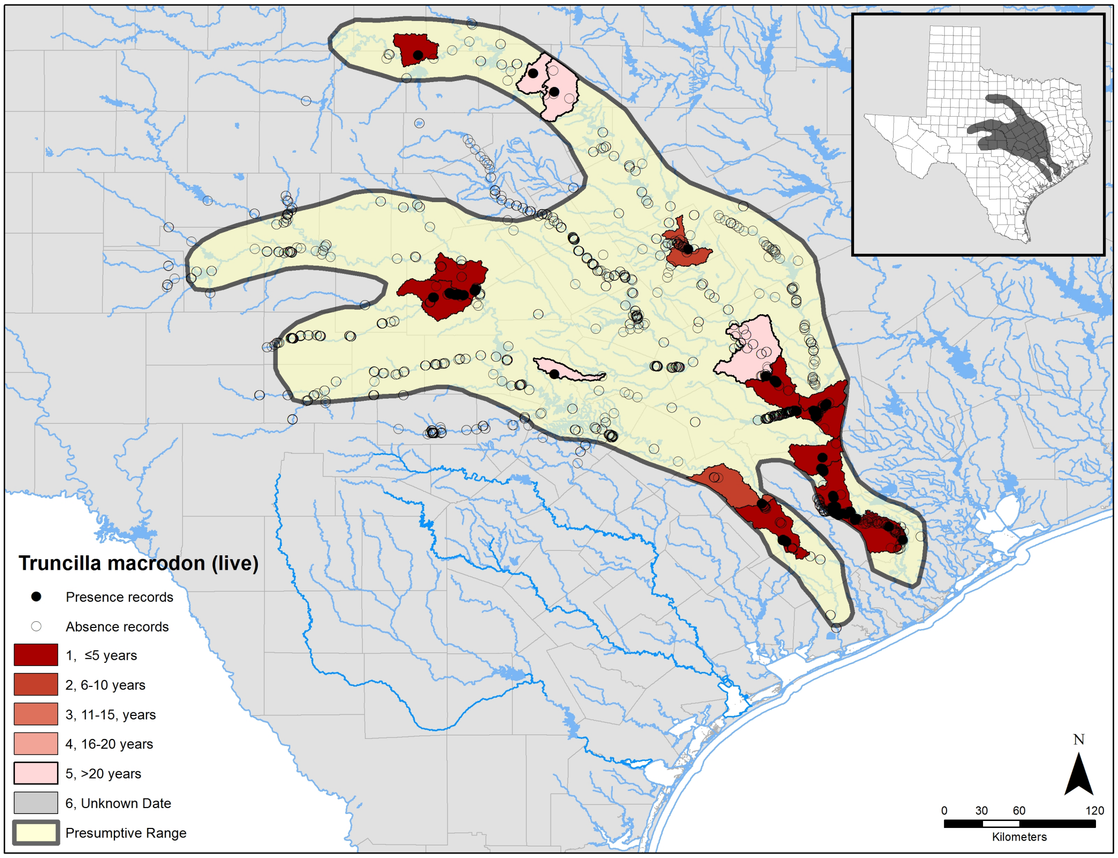

Figure 2. Conservation assessment map for *Truncilla macrodon* (Texas fawnsfoot) populated with presence/absence records for only live individuals. Shaded circles denote presence and unshaded circles indicate absence for *T. macrodon*. Survey sites shown of live individuals are from 1973 to present and are taken from the present study plus those obtained from academic, state, and federal agencies. Survey sites shown of absence data are from 1890 to present and are taken from the same sources listed above. HUCs are colored based on date of sampling.

Task 4: Phylogenetic relationships for Genus *Quadrula* in Texas

Section Summary

Species are a fundamental unit of biology and the application of rigorous approaches to delimiting them is essential to many aspects of basic and applied biological research. This study demonstrates the utility of integrative approach to species delimitation that considers molecular, distribution, and morphology data to evaluate evolutionary relationships within and among several imperiled freshwater mussel taxa of the Quadrulini. We examined genetic relationships using three genes (COI, ND1, and ITS1) representing 8 genera and 20 species in the Quadrulini and evaluated morphological variation throughout the ranges of 8 species in two species complexes. Our results support that the following 12 nominal taxa investigated in this study be assigned to the genus *Cyclonaias*: *C. aurea*, *C. asperata, C. houstonensis, C. infucata, C. kleiniana, C. mortoni, C. nodulata, C. petrina, C. pustulosa, C. refulgens, C. succissa,* and *C. tuberculata.* Additionally, congruence across all lines of evidence (i.e., morphology, geography, and genetics) indicates that current taxonomy overestimates species-level diversity in the *'pustulosa'* species complex while underestimating diversity in the *'petrina* 'species complex. We revise species-level classifications by synonymizing four taxa (*C. aurea*, *C. houstonensis, C. mortoni, and C. refulgens*) considered either species or subspecies under *Cyclonaias pustulosa* and provide evidence for a previously unrecognized species from the *Cyclonaias petrina* complex that is endemic to the Guadalupe River basin.

Introduction

Accurate taxonomy is not merely a semantics issue and our abilities to accurately delimit and classify biodiversity have profound implications on the inference of biological characteristics, ecological responses, and conservation priorities (Barraclough and Nee 2001; Mace 2004). Methods used to diagnose genera and species continue to advance and often reflect different interpretations dependent on types of data available. Modern systematics studies benefit from an integrative taxonomic approach that aims to draw inference from multiple independent lines of evidence (Dayrat 2005; Leache et al. 2009; Knowles and Carstens 2007; Padial et al. 2010; Schlick-Steiner et al. 2010; Carstens et al., 2013) and often reveal that morphological characters used for classification at the generic and species levels are not diagnostic (e.g., Huang and Knowles 2016; Pfeiffer et al. 2016; Perkins et al. 2017).

Freshwater mussels (Bivalvia: Unionidae) are among the most critically endangered assemblages on Earth. In the United States, at least 10% of the fauna is extinct and 65% of the remaining species are considered imperiled (Williams et al. 1993; Haag 2012; Haag and Williams 2014). Conservation efforts focused on freshwater mussels are complicated by various taxonomic uncertainties, and often stem from a lack of discrete morphological characters capable of diagnosing species or determining evolutionary lineages (Shea et al. 2011). The application of molecular phylogenetics have dramatically improved the delimitation of freshwater mussel species boundaries by revealing morphologically cryptic diversity (e.g., Roe and Lydeard 1998; King et al. 1999; Lydeard et al. 2000; Jones et al. 2006; Pfeiffer et al. 2016) and demonstrating that some morphology-based taxonomy has over-inflated diversity estimates (Mulvey et al. 1997; Inoue et al. 2013; Lane et al. 2016).

The systematics of the Tribe Quadrulini, specifically the genus *Quadrula*, has long been a source of taxonomic debate and confusion (Simpson 1900; 1914; Ortmann 1912; Frierson 1927; Vidrine 1993; Howells et al. 1996; Serb et al. 2003; Graf and Cummings 2007; Campbell and Lydeard 2012b). Recent taxonomic treatments have recognized as many as eight genera in Quadrulini: *Amphinaias*, *Cyclonaias*, *Megalonaias*, *Quadrula*, *Quincuncina*, *Rotundaria*, *Theliderma*, *Tritigonia*, and *Uniomerus* (e.g., Williams et al. 1993; 2008; 2014; Turgeon et al. 1988; 1998; Serb et al. 2003; Graf and Cummings 2007; Campbell and Lydeard 2012b). Based on the molecular phylogeny of Serb et al (2003), Graf and Cummings (2007) resurrected *Amphinaias* and *Theliderma* to distinguish the 'Pustulosa' and 'Metanevra' groups, respectively, from the remaining *Quadrula sensu stricto* group. Subsequent phylogenetic studies recovered *Cyclonaias* nested within the 'Pustulosa' group (Campbell et al. 2005; Campbell and Lydeard 2012a; 2012b) and introduced the generic epithet *Rotundaria* (Rafinesque, 1820). These taxonomic incongruences are problematic for conservation efforts that rely on classifications that reflect common ancestry. We set out to better understand the supraspecific relationships within the Quadrulini and establish a generic-level classification that reflects evolutionary history.

The species boundaries within *Quadrula sensu lato* remain uncertain as well and are complicated by a variety of morphological and geographic forms that have perplexed systematists for decades due to high levels of intraspecific variation in shell morphology that often overlaps between species (Valentine and Stansbery, 1971; Neck, 1982; Vidrine et al., 1993; Howells et al., 2002; Serb et al., 2003; Williams et al. 2008). Several taxa occupying Gulf drainages and lower sections of the Interior Basin have been recognized as either distinct species or subspecies of *Q. pustulosa* during recent taxonomic treatments (Turgeon et al. 1988; 1998; Vidrine 1993; Williams et al. 1993; 2008; 2014; Howells et al. 1996; Graf and Cummings 2007). Phylogenetic studies have revealed *Q. aurea*, *Q. mortoni*, *Q. refulgens*, *Q. pustulosa*, and *Q. succissa* to be members of a species complex but none have included *Q. houstonensis* (Serb et al. 2003; Szumowski et al. 2012). Both studies also revealed the close relationship of *Q. nodulata* and *Q. petrina* and advocated for denser phylogeographic sampling before delineating species boundaries. Of particular importance is the taxonomic validity of 3 species being considered for protection under the Endangered Species Act (USFWS 2011): *Quadrula aurea*; *Quadrula houstonensis*; and *Quadrula petrina*.

Here we implement an integrative taxonomic approach utilizing multilocus sequence data, morphometric analyses, and geographic distributions to investigate the species boundaries of 12 species in the 'Pustulosa' group. Additionally, we used our findings to revise generic-level classification within Quadulini, synonymize geographically isolated taxa, and diagnose previously undescribed diversity to better guide conservation efforts.

Methods

Taxon sampling and molecular data

Our taxon sampling concentrated on the following taxa: *Q. asperata, Q. aurea*, *Q. houstonensis*, *Q. infucata, Q. kleiniana, Q. mortoni, Q. nodulata*, *Q. petrina, Q. pustulosa, Q. refulgens,* and *Q. succissa*. Efforts were made to sample throughout the range of each species including type localities. Outgroups from within Quadrulini and two closely related tribes (Amblemini and Pleurobemini) were selected based on relationships resolved in previous phylogenetic studies (Serb et al., 2003; Campbell and Lydeard, 2012a; 2012b; Lopes-Lima et al 2017).

We utilized two protein-coding mitochondrial genes (mtDNA) and one nuclear gene (nDNA) for phylogenetic reconstruction: *cytochrome c oxidase subunit 1* (CO1), *NADH dehydrogenase subunit 1* (ND1), and *internal transcribed spacer 1* (ITS1). Tissue samples were preserved in 95% ethanol and DNA was extracted using a modified plate extraction protocol (Ivanova et al., 2006). Primers used for polymerase chain reaction (PCR) and sequencing were as follows: CO1 dgLCO-1490-GGTCAACAAATCATAAAGAYATYGG and CO1 dgHCO-2198-TAAACTTCAGGGTGACCAAARAAYCA (Meyer, 2003); ND1 Leu-uurF-TGGCAGAAAAGTGCATCAGATTAAAGC and LoGlyR-CCTGCTTGGAAGGCAAGTGTACT (Serb et al., 2003); ITS1-18S-AAAAAGCTTCCGTAGGTGAACCTGCG and ITS1-5.8S-AGCTTGCTGCGTTCTTCATCG (King et al., 1999). The PCR protocol for plate amplifications was conducted in a 12.5 µl mixture: distilled deionized water (4.25 µl), MyTaq™ Red Mix (6.25 µl) (Bioline), primers (0.5 µl) and DNA template (20 ng). Bidirectional sequencing was performed at the Interdisciplinary Center for Biotechnology

Research at the University of Florida on an ABI 3730 (Life Technologies). Geneious v 9.1.5 (Kearse et al., 2012) was used to edit chromatograms and assemble consensus sequences. The mtDNA genes were aligned in Mesquite v 3.2.0 (Maddison and Maddison, 2017) using the L-INS-i method in MAFFT v 7.299 (Katoh and Standley, 2013) and translated into amino acids to ensure absence of stop codons and gaps. The ITS1 alignment was performed using the E-INS-i method in MAFFT due to the presence of indels.

