



Native freshwater mussel distribution in tributaries of the Colorado River downstream of Longhorn Dam near Austin, Texas, USA

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Abstract

Native freshwater mussels are sentinel species of ecological integrity and are facing significant declines due to anthropogenic factors such as habitat loss/degradation, flow management/attenuation, pollution, and invasive species. In Texas, 18 of the 52 recognized taxa are currently listed as threatened or endangered at the state or federal level. To understand the condition of freshwater mussel populations in the rapidly urbanizing area of Austin, we conducted surveys to characterize unionid species presence, distribution, density, and diversity in tributaries of the Colorado River downstream of the Longhorn Dam. This study documented the presence of 13 species of unionids in 30 of the 89 sites surveyed. The majority (92%) of which belonged to only five species: *Lampsilis teres*, *Pyganodon grandis*, *Toxolasma texasiense*, *Uniomerus tetralasmus*, and *Utterbackia imbecillis*. Less represented species with live specimens included *Potamilus fragilis*, *Quadrula quadrula*, and *Toxolasma parvum*. Of particular interest, one live Texas Fatmucket (*Lampsilis bracteata*), a federally endangered species, was observed and confirmed with DNA analysis. Four additional species were only observed as discrete valves: *Amblema plicata*, *Cyclonaias pustulosa*, *Cyrtonaias tampicoensis*, and *Uniomerus declivis*. Although relative densities of live individuals were highest in Decker, Dry East, and Cottonwood watersheds, diversity metrics were favorable in Wilbarger, Rinard, and Onion watersheds. We were unable to effectively model selected site environmental attributes with mussel detection, suggesting that additional attributes (e.g., nutrients, riparian cover, percent impervious cover) need to be factored into understanding local population dynamics. This study provides a foundation for the City of Austin's understanding of freshwater mussel species occurrence and distribution to inform future studies and policies relevant to the success of native freshwater mussels within Austin area waterways.

Key words: Freshwater mussels, unionid survey, Unionidae distribution, Texas Fatmucket

Introduction

Urban growth has led to changing landscapes across the United States directly altering watershed hydrology and the biological communities that depend on these systems. This ecological degradation in urban areas has become known as “urban stream syndrome” where similar responses can be seen in highly developed watersheds across the country and internationally (Walsh et al. 2005, Booth et al