Phylogenetic and phylogeographic analyses

We estimated phylogenetic relationships using a three gene concatenated dataset (i.e., CO1, ND1, ITS1) for members of Quadrulini using maximum likelihood (ML) searches in IQ-TREE v 1.5.2 (Nguyen et al., 2015) and Bayesian inference (BI) in BEAST v 2.4.4 (Bouckaert et al., 2014). Partitions and substitution models for IQ-TREE and BEAST2 were determined by PartitionFinder v1.1.1 (Lanfear et al., 2012). ML analyses included an initial tree search before implementing 1000 ultrafast bootstrap (BS) replicates to estimate nodal support (Minh et al. 2013). BI analyses executed a total of $2*10^8$ generations sampling trees every 1000 generations with an initial 25% burnin. A relaxed log-normal molecular clock was used on all partitions considering the standard deviation of log rate on branches and the coefficient of variance were greater than 0.1 for all partitions (Drummond and Bouckaert, 2015). The relaxed log-normal molecular clock was fixed at 0.34 for the 1st codon position of CO1 (Marko, 2002) and remaining partitions were estimated by BEAST2. Yule process was used as the species tree prior. To ensure adequate sampling, effective sample size (ESS) of all parameters was assessed in Tracer v.1.6 (Rambaut et al., 2014). We used SumTrees in DendroPy v 4.2.0 (Sukumaran and Holder 2010) to estimate a consensus tree with an initial 25% burnin. We tested for a significant difference between ML and BI topologies using K-H (Kishino and Hasegawa 1990), S-H (Shimodaira and Hasegawa 2000), and approximately unbiased (AU) tests (Shimodaira and Goldman 2002). A significance level of α=0.05 was assumed when interpreting output.

Phylogeographic structure was assessed to visualize the geographic distribution of genetic diversity within and between the members of two species complexes: the *'pustulosa' species* complex (*Q. aurea, Q. houstonensis, Q. mortoni, Q. pustulosa, Q. refulgens,* and *Q. succissa*) and the *'petrina'* species complex (*Q. nodulata* and *Q. petrina)*. TCS haplotype networks were generated from mtDNA and nDNA independently for each group using PopART 1.7 (Clement et al., 2002). Samples with only mtDNA sequences were included in the mtDNA haplotype networks to increase sample sizes.

To further investigate evolutionary relationships, intraspecific and interspecific uncorrected p-distances were calculated in MEGA7 (Kumar et al., 2016) for CO1, ND1, and ITS1 independently. Sequences were grouped according to drainage of collection as follows: *Q. aurea* (Guadalupe and Nueces), *Q. houstonensis* (Colorado and Brazos), *Q. mortoni* (Trinity, Neches, and Sabine), *Q. nodulata* (Neches, Red, Sabine, and Mississippi), *Q. petrina* (Colorado), *Q.* sp. cf *petrina* (Guadalupe), *Q. pustulosa* (Red and Mississippi), *Q. refulgens* (Pascagoula and Pearl), *Q. succissa* (Escambia, Yellow, and

Choctawhatchee) (Fig. 1; Fig 2). Gaps and missing data were treated by pairwise deletion between taxa and each taxon was evaluated for diagnostic nucleotides at each mtDNA locus.

Morphometric analyses

We collected two morphometric datasets using external shell dimensions for the members of the *'pustulosa'* and *'petrina'* species complexes. All specimens used in genetic analyses along with additional individuals encountered during field surveys were measured. Specimen groups followed those described above for both haplotype networks and pairwise distance calculations. Three measurements were taken for morphological analyses: maximum length, height, and width to the nearest 0.01 mm using digital calipers. Measurement values were $\log_e$-transformed to produce a scale-invariant matrix while preserving information about allometry (Jolicoeur, 1963; Strauss, 1985; Kowalewski et al., 1997). Log_e-transformed variables were converted into three ratios: height/length, width/length, and width/height. We examined morphological variation through principal components analyses (PCA) in the ggbiplot package (Vu, 2011) and canonical variates analyses (CVA) in the package Morpho (Schlager and Jefferis, 2016) using R v 3.3.1. The PCA analyses were performed to test whether morphological groupings were apparent without *a priori* assignment to a specific group. Canonical variate scores were used for cross-validated discriminant analyses (DA) to test whether morphometric data could assign individuals to geographic groups for the '*petrina*' complex or currently recognized species for the '*pustulosa*' complex. Additionally, we analyzed morphological variation of $\log_e$-transformed variables between the two *Q. petrina* clades (Colorado and Guadalupe drainages) using a permutational multivariate analysis of variance (MANOVA) in the R package vegan (Oksanen et al., 2016) using 1000 iterations. A significance level of α=0.05 was assumed when assessing the statistical significance of all tested hypotheses.

Results

Taxon sampling and molecular analyses

Our three gene molecular matrix consisted of 217 individuals representing 8 genera and 20 species (Table 1). Each taxon was represented by CO1 (avg. ≈ 642 nucleotides [nt]), ND1 (avg. ≈ 797 nt), and ITS1 (951 nt with avg. ≈ 49.13% gaps) and the concatenated three gene alignment consisted of 2397 nt. Protein coding mtDNA genes did not contain any gaps or stop codons. The large proportion of gaps in the ITS1 alignment was primarily caused by a partial duplication in the gene region (294-298 nt) in *Cyclonaias tuberculata,* which was previously reported (Campbell et al., 2012b). Five partitions and nucleotide substitution models were selected by Partitionfinder for implementation in both IQ-TREE and BEAST: CO1 and ND1 1st position- TrNef+I+G, CO1 and ND1 2nd position- HKY+I+G, CO1 3rd position- HKY+G, ND1 3rd position- TrN+G, and ITS1-K80+I+G. Convergence of BEAST runs was supported by ESS>200 for all parameters except ITS1 likelihood (ESS=168) and proportion of invariant sites at CO1 and ND1 2nd position (ESS=55). All topological tests (KH, SH, and AU) found significant support for the ML topology ($p<0.05$) compared to the BI topology. We present ML phylogenetic reconstruction of the concatenated 3 gene matrix containing ML and BI nodal support values (Fig. 3).

Our species-level analyses resolved a paraphyletic *Q. petrina*, with *Q. nodulata* nested between two reciprocally monophyletic and geographically isolated *Q. petrina* clades (Colorado and Guadalupe drainages). In contrast, five of the six recognized species in the *Q. pustulosa* species complex were not monophyletic in the optimal topology. Specifically, *Q. succissa* was resolved sister to a clade containing *Q. aurea, Q. houstonensis*, *Q. mortoni*, *Q. pustulosa,* and *Q. refulgens*. For the *'petrina'* complex, a total of 80 and 55 individuals were included in the mtDNA and ITS1 haplotype networks, respectively (Fig. 4). Three groups are clearly depicted in both networks: *Q. petrina* from the Colorado River, *Q. petrina* from the Guadalupe River, and *Q. nodulata*. For the *'pustulosa'* species complex, 263 and 114 individuals were included in the mtDNA and ITS1 haplotype networks, respectively (Fig. 5). *Quadrula succissa* was molecularly diagnosable from other taxa and clearly divergent in both the mtDNA and ITS1 haplotype networks. All other species shared ITS1 haplotypes and showed weak phylogeographic structuring among mtDNA haplotypes. We observed no overlap between intraspecific variation and interspecific divergence among members of the *'petrina'* complex (Fig. 6). Additionally, all three clades contained diagnostic nucleotides: *Q. petrina* from the Colorado River (CO1/ND1 = 4/16), *Q. petrina* from the Guadalupe River (CO1/ND1 = 4/16), and *Q. nodulata* (CO1/ND1 = 6/5). Uncorrected p-distances show a high degree of overlap between intraspecific variation and interspecific divergence among members of the *Q. pustulosa* complex with the exception of *Q. succissa* (Fig. 6), which also exhibited diagnostic nucleotides (CO1/ND1 =3/4). None of the other taxa were molecularly diagnosable.

Morphometric analyses

We measured a total of 3800 individuals from museum and field collections representing members of the *'petrina'* (1387) and *'pustulosa'* (2413) complexes: *Q. petrina* from the Colorado (527), *Q. petrina* from the Guadalupe (849), *Q. nodulata* (11), *Q. aurea* (868), *Q. houstonensis* (604), *Q. mortoni* (796), *Q. pustulosa* (95), *Q. refulgens* (10), and *Q. succissa* (40). PCA eigenvalues explained 99.6% and 100% of the total variability between members of the *Q. petrina* and *Q. pustulosa* complexes, respectively (Fig. 4; Fig. 5). The PCA for the *Q. petrina* complex revealed high levels of morphological variation among individuals within three distinct groups: Colorado River *Q. petrina*; Guadalupe River *Q. petrina*; *Q. nodulata*. Cross-validated DA scores provided an overall classification accuracy of 80.1% (Colorado River *Q. petrina* = 77.8%; Guadalupe River *Q. petrina* = 81.3%; *Q. nodulata* = 100%). Additionally, permutational MANOVA depicted significant differentiation between *C. petrina* from the Colorado and Guadalupe Rivers (α=0.000999). PCA for the *Q. pustulosa* complex illustrated high levels of morphological overlap between currently recognized species. Cross-validated DA scores provided an overall classification accuracy of 50.48%. Visualization of the PCA plot and DA scores provides a marginal signal for two groups: *Q. houstonensis* (47.2%), *Q. mortoni* (25.9%), *Q. pustulosa* (61.1%), and *Q. refulgens* (40.0%); and *Q. aurea* (74.3%) and *Q. succissa* (50.0%).

Discussion

In this study, we use an integrative approach that considers molecular, distribution, and morphology data to evaluate evolutionary relationships within and among several genera of the Quadrulini. Our phylogenetic analyses revealed that morphological and anatomical characters considered to be synapomorphic at the genus-level may have misguided prior taxonomy. We use our findings to revise generic-level classifications (Fig. 3; Table 1). Congruence across all lines of evidence indicates that current taxonomy overestimates species-level diversity in the *'pustulosa'* complex while underestimating diversity in the *'petrina'* complex. These findings will have profound impacts on future conservation and management efforts, especially for the three species (*Q. aurea*, *Q. houstonensis,* and *Q. petrina*) under consideration for listing by the US Fish and Wildlife Service (USFWS 2011).

Discussion of generic-level relationships

Several recent molecular phylogenies have helped resolve the supraspecific relationships of the Quadrulini (e.g., Serb et al. 2003; Campbell and Lydeard 2012b) but interpretations of these relationships have led to several incongruent generic-level classifications (e.g., Serb et al. 2003; Graf and Cummings 2007; Williams et al. 2008; 2014; Campbell and Lydeard 2012b). Our phylogenetic analyses support the recognition of six genera within Quadrulini: *Cyclonaias*, *Megalonaias*, *Quadrula*, *Theliderma*, *Tritogonia*, and *Uniomerus* (Fig. 3; Table 1). Similar to previous molecular studies, *Theliderma* was recovered as sister to *Tritogonia verrucosa* and *Quadrula s.s.* (e.g., Serb et al. 2003; Campbell and Lydeard 2012b). *Tritogonia* has been treated either as a synonym of *Quadrula s.s.* (e.g., Serb et al. 2003; Williams et al. 2008) or as a monotypic genus (Graf and Cummings 2007; Watters et al. 2009). We recognize *Tritogonia* as a monotypic genus distinct from *Quadrula s.s.* given its sexual dimorphic and elongate shell (Simpson 1900; 1914; Watters et al. 2009) but recognize that these relationships warrant further investigation.

The genus *Cyclonaias* has long been considered monotypic and distinguished from *Quadrula*, *Theliderma*, and *Tritogonia* by only brooding larvae in the outer two gills (Simpson 1900; 1914; Ortmann 1912; 1919; Walker 1918; Williams et al. 2008; Watters et al. 2009). However, *C. tuberculata* has been reported to brood larvae in all 4 gills and subsequently described the genus *Cyclonaias* as "recalcitrant" and playing "havoc with classification" due to the variability in brooding morphology (Frierson 1927). Furthermore, at least three other species, *Q. apiculata, Theliderma cylindrica,* and *T. verrucosa* have been reported to brood larvae in two or four gills (Simpson 1914; Yeager and Neves 1986; Williams et al. 2008). Phylogenetic relationships do not support previous classifications based on larval brooding morphology indicating that the number of gills involved in larval brooding can vary and may not represent shared ancestral states among genera and species of the Quadrulini (Fig. 3; also see Campbell and Lydeard 2012b).

In our phylogenetic analyses, *C. tuberculata* was recovered within a well-supported clade (BS/PP=97/94) that included taxa previously assigned to *Amphinaias* (Graf and

Cummings 2007), *Quadrula* (Simpson 1914; Williams et al. 1993; Turgeon et al. 1988; 1998; Serb et al. 2003; Williams et al. 2008; 2014), *Quincuncina* (Graf and Cummings 2007), and *Rotundaria* (Campbell and Lydeard 2012a; 2012b)(Fig. 3). Several genus- or subgenus-level names have recently been used for this group but no consensus has been reached. Graf and Cummings (2007) resurrected *Amphinaias* based on the molecular phylogeny of Serb et al. (2003) and the morphological groups of Simpson (1900). Campbell and Lydeard (2012b) resolved *C. tuberculata* nested within a paraphyletic *Amphinaias* and subsequently resurrected the epithet *Rotundaria* (Agassiz, 1852) to represent this clade based on statements in Valenciennes (1827). However, Valenciennes (1827) did not explicitly state that *C. tuberculata* was the type of *Rotundaria*. Ortmann and Walker (1922) clarified this issue pointing out that Herrmannsen (1848) designated *Obovaria subrotunda* as the type of *Rotundaria*, relegating *Rotundaria* a junior synonym of *Obovaria* and recognized *Cyclonaias tuberculata*. Therefore, treatment of Rafinesque's type of *Unio tuberculata* as the type species of *Rotundaria* is invalid.

The type species of *Amphinaias*, *A. couchiana* (Lea, 1860), could not be included in this analysis and is thought to be extinct (Williams et al. 1993; Howells et al. 1996; Turgeon et al. 1998; Serb et al. 2003). Morphologically, *A. couchiana* most closely resembles members of *Quadrula s.s.* and has been allied with this group in previous assessments (Simpson 1900; 1914; Strecker 1931) and herein we support the combination *Quadrula couchiana*. Regardless of the generic placement of *Unio couchiana*, the inclusion of *C. tuberculata* in the clade representing the 'Pustulosa' group makes *Cyclonaias* the oldest name available. The priority of *Cyclonaias* applies to the generic epithet *Pustulosa* (Frierson, 1927) as well. Accordingly, we support that the following 12 species included in this study be assigned to the genus *Cyclonaias*: *C. aurea*, *C. asperata, C. houstonensis, C. infucata, C. kleiniana, C. mortoni, C. nodulata, C. petrina, C. pustulosa, C. refulgens, C. succissa,* and *C. tuberculata* (see Table 1).

Integrative approach to species delimitation

Here, our primary goal was to investigate currently recognized species in the genus *Cyclonaias* using multiple molecular-based analyses and additional lines of evidence (e.g., morphometrics) to delimit species within an integrative taxonomic framework (Dayrat 2005; Leache et al. 2009; Knowles and Carstens 2007; Padial et al. 2010; Schlick-Steiner et al. 2010; Carstens et al., 2013). Phylogenetic relationships of our concatenated molecular matrix and broad geographic sampling identified nine well-supported species-level clades within *Cyclonaias*, including two species complexes containing taxa of immediate conservation concern (Fig. 3; Fig. 4; Fig. 5). Both BI and ML analyses resolved *C. petrina* as paraphyletic in respect to *C. nodulata.* The two divergent *C. petrina* clades correspond to individuals sampled from the Colorado and Guadalupe rivers, with the Colorado River clade being sister to *C. nodulata*. This provides credible evidence that species-level diversity is underestimated in this complex. mtDNA sequence divergence exhibited a clear gap between intraspecific variation and interspecific divergence among the three geographically isolated clades (Fig. 6), indicative of species-level divergence and similar to values reported for several other freshwater mussel species (e.g., Roe and Lydeard 1998; Serb et al. 2003; Jones et al., 2006; Campbell et al. 2008; Inoue et al. 2014; Pfeiffer et al., 2016; Perkins et al. 2017). Sequence divergence at ITS1 was lower relative to both mtDNA loci but consistent with patterns observed in previous studies utilizing these genes (e.g., Pfeiffer et al. 2016; Perkins et al. 2017). Morphometric analyses also suggest clear separation of *C. nodulata* and the Colorado and Guadalupe *C. petrina* clades (Fig 4).

Prior to our study, little information was available regarding phylogenetic relationships between members of the *'pustulosa* 'complex. Previous researchers have questioned the validity of taxa in the *'pustulosa'* complex due to difficulties distinguishing between morphologic forms, geographic variants, and distinct species (e.g., Strecker 1931; Turgeon et al. 1988; 1998; Vidrine 1993; Williams et al. 1993; 2008; 2014; Howells et. al 1996; Graf and Cummings 2007; Watters et al. 2009). For our assessment, we allowed geographic distributions based on current taxonomy to represent the null species hypotheses. Our molecular and morphometric data indicate that current taxonomy overestimates species-level diversity in the *'pustulosa'* complex. In fact, our data show greater genetic divergence and morphological distinctiveness between the two geographically isolated populations of *C. petrina* than between all *C. aurea*, *C. houstonenis*, *C. mortoni*, *C. pustulosa*, and *C. refulgens* sampled. All five taxa previously recognized as species or subspecies in the *'pustulosa'* complex exhibited extensive paraphyly (Fig. 5) with no clear distinction between intraspecific variation and interspecific divergence at mtDNA loci (Fig. 6) or clear signal for diagnosis using morphological characters (Fig. 5). With the exception of *C. succissa*, relationships among mtDNA haplotypes show weak associations with currently recognized taxonomy and several nominal taxa share of ITS1 haplotypes (Fig. 5). Additionally, morphometric analyses depicted limited ability to distinguish between members of the *'pustulosa'* complex using shell measurements. Specifically, *C. houstonensis*, *C. mortoni*, *C. pustulosa*, and *C. refulgens* were all indistinguishable. Both *C. aurea* and *C. succissa* were found to be significantly more compressed than other members of the complex yet only 74% of individuals identified morphologically as *C. aurea* were correctly binned, where 25% were assigned to *C. succissa*. However, our molecular-based analyses do not support the recognition of *C. aurea* as a distinct species and we suspect that the observed morphological differences in *C. aurea* may be a product of ecophenotypic variation, a common phenomenon in freshwater mussels (Ortmann 1920; Eagar 1954; Zieritz et al. 2010; Inoue et al. 2013; Bourdeau et al. 2015; Fassatoui et al. 2015; Zajac et al 2017).

Implications for taxonomy and conservation

Our study is the first to analyze extensive phylogeographic and morphometric variation in the *C. pustulosa* and *C. petrina* species complexes and joins a growing number of empirical studies showing that patterns of diversity in freshwater mussels are complex and do not always match expectations based on morphological characters or geographic distributions (e.g., Inoue et al. 2014; Pfeiffer et al. 2016; Perkins et al. 2017; Smith et al. 2017). Considering the lack of diagnosability across multiple lines of evidence, it is our recommendation that *C. aurea, C. houstonensis, C. mortoni,* and *C. refulgens* be designated as synonyms of *C. pustulosa.* This expands the distribution of *C. pustulosa* from the Pascagoula River drainage west to the Nueces River drainage in South Texas. Our phylogeographic assessment shows geographic structuring of populations within *C.*

pustulosa sensu lato, which provides resources managers with valuable information for future recovery efforts, especially those involving propagation, augmentation, translocation, and reintroduction (see Jones et al. 2006). Additionally, our findings provide compelling evidence for recognition of an undescribed species in the *C. petrina* species complex that is endemic to the Guadalupe River. These taxonomic treatments will have profound impacts conservation and management efforts, especially the three species (*C. aurea*, *C. houstonensis,* and *C. petrina*) under consideration for listing by the US Fish and Wildlife Service (USFWS 2011).

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Table 1. Taxa sampled drainage of collection, and number of sequences for all individuals included in our molecular analyses.

Taxa	Drainage	CO1 & ND1	ITS1
Tribe Amblemini			
Amblema plicata	Colorado River	2	2
Tribe Pleurobemini			
Elliptio crassidens	Ohio River	1	1
	Pearl River	1	1
Tribe Quadrulini			
Cyclonaias aurea	Guadalupe	30	9
	Nueces	39	7
Cyclonaias asperata	Mobile	6	6
Cyclonaias houstonensis	Brazos	18	12
	Colorado	14	7
Cyclonaias howmanni	Guadalupe	33	27
Cyclonaias infucata	Apalachicola	16	16
	Ochlockonee	5	5
Cyclonaias kleiniana	Suwannee	4	4
Cyclonaias mortoni	Neches	26	10
	Sabine	8	6
	San Jacinto River	9	0
	Trinity	15	9
Cyclonaias nodulata	Mississippi River	5	1
	Neches	3	0
	Ouachita	4	4
	Red	1	0
Cyclonaias petrina	Colorado	33	23
Cyclonaias pustulosa	Neosho	4	2
	Ohio	9	5
	Osage	4	2
	Ouachita	16	8
	Red	26	11
	St. Croix River	5	3
	St. Francis	12	5
Cyclonaias refulgens	Pascagoula	5	3
	Pearl	5	2
Cyclonaias succissa	Choctawhatchee	33	9
	Escambia	13	2
	Yellow	3	3
Cyclonaias tuberculata	Tennessee River	3	3
Megalonaias nervosa	Guadalupe River	1	1
	Ohio River	1	1
Quadrula apiculata	Rio Grande	1	1
Quadrula quadrula	Ohio	1	1
Theliderma metanevra	Ohio River	1	1
	Tennessee	1	1
Tritigonia verrucosa	Ohio	3	1
	Red	1	1
Uniomerus tetralasmus	Bayou Pierre	1	1
	Colorado	1	1

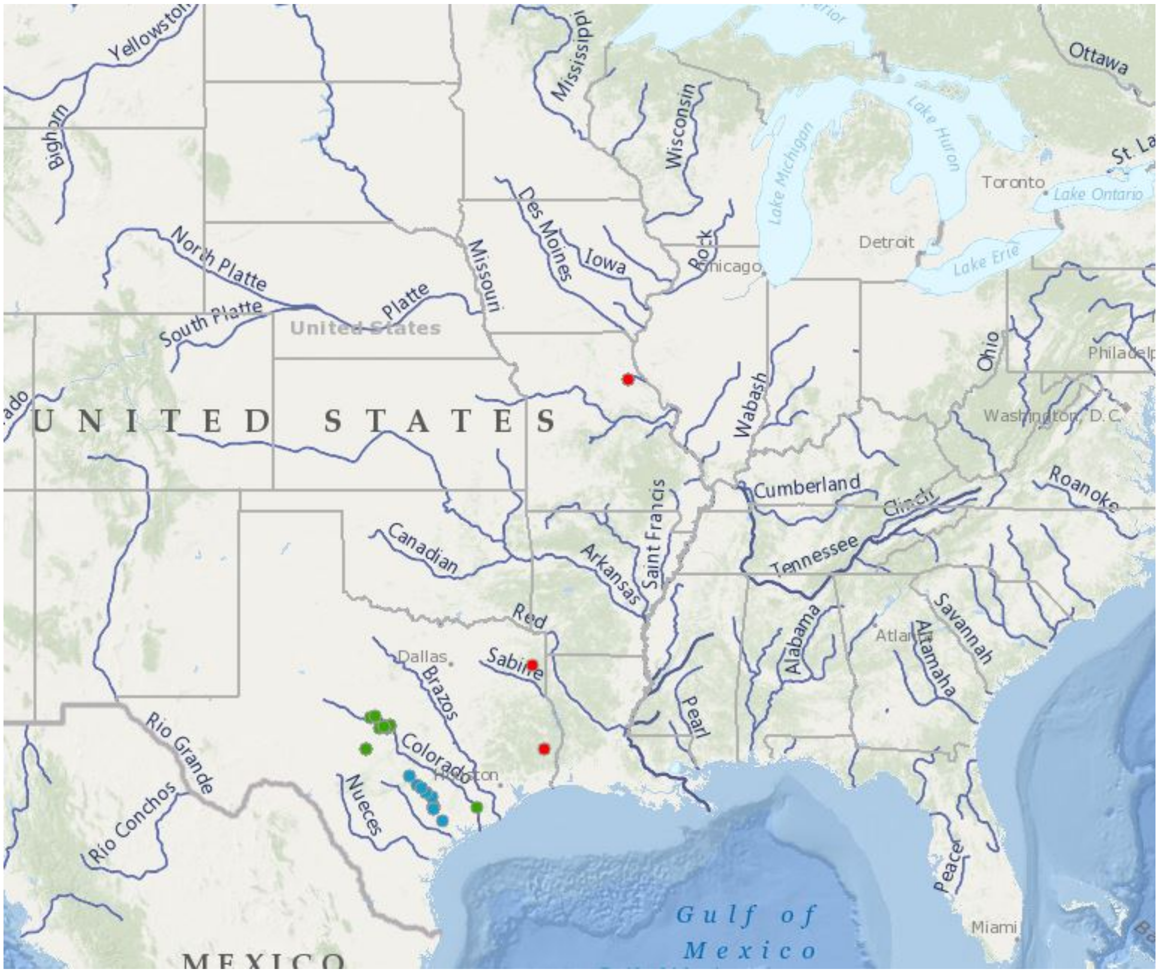

Figure 1. Map showing sampled localities for members of the *C. petrina* species complex. Red (*Cyclonaias nodulata*), green (*Cyclonaias petrina*), and blue (*Cyclonaias sp. cf. petrina*).

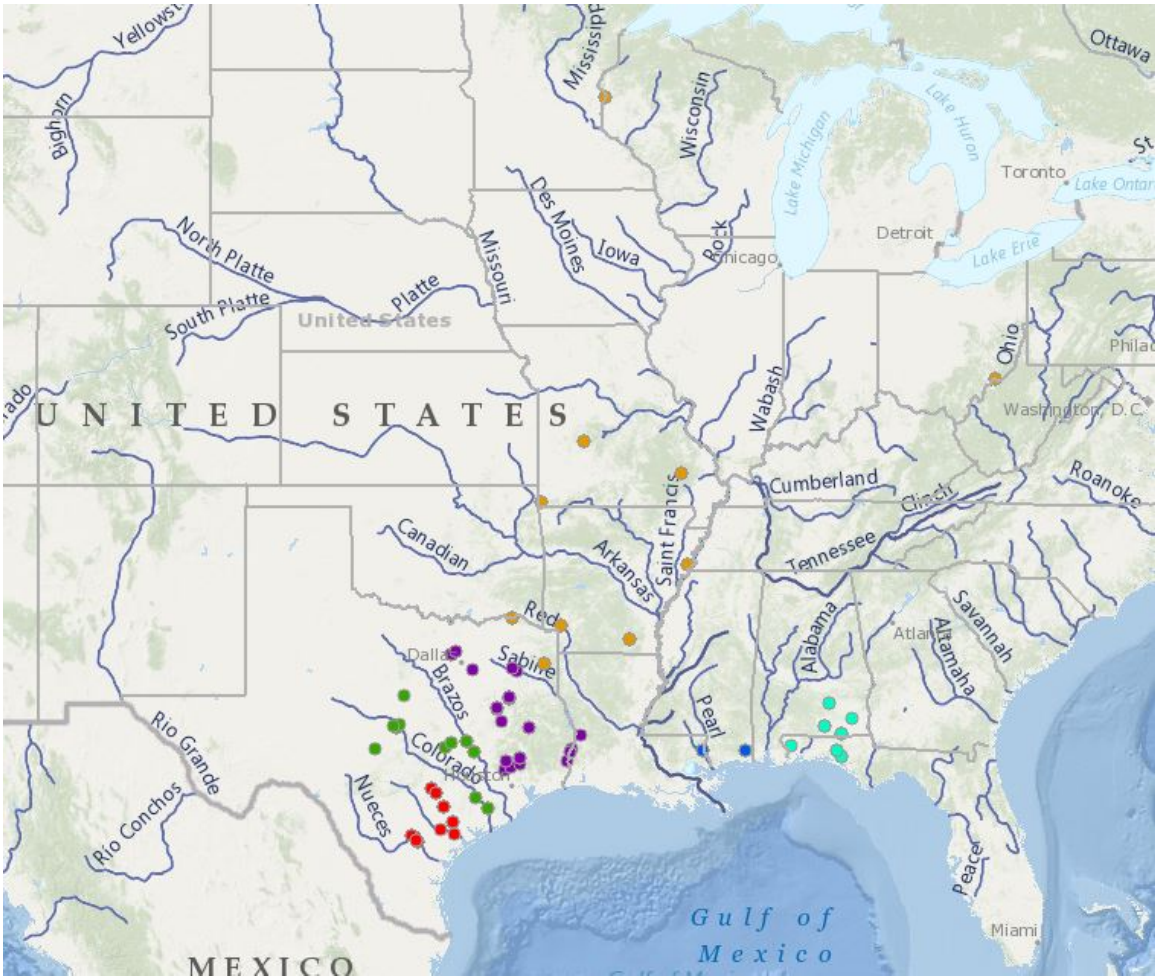

Figure 2. Map showing sampled localities for members of the *C. pustulosa* species complex. Red (*Cyclonaias aurea*), green (*Cyclonaias houstonensis*), purple (*Cyclonaias mortoni*), orange (*Cyclonaias pustulosa*), blue (*Cyclonaias refulgens*), and cyan (*Cyclonaias succissa*).

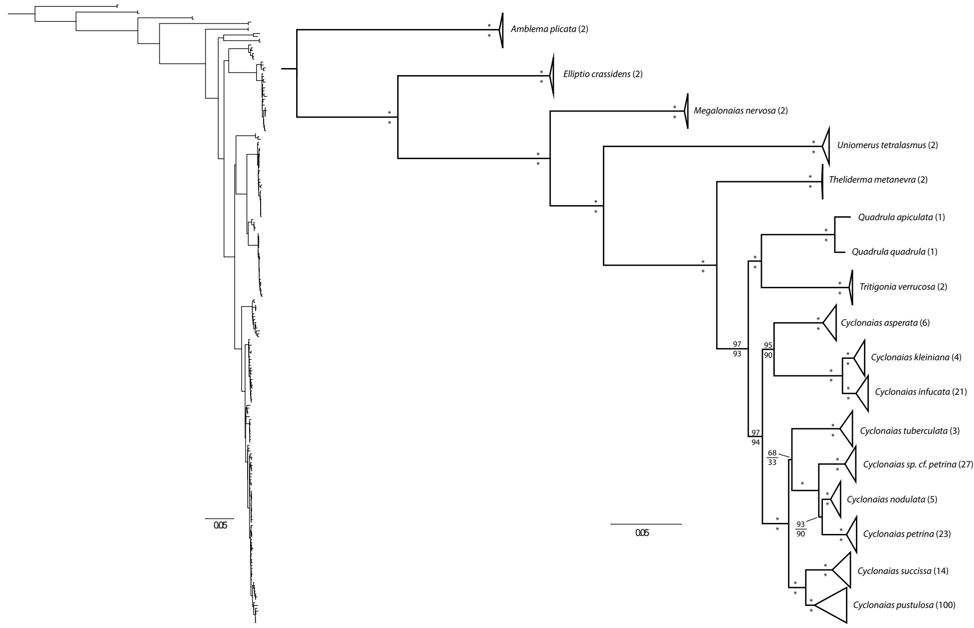

Figure 3. Maximum likelihood (ML) phylogeny based on concatenated mtDNA and nDNA dataset of the Quadrulini with all terminals shown (left) and collapsed into single species-level clades (right). Asterisks above and below nodes represent ≥ 99% bootstrap and 0.99 posterior probability support, respectively. Numbers in parentheses after taxon names indicate sample sizes.

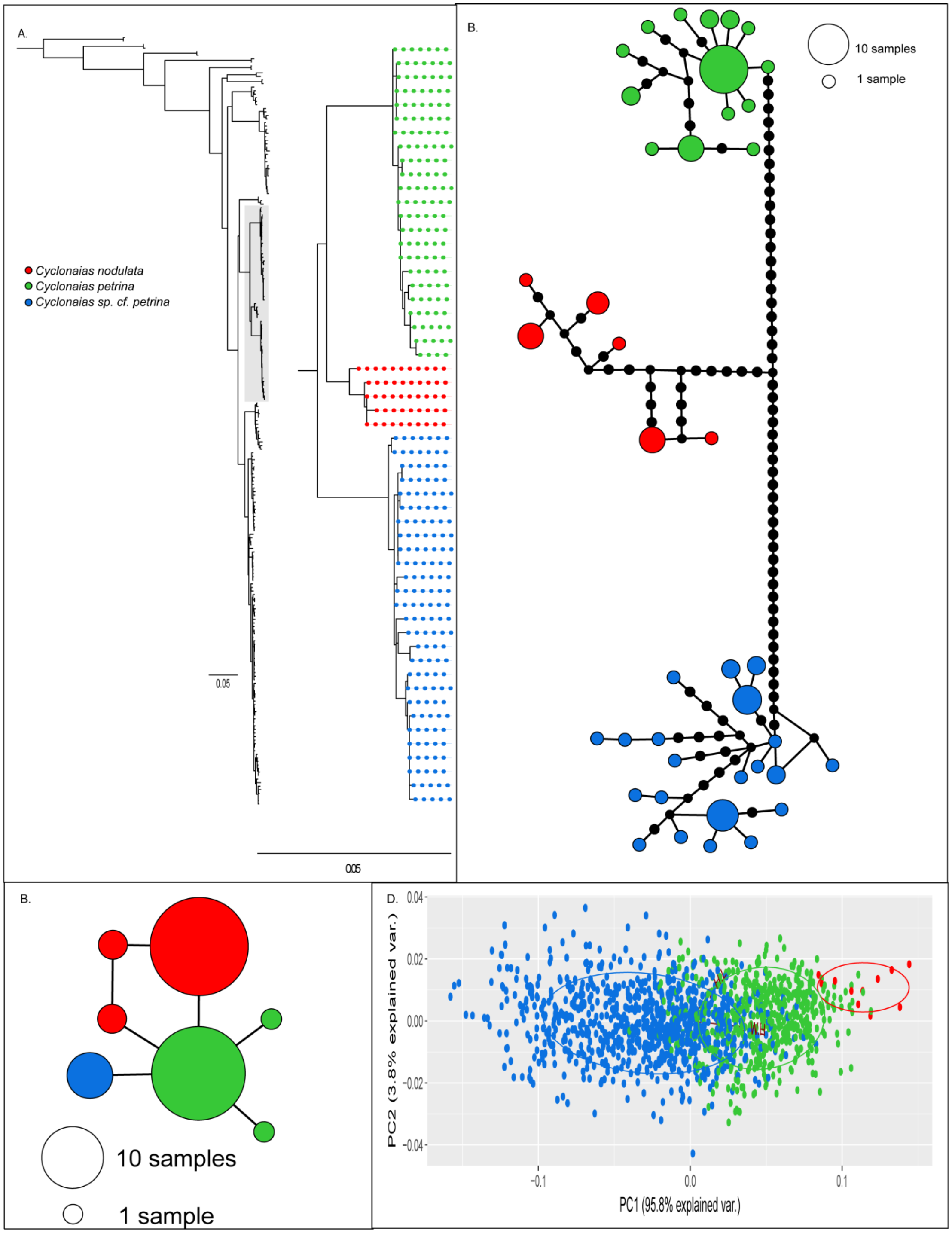

Figure 4. Comparison of results for members of the *Cyclonaias petrina* species complex. A. Fully-resolved and expanded phylogeny based on CO1, ND1, and ITS1 sequences; B) ITS1 haplotype networks; C) CO1+ND1 haplotype networks; and D) PCA plots. Red (*Cyclonaias nodulata*), green (*Cyclonaias petrina*), and blue (*Cyclonaias sp. cf. petrina*).

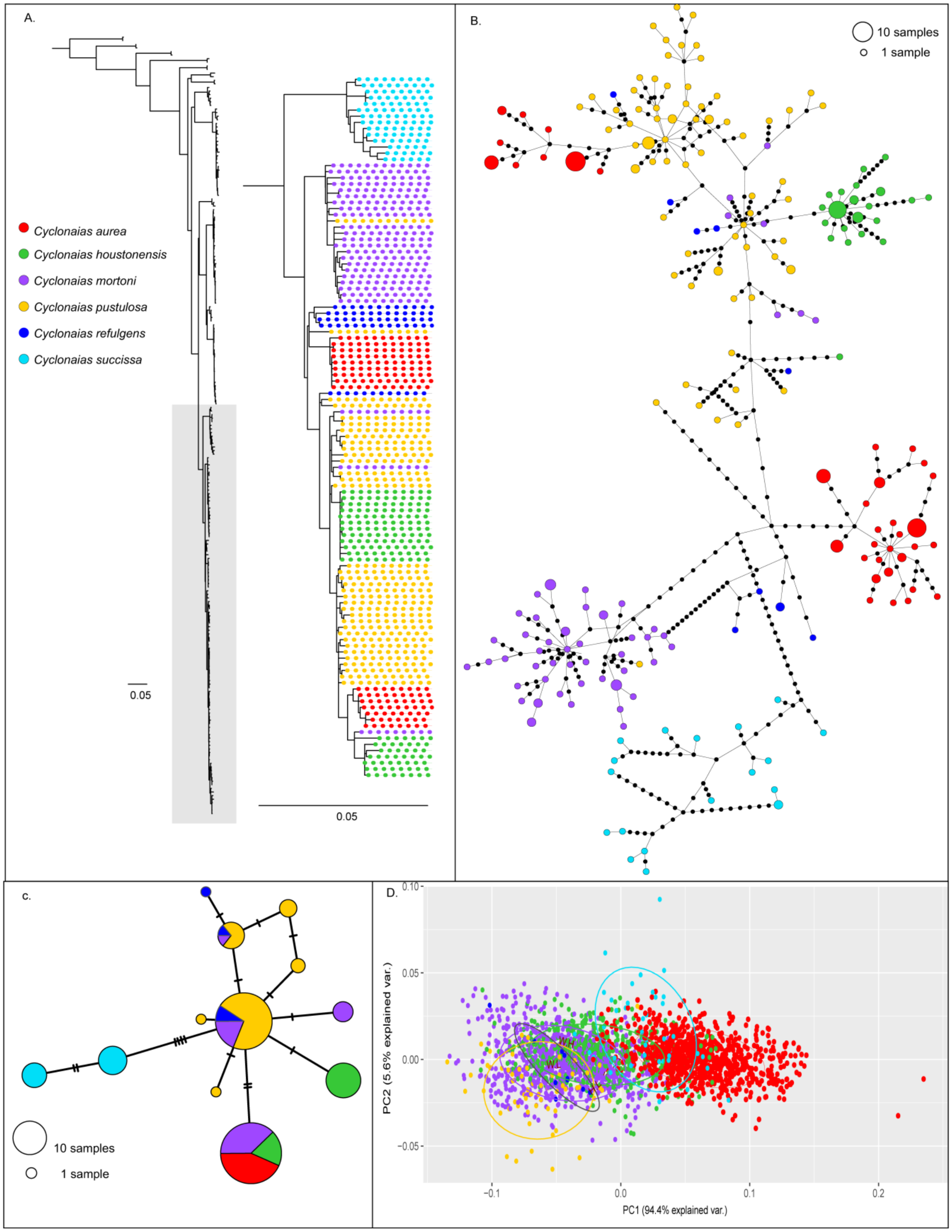

Figure 5. Comparison of results for members of the *Cyclonaias pustulosa* species complex. A. Fully-resolved and expanded phylogeny based on COI, NDI, and ITS1 sequences; B) CO1+ND1 haplotype networks; C); ITS1 haplotype network and D) PCA plots. Red (*Cyclonaias aurea*), green (*Cyclonaias houstonensis*), purple (*Cyclonaias mortoni*), orange (*Cyclonaias pustulosa*), blue (*Cyclonaias refulgens*), and cyan (*Cyclonaias succissa*).

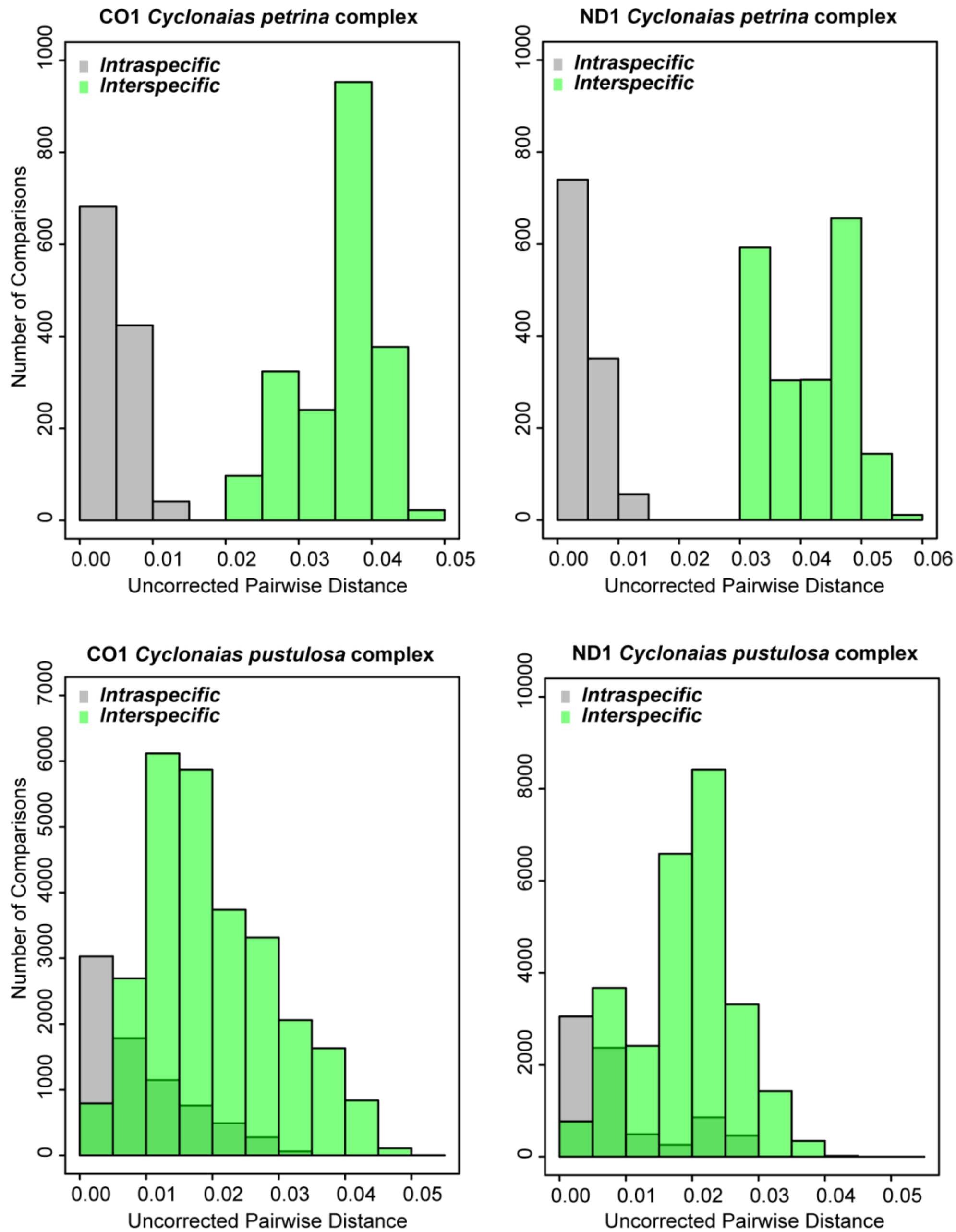

Figure 6. Histograms illustrating the distribution of all intraspecific and interspecific pairwise uncorrected-p distances for *Cyclonaias petrina* complex (top) and *Cyclonaias pustulosa* complex (bottom) based on CO1 (Left) and ND1 (right).

Appendix A: Contracts

STATE OF TEXAS
INTERAGENCY COOPERATION CONTRACT
FOR
ENDANGERED SPECIES RESEARCH PROJECTS ON FRESHWATER MUSSELS

This Interagency Cooperation Contract ("Contract") is entered into between Texas A&M AgriLife Research, a member of the Texas A&M University System and an agency of the State of Texas with principal offices located at 400 Harvey Mitchell Pkwy., Suite 300, College Station, Texas 77845-4375 ("Agency") and the Texas Comptroller of Public Accounts, an agency of the State of Texas ("Comptroller"). Agency and Comptroller are hereinafter collectively referred to as the "Parties."

RECITALS

Whereas, Senate Bill No. 1 of the 82nd Texas Legislature, First Called Session (2011), authorizes Comptroller to promote compliance with federal law protecting endangered or candidate species in a manner consistent with this state's economic development and fiscal stability;

Whereas, understanding the need for more and better science on species in Texas, the 83rd Texas Legislature through its passage of House Bill No. 1025, Section 42, provided $5,000,000 to Comptroller to support high quality species research through state-funded universities;

Whereas, on January 31, 2014, Comptroller issued Request for Proposals No. 207c ("RFP") from Texas state public universities to conduct endangered species research projects on freshwater mussels;

Whereas, on February 28, 2014, Comptroller clarified its Request for Proposals through its official responses to written questions (collectively, the "RFP"),

Whereas, Agency submitted a proposal on or before March 14, 2014, in response to Comptroller's RFP ("Proposal");

Whereas, Agency was selected as Successful Respondent to receive an award under this RFP to perform research projects on freshwater mussels with respect to Region 1 and Region 3 as described in the RFP;

Whereas, in consideration of Agency's compliance with all requirements of this Contract and the terms of the RFP, Comptroller awards this Contract to Agency; and

Whereas, any capitalized term not defined herein shall have the meaning assigned to such term in the RFP.

Now, therefore, in consideration of the foregoing, the parties to this Contract hereby agree as follows:

1. Legal Authority

This Contract is entered into pursuant to the authority granted in and in compliance with the provisions of Chapter 771 of the Texas Government Code (the "Interagency Cooperation Act") and Chapter 403, Subchapter Q of the Texas Government Code.

2. Deliverables; Statement of Services to be Performed

Agency shall provide all of the services and personnel described in Attachment A to this Contract, which is attached hereto and incorporated herein for all purposes. In addition, Agency shall provide all services reasonably related to those specified in Attachment A.

Agency shall retain full control over personnel, including all subcontractors, equipment, supplies, and other items Agency selects as necessary to provide all of the services described in Attachment A.

Agency shall submit such records, information, and reports in such form and at such times as may be required by Comptroller; these reports shall include, but are not limited to, the reports specified in Attachment A.

3. Payments

Total payments to Agency under this Contract shall not exceed **FIVE HUNDRED NINETY-NINE THOUSAND NINE HUNDRED TWENTY-EIGHT AND 00/100 DOLLARS ($599,928.00)**, including all costs and expenses of every kind. Comptroller's payments under this Contract are limited to reimbursements of reasonable, authorized costs and out-of-pocket expenses incurred pursuant to the budget provided in Attachment B, which is attached hereto and incorporated herein for all purposes. No other amounts shall be paid. Agency shall submit an invoice, accompanied by all statements and invoices for services and expenses incurred and any other supporting documentation, to Comptroller on a quarterly basis in arrears for approval prior to payment. Supporting documentation for expenses shall include, but is not limited to, documentation regarding number of hours worked by Agency's personnel and authorized out-of pocket expenses, including travel. When submitting invoices to Comptroller for reimbursement, Agency must certify the following: that the invoices have been carefully reviewed for detailed description of the services performed; that the services have been performed in compliance with this Contract; that the amount of the invoice and all previous invoices together do not exceed the contractual cap of the Contract or Agency's negotiated fees; that the charges and expenses shown on the invoices are reasonable and necessary; and that all appropriate and required supporting documentation for all actual costs incurred is submitted with the invoice corresponding to such costs. Comptroller reserves the right to request additional detail or documentation to support each such quarterly invoice; Agency shall respond to such requests within ten (10) calendar days. Agency expressly acknowledges and agrees that Comptroller shall not be liable to Agency for the payment of any invoiced expense for which Agency has not provided supporting documentation in a timely fashion. All authorized travel expenses shall be limited to and reimbursed in accordance with State of Texas approved rates for mileage, per diem, airfare and lodging. In-state travel directly related to the delivery of services under the Contract shall be permitted, but Agency's reimbursement for travel expenses shall be limited by to only the amount set forth in the State of Texas approved rates for mileage, per diem, airfare and lodging. Agency must receive Comptroller's express prior written approval for any out-of-state travel. Payment will be made according to the provisions of the Texas Prompt Payment Act, to the extent applicable.

4. Contingency of Funding

Comptroller's performance of its obligations under this Contract is contingent upon and subject to availability of and actual receipt by Comptroller of sufficient and adequate funds from the sources contemplated by this Contract. This Contract is subject to immediate cancellation or termination, without penalty to Comptroller, subject to the availability and receipt of these funds. In addition, Comptroller's authority and appropriations are subject to the actions of the Texas Legislature. If Comptroller becomes subject to a legislative change, revocation of statutory authority or lack of funds that would render the services to be provided under this Contract impossible or unnecessary, Comptroller may terminate this Contract without penalty to Comptroller or the State of Texas. In the event of a termination or cancellation under this Section, Comptroller shall not be liable for damages or losses caused or associated with such termination or cancellation but shall reimburse Agency for reasonable costs and non-cancellable commitments incurred up through and including the date of termination.

5. Term; Termination; Amendment

A. This Contract is effective upon the date signed by Comptroller, after having first been signed by Agency, shall terminate on May 31, 2017, unless otherwise sooner terminated as provided herein. Comptroller, in its sole discretion, may elect to extend the term depending on the progress of the research project.

B. Both Parties shall have the option to immediately terminate this Contract upon thirty (30) days' written notice to the other Party for cause or convenience, or if it is determined that termination of the Contract is in the best interests of the State of Texas. Comptroller shall have no liability whatsoever to any other party, person, agency, or entity upon termination of this Contract for any reason, whether for cause or for convenience.

C. In the event Agency fails to implement the project(s) outlined in its Proposal with respect to Region 1 and Region 3 to Comptroller or comply with any material provisions in this Contract, in addition to the remedies specified in this Contract, Agency may be liable to Comptroller for an amount not to exceed the award amount of this Contract and may be barred from applying for or receiving additional research grant program funds administered by Comptroller until repayment to Comptroller is made and any other compliance or audit finding is satisfactorily resolved.

D. This Contract may be amended only in writing by an instrument signed by Comptroller and Agency; however, Comptroller expressly reserves the right in its sole discretion, to amend this Contract unilaterally with ten (10) business days written notice to ensure compliance of this Contract or either Party with state or federal law or other regulation.

6. Personnel and Subcontracting

Agency shall assign only qualified personnel to perform the services set forth under this Contract, including subcontractors, with Agency being required to obtain Comptroller's prior written approval of all subcontractors. Agency may substitute appropriate key personnel to accomplish its duties so long as the substituted personnel are equally qualified and skilled in the tasks necessary to accomplish the tasks and services required; however, Agency shall provide to Comptroller prior written notice of any proposed change in key personnel involved in providing services under this Contract. Agency, in subcontracting any of its performance hereunder, shall legally bind its subcontractors to perform and, to the extent applicable, make such subcontractors subject to the duties, requirements, and obligations of Agency under this Contract. No subcontracting shall release Agency from its responsibility for its obligations under this Contract, and Agency shall be the sole contact for Comptroller. Agency shall be responsible for the work and activities of each subcontractor, including compliance with the terms of this Contract. Agency shall be responsible for all payments to its subcontractors.

7. Property Rights

For the purposes of this Contract, the term "Work" is defined as all reports, work papers, work products, videos, content, materials, approaches, designs, specification, systems, documentation, methodologies, concepts, intellectual property, or other property developed, produced or generated in connection with the services provided under this Contract. Agency owns and will continue to own all right, title and interest and all proprietary rights in and to the Work and any and all documentation or other products and results of the services rendered by Agency, including all trade secret, copyright, patent, trademark, and other proprietary rights.

Agency hereby grants Comptroller a perpetual, royalty-free, nonexclusive, irrevocable, transferable, worldwide license for governmental purposes to use, reproduce, distribute, display, and perform the Work and to prepare derivative works based thereon. Comptroller shall be deemed to have paid all non-commercial licenses, support, maintenance, subscription, and other fees of any kind, and Agency understands and agrees to this provision. Comptroller shall further have the right to share all Work with the U.S. Fish and Wildlife Service.

In the event that either party intends to use, reproduce, display, or perform such Work for commercial purposes, the parties agree in good faith to negotiate the applicable licenses.

No later than the first calendar day after the termination or expiration of this Contract or no later than thirty (30) days after receipt of Comptroller's request, Agency shall deliver to Comptroller all completed, or partially completed, Work and any and all documentation or other products and results of these services.

8. Records Retention and Right to Audit

A. Retention of Records. Agency shall create, maintain and retain sufficient records to adequately document any and all transactions related the services provided under this Contract at a level of detail in accordance with Agency's own record-keeping requirements. Agency shall retain fiscal records and supporting documentation for all expenditures related to this Contract at its principal office adequate to ensure that claims for reimbursement are in accordance with applicable Comptroller and State of Texas requirements. Agency shall maintain all such documents and other records relating to this Contract for a period of four (4) years after the date of submission of the final invoice or until a resolution of all billing questions, whichever is later.

B. Access to Records. Agency shall give the Auditor of the State of Texas, Comptroller, or any of their duly authorized representatives, during normal business hours, access to and the right to examine all books, accounts, records, reports, files, other papers, things, or property belonging to or in use by Agency pertaining to this Contract. Such rights to access shall continue as long as the records are retained by Agency. Agency shall cooperate with auditors and other authorized representatives of Comptroller and the State of Texas and shall provide them with prompt access to all such property as requested by Comptroller or the State of Texas. By example and not as exclusion to other breaches or failures, Agency's failure to comply with this Section shall constitute a material breach of this Contract and shall authorize Comptroller to immediately terminate this Contract. Agency understands that Comptroller is bound by provisions of the Texas Public Information Act, Chapter 552 of the Texas Government Code (the "TPIA") as interpreted by judicial opinions and Attorney General Opinions issued under the statute. Agency is required to make any information created or exchanged with Comptroller pursuant to this Contract, and not otherwise excepted from disclosure under the Texas Public Information Act or by terms of this Contract, available in a format that is accessible by the public at no additional charge to the state. In order to comply with such requirement, any information created or exchanged with the Committee shall be in Word, Excel, or pdf format, as applicable.

C. Right to Audit. Comptroller may require independent audits by a qualified certified public accounting firm of the Agency's books and records or the State's property. The independent auditor shall provide Comptroller with a copy of such audit at the same time it is provided to Agency. Comptroller retains the right to issue a request for proposals for the services of an independent certified public accounting firm under this Contract. In addition to and without limitation on the other audit provisions of this Contract, pursuant to Section 2262.154 of the Texas Government Code, the state auditor may conduct an audit or investigation of the Agency or any other entity or person receiving funds from the state directly under this Contract or indirectly through a subcontract under this Contract. The acceptance of funds by Agency or any other entity or person directly under this Contract or indirectly through a subcontract under this Contract acts as acceptance of the authority of the state auditor, under the direction of the legislative audit committee, to conduct an audit or investigation in connection with those funds. Under the direction of the legislative audit committee, Agency or other entity that is the subject of an audit or investigation by the state auditor must provide the state auditor with access to any information the state auditor considers relevant to the investigation or audit. This Contract may be amended unilaterally by Comptroller to comply with any rules and procedures of the state auditor in the implementation and enforcement of Section 2262.154 of the Texas Government Code. Agency understands that: (1) the acceptance of funds under this Contract acts as acceptance of the authority of the state auditor to conduct an audit or investigation in connection with those funds; (2) Agency further agrees to cooperate fully with the state auditor in the conduct of the audit or investigation, including providing all records requested; (3) Agency shall ensure that this paragraph concerning the authority to audit funds received indirectly by Agency's subcontractors through Agency and the requirement to cooperate is included in any subcontract it awards; and (4) the state auditor shall at any time have access to and the right to examine, audit, excerpt, and transcribe any pertinent books, documents, working papers, and records of Agency relating to this Contract.

9. Notices; Liaison Personnel

Any notice relating to this Contract, which is required or permitted to be given under this Contract by one party to the other party shall be in writing and shall be addressed to the receiving party at the address specified below. The notice shall be deemed to have been given immediately if delivered in person to the recipient's address specified below. It shall be deemed to have been given on the date of certified receipt if placed in the United States mail, postage prepaid, by registered or certified mail with return receipt requested, addressed to the receiving party at the address specified below. Registered or certified mail with return receipt is not required for copies.

1. The address of Comptroller for all purposes under this Contract and for all notices hereunder shall be:

Comptroller: Texas Comptroller of Public Accounts
Legislative Affairs Division
Central Services Building
1711 San Jacinto Boulevard, # 2013
Austin, Texas 78701

Contact Person: Lisa Elledge
Natural Resource Policy Advisor
Legislative Affairs Division
Email: Lisa.Elledge@cpa.state.tx.us
Phone: (512) 475-5606

2. The address of Agency for all purposes under this Contract and for all notices hereunder shall be:

Agency: Texas A&M AgriLife Research
Sponsored Research Services
400 Harvey Mitchell Pkwy., Suite 300
College Station, Texas 77845-4375

Contact Person: Jane Zuber
Director, Contracts and Grants
Sponsored Research Services
400 Harvey Mitchell Pkwy., Suite 300
College Station, Texas 77845-4375
Email: jzuber@tamus.edu
Phone: (979) 845-8615

Comptroller and Agency shall maintain specifically identified liaison personnel for their mutual benefit during the term of the Contract for day-to-day communications. The liaison(s) shall be identified in writing following the execution of this Contract. Subsequent changes in liaison personnel shall be communicated by the respective Parties in writing.

10. Release of Information; Confidential Information; Information Security Requirements

No release of any information pertaining to this Contract and the RFP, including but not limited to media releases or releases for educational or academic purposes, such as seminars, lectures, abstracts, articles, case studies, course materials, or written or electronic handouts, shall be made without express, prior written consent of Comptroller and in accordance with Comptroller's explicit written instructions. Notwithstanding the foregoing or any provisions of this Contract or RFP to the contrary, the parties understand that Comptroller and Agency are bound by the provisions of the Texas Public Information Act and Attorney General Opinions issued thereunder. Within three (3) days of receipt, Agency shall refer to Comptroller any third party requests received directly by Agency for information related to this Contract or the RFP.

Any information collected by Agency, or its subcontractors, from a private landowner relating to the specific location, species identification, or quantity of any animal or plant life may only be disclosed to the landowner or participant that provided such information or Comptroller and shall not be disclosed to any other person, including any state or federal agency, without the express written consent of the landowner. Further, pursuant to Section 403.454 of the Texas Government Code, this information is not subject to disclosure under the TPIA without express written consent of the landowner.

Agency shall take all necessary and appropriate action within its abilities to safeguard all sensitive data and other confidential information, including any information made confidential pursuant to Section 403.454 of the Government Code ("Confidential Comptroller Information") from unauthorized disclosure. Whenever communications with Agency necessitate the transmission of Confidential Comptroller Information, Agency shall transmit the information electronically and such electronic transmission shall, at a minimum, be made in 128 AES bit encryption to protect it from unauthorized disclosure.

Each individual who will require access to or may be exposed to Confidential Comptroller Information must sign a Confidential Treatment of Information Acknowledgement form (CTIA). Agency shall access Comptroller's systems or Confidential Comptroller Information only for the purposes for which it is authorized.

Agency shall ensure that any sensitive or confidential Comptroller Information in the custody of Agency is properly sanitized or destroyed when the information is no longer required to be retained by Comptroller or Agency in accordance with this Contract. Electronic media used for storing any sensitive or confidential Comptroller Information must be sanitized by clearing, purging or destroying in accordance with NIST Special Publication 800-88 Guidelines for Media Sanitization. Agency must maintain a record documenting the removal and completion of all sanitization procedures with the following information:

- Date and time of sanitization/destruction;
- Description of the item(s) and serial number(s) if applicable;
- Inventory number(s); and
- Procedures and tools used for sanitization/destruction.

No later than sixty (60) days from contract expiration or termination or as otherwise specified in this contract, Agency must complete the sanitization and destruction of the data and provide to Comptroller all sanitization documentation.

Agency shall not access, process, store, or transmit IRS Federal Taxpayer Information unless expressly authorized by this Contract. Agency shall comply with IRS Publication 1075 requirements if it accesses, processes, stores, or transmits IRS Federal Taxpayer Information.

11. Force Majeure

Except as otherwise provided, neither Agency nor Comptroller shall be liable to the other for any delay in, or failure of performance of any requirement contained in this Contract caused by force majeure. The existence of such causes of delay or failure shall extend the period of performance until after the causes of delay or failure have been removed, provided the non-performing party exercises all reasonable due diligence to perform. Force majeure is defined as acts of God, war, terrorist attacks, fires, explosions, earthquakes, hurricanes, floods, failure of transportation, or other causes that are beyond the reasonable control of either party and that by exercise of due foresight such party could not reasonably have been expected to avoid, and which, by the exercise of all reasonable due diligence, such party is unable to overcome. Each party must inform the other in writing with proof of receipt within ten (10) business days of the existence of such force majeure or otherwise waive this right as a defense.

12. Indemnification

TO THE EXTENT ALLOWED BY THE CONSTITUTION AND LAWS OF THE STATE OF TEXAS,AGENCY SHALL INDEMNIFY AND HOLD HARMLESS THE STATE OF TEXAS AND COMPTROLLER, AND/OR THEIR OFFICERS, AGENTS, EMPLOYEES, REPRESENTATIVES, CONTRACTORS, ASSIGNEES, AND/OR DESIGNEES FROM ANY AND ALL LIABILITY, ACTIONS, CLAIMS, DEMANDS, OR SUITS, AND ALL RELATED COSTS, ATTORNEY FEES, AND EXPENSES ARISING OUT OF, OR RESULTING FROM ANY ACTS OR OMISSIONS OF AGENCY OR ITS AGENTS, EMPLOYEES, SUBCONTRACTORS, ORDER FULFILLERS, OR SUPPLIERS OF SUBCONTRACTORS IN THE EXECUTION OR PERFORMANCE OF THIS CONTRACT. THE DEFENSE SHALL BE COORDINATED BY AGENCY WITH THE OFFICE OF THE ATTORNEY GENERAL WHEN TEXAS STATE AGENCIES ARE NAMED DEFENDANTS IN ANY LAWSUIT AND AGENCY MAY NOT AGREE TO ANY SETTLEMENT WITHOUT FIRST OBTAINING THE CONCURRENCE FROM THE OFFICE OF THE ATTORNEY GENERAL. AGENCY AND COMPTROLLER AGREE TO FURNISH TIMELY WRITTEN NOTICE TO EACH OTHER OF ANY SUCH CLAIM.

13. Assignment

Without the prior written consent of Comptroller, Agency may not transfer or assign any rights or duties under or any interest in this Contract.

14. Dispute Resolution

The parties shall use the dispute resolution process provided for in Chapter 2260 of the Texas Government Code to resolve any disputes under this Contract.

15. Actual or Potential Conflicts of Interest Prohibited

Agency represents that it and its personnel, including the Agency's subcontractors, have no actual or potential conflicts of interest in performing this Contract and related activities, and performance of this Contract will not create any appearance of impropriety. Agency shall notify Comptroller immediately upon learning of any potential or actual conflicts of interest.

16. No Waiver

This Contract shall not constitute or be construed as a waiver of any of the privileges, rights, defenses, remedies, or immunities available to either Agency or the State of Texas or otherwise available to either Agency. The failure to enforce or any delay in the enforcement of any privileges, rights, defenses, remedies, or immunities available to either Agency under this Contract or under applicable law shall not constitute a waiver of such privileges, rights, defenses, remedies, or immunities or be considered as a basis for estoppel. Either Agency does not waive any privileges, rights, defenses, or immunities available to either Agency or the State of Texas, or otherwise available to either Agency, by entering into this Contract or by its conduct prior to or subsequent to entering into this Contract. **The modification of any privileges, rights, defenses, remedies, or immunities available to Comptroller must be in writing, must reference this section, and must be signed by Comptroller to be effective, and such modification of any privileges, rights, defenses, remedies, or immunities available to Comptroller shall not constitute waiver of any subsequent privileges, rights, defenses, remedies, or immunities under this Contract or under applicable law.**

17. Entire Agreement

This Contract and its accompanying attachments contain the entire agreement between the parties relating to the rights granted and the obligations assumed in it. Any oral representations or modifications concerning this Contract shall be of no force or effect unless contained in a subsequent writing signed by both parties.

18. Severability Clause

In the event that any provision of this Contract is later determined to be invalid, void, or unenforceable, then the remaining provisions of this Contract shall remain in full force and effect and shall in no way be affected, impaired, or invalidated.

19. Governing Law; Venue

This Contract is governed by and construed under and in accordance with the laws of the State of Texas. Any and all obligations under this Contract are due in Travis County and venue is proper only in such county.

20. Certifications

The undersigned Parties do hereby certify that: (1) the services specified above are necessary and essential and are properly within the statutory functions and programs of the affected agencies of State Government, (2) the proposed arrangements serve the interest of efficient and economical administration of those agencies, (3) the services, supplies or materials contracted for are not required by Section 21 of Article 16 of the Constitution of Texas to be supplied under contract to the lowest responsible bidder, and (4) this Contract neither requires nor permits either Party to exceed its duties and responsibilities or the limitations of its appropriated funds.

Agency further represents and warrants that it has obtained all necessary permits, approval, and permissions from applicable state and federal agencies in order to conduct the proposed research project. Agency is solely responsible for obtaining all applicable permits, approval, and permissions and Agency's failure to do so may result in termination of this Contract.

21. Signatories

The undersigned signatories represent and warrant that they have full authority to enter into this Contract on behalf of the respective Parties. This Contract may be executed in one or more counterparts, each of which is an original, and all of which constitute only one agreement between the Parties.

TEXAS COMPTROLLER OF PUBLIC ACCOUNTS

By: ____________________
Martin A. Hubert
Deputy Comptroller

Date: 6/4/14

TEXAS A&M AGRILIFE RESEARCH

By: ____________________
Craig Nessler *AK*
Director

Date: 6/3/2014

ATTACHMENT A

STATEMENT OF SERVICES TO BE PERFORMED

A. Agency shall perform all of the services described in this Attachment A, or otherwise required by this Contract, ("services"). These services include, but are not limited to, the furnishing of all personnel, supplies, and other items necessary to provide those services in compliance with this Contract. Agency shall provide all services in accordance with the Standards of Performance established by Comptroller for these services. Agency shall review and implement Comptroller recommendations, as Comptroller adopts them from time to time, so that the services may be expeditiously and satisfactorily completed. Agency shall meet with Comptroller personnel at such times as Comptroller may reasonably request to discuss the progress of services and any other matters that may arise in regard to this Contract.

B. Agency shall provide all of the following services to achieve technical research needs identified in RFP 207c Endangered Species Research Projects for Freshwater Mussels, Part III, Scope of Work for Regions one (1) and three (3):

1. Conduct research studies and address the status of freshwater mussels in Region one (1) in all or part of the Brazos, Colorado, Guadalupe, San Antonio and Nueces River Systems.

2. Freshwater mussel studies in Region one (1) shall include: the Texas Fatmucket, *Lampsilis bracteata;* Texas Hornshell, *Popenaias popeii;* Golden Orb, *Quadrula aurea;* Smooth Pimpleback, *Quadrula houstonensis;* False Spike, *Quadrula mitchelii;* Texas Fawnsfoot, *Truncilla macrodon.*

3. If *Quadrula aurea, Q. houstonensis, Q. mitchelli,* and *Q. petrina* (or any combination of these species) are found in Region one (1), a genetic analysis should be conducted to resolve the taxonomic status of Texas pimplebacks.

4. Conduct research studies and address the status of freshwater mussels in Region three (3) in all of part of the Rio Grande River System, including the Devil's River and Pecos River.

5. Freshwater mussel studies in Region three (3) shall include: the Texas Hornshell, *Popenaias popeii;* False Spike, *Quadrula mitchelii;* and Mexican Fawnsfoot, *Truncilla cognate.*

6. Deposit data and research results, using Fish and Wildlife Services (FWS) guidelines, in a registry or provide to FWS.

C. Agency shall provide the following services during the period of this Contract and all services reasonably related to them. Comptroller may request additional records, information or reports related to the services hereinafter described and funded by Comptroller pursuant to Attachment B. These services are as follows:

The minimum deliverables are summarized in the following chart:

Deliverables and Milestones	Schedule
1. Kick-off meeting	TBD
2. Select Technical Advisory Panel (TAP)	TBD
3. Submit TAP approval of research methodology	July 30, 2014

4.	Quarterly progress report emailed to Comptroller	On or before ten days following the end of each state fiscal quarter*
5.	Conference with TAP	Quarterly
6.	Written response to TAP comments	On or before the end the month following the state fiscal quarter
7.	Annual Progress Report	On or before December 31st of each year
8.	Collect permits and permisions needed to conduct research	August 31, 2014
9.	Written review of literature for research species	August 31, 2014
10.	Data collection complete	October 31, 2016
11.	Notice of completion of research	November 30, 2016
12.	Data and research results deposited in a registry or provided to Fish and Wildlife Services	December 30, 2016
13.	Final report due**	April 30, 2017
14.	Close-out meeting	May 31, 2017

*The state fiscal quarters are the last day of February, May, August, and November.

**Final Report includes:
1. GIS analysis of suitable habitats
2. Mapped location and population levels for all species found
3. Detailed species status assessments
4. Detailed habitat Assessment
5. Comments on species management

ATTACHMENT B

BUDGET

Budget Category	Amount
Personnel	$145,109.00
Travel	$26,667.00
Supplies	$20,667.00
Contractual	$336,592.00
Other	$16,354.00
Total Direct Cost	$545,389.00
Indirect Cost	$54,539.00
Total Cost	**$599,928.00**

ATTACHMENT C

NONDISCLOSURE AGREEMENT

To the extent permitted under the Constitution and laws of the State of Texas, and in consideration of the Texas Comptroller of Public Accounts ("Comptroller") entering into an Interagency Cooperation Contract ("Contract") with the Texas A&M Agrilife Research, a member of the Texas A&M University System and an agency of the State of Texas ("Agency") regarding proposed research project and because of the sensitivity of certain information which may be provided to Agency during this project, Agency agrees that all information received from Comptroller or gathered, produced, collected or derived from or related to the project or provided to Agency as a result of the project or related services ("Confidential Information") must remain confidential subject to release only upon prior written approval of Comptroller, and more specifically Agency agrees as follows:

1. The Confidential Information may be used by Agency only to assist it in connection with the proposed project or any resulting contract with Comptroller.
2. Agency agrees to maintain the confidentiality of any and all Confidential Information related to this project or any resulting contract in the same manner that it protects the confidentiality of its own proprietary products of like kind.
3. Information collected from private land owners in connection with the project is considered Confidential Information pursuant to Section 430.454 of the Texas Government Code and is not subject to disclosure under the Texas Public Information Act.
4. The Confidential Information may not be copied, reproduced, disclosed or distributed without Comptroller's prior written approval.
5. All Confidential Information made available to Agency including copies thereof must be returned to Comptroller upon the first to occur of: (a) completion of the project or any resulting contract; or (b) request by Comptroller.
6. The foregoing does not prohibit Agency's use of information (including, but not limited to, ideas, concepts, know-how, techniques and methodologies) (a) previously known to it, (b) independently developed by it, (c) acquired by it from a third party, or (d) which is or becomes part of the public domain through no breach by respondent of this Nondisclosure Agreement ("NDA").
7. This NDA shall become effective as of the date Confidential Information is first made available to Agency and shall survive the project and any resulting contract and be a continuing requirement.
8. By its execution of this NDA, Agency agrees that the measure of damages in the event of a default may be difficult or impossible to determine and may vary with the nature of the default involved. Agency further agrees that the amounts of liquidated damages set out below represent Comptroller's best estimate, at effective date of this NDA, of actual damages which may be suffered by Comptroller as a direct result of a breach of contract and are not intended to be a penalty. Comptroller in its reasonable discretion may reduce the amount of liquidated damages set out below depending on the nature or severity of the default. In lieu of the liquidated damages and in its reasonable discretions, Comptroller may pursue monetary damages as available to Comptroller under the NDA and any applicable law. The breach of this NDA by Agency shall entitle Comptroller to immediately terminate the project or any resulting contract upon written notice to respondent for such breach. Regardless of whether Comptroller elects to terminate the project or any resulting agreement upon the breach hereof, Comptroller may require Agency to pay to Comptroller the sum of $5,000 as liquidated damages for each substantial breach involving release of Confidential Information. Comptroller does not waive any right to seek additional relief, either equitable or otherwise, concerning any breach of this NDA.

TEXAS A&M AGRILIFE RESEARCH

By: ______________________

Craig Nessler dK
Director

Date: 6/3/2014

AMENDMENT NO. 3
TO STATE OF TEXAS
INTERAGENCY COOPERATION CONTRACT
FOR ENDANGERED SPECIES RESEARCH PROJECTS ON FRESHWATER MUSSELS

This Amendment No. 3 ("Amendment") to the Interagency Cooperation Contract is entered into by and between the Texas Comptroller of Public Accounts ("Comptroller"), and Texas A&M AgriLife Research, a member of the Texas A&M University System and an agency of the State of Texas with principal offices located at 400 Harvey Mitchell Pkwy., Suite 300, College Station, Texas 77845-4375 ("Agency").

I. Recitals

WHEREAS, on June 4, 2014, Comptroller and Agency entered into the Interagency Cooperation Contract, as amended by Amendments No. 1 dated February 13, 2015, and as further amended by Amendment No. 2 dated September 18, 2015 (the "Contract");

WHEREAS, Comptroller and Agency desire to further amend the Contract to add a new task to the Statement of Services to be Performed and to create a standalone budget for such task; and

WHEREAS, capitalized terms used herein and not otherwise defined herein shall have the meaning set forth in the Contract unless modified herein.

NOW, THEREFORE, in consideration of mutual covenants and agreements herein contained, Comptroller and Agency hereby agree to the following amendment to this Contract:

II. Amendments

1. Attachment A, Statement of Services To Be Performed, is hereby amended by adding a new Task 7 as follows:

   "7. Conduct additional comprehensive surveys in the Devils River and Pecos River and evaluate the conservation status of freshwater mussels in Region three (3). Conduct timed search surveys at a subset of locations in the Pecos River, between Barstow, Texas and the confluence with the Rio Grande, and in the Devils River. Collect data to determine the species' redundancy and resiliency throughout the Rio Grande drainage. Genetic samples for freshwater mussels in Region three (3) will be collected from the Pecos River and Devils River populations and stored for potential future analysis to determine species representation. Submit preliminary findings to Comptroller and U.S. Fish and Wildlife Service on a weekly basis. Submit final supplemental reports on the Texas Hornshell to Comptroller and U.S. Fish and Wildlife Service by the deadlines specified below in Deliverables and Milestones for this Contract."

2. The Contract is hereby amended by adding Attachment B-1 creating a standalone budget and milestone report for Task 7. Attachment B-1 is attached hereto as Exhibit 1.

3. The first sentence of Section 3, Payments, is deleted in its entirety and replaced with the following language:

   "Total payments to Agency under this Contract shall not exceed **SIX HUNDRED SEVENTY-THREE THOUSAND SIX HUNDRED TWENTY-EIGHT AND NO/100 DOLLARS ($673,628.00)**, including all costs and expenses of every kind."

III. Conditions

1. Except as provided in this Amendment, execution and delivery of this Amendment shall not amend, modify, or supplement any provision of, or constitute a consent to, or waiver of, any noncompliance with the provisions of the original Contract and except as specifically provided in this Amendment, the Contract shall remain in full force and effect.

2. This Amendment shall become effective as of the date of the signature of Comptroller, after having first been signed by Agency.

IN WITNESS WHEREOF, the parties have executed this Amendment in duplicate originals, each of which shall constitute only one instrument.

TEXAS COMPTROLLER OF PUBLIC ACCOUNTS

By: ______________________
Mike Reissig
Deputy Comptroller

Date: 4-27-16

TEXAS A&M AGRILIFE RESEARCH

By: Michele Lacey
Michele Lacey
Director, Contracts and Grants

Date: 4/22/16

CMD #15-5283-2RR

Exhibit 1 to Amendment No. 3

ATTACHMENT B-1

BUDGET: TASK 7

The minimum deliverables for Task 7 are summarized in the following chart:

	Deliverables and Milestones	Schedule
1.	Submit preliminary findings on comprehensive Devils River and Pecos River surveys to Comptroller and U.S. Fish and Wildlife Service	Weekly
2.	Complete all work on the Pecos River survey and analysis. Submit final supplemental report* to Comptroller and U.S. Fish and Wildlife Service on Texas Hornshell	June 1, 2016
3.	Complete all work on the Devils River survey and analysis. Submit final supplemental report* to Comptroller and U.S. Fish and Wildlife Service on Texas Hornshell	July 1, 2016

*Final supplemental report includes:

1. GIS analysis of suitable habitats
2. Mapped location and population levels for all species found
3. Detailed species status assessments
4. Detailed habitat assessment
5. Comments on species management

TASK 7 BUDGET

DESCRIPTION	BUDGET
Salaries	$23,849
Benefits	$6,151
Travel	$25,000
Supplies	$2,000
Sub Award	$10,000
Total Direct Costs	$67,000
Total Indirect Costs	$6,700
Total Task Costs	$73,700

Only expenditures incurred as a direct result of work performed under Task 7 may be billed to the above listed budget for Task 7. There shall be no comingling of funds between the Task 7 Budget and the original Budget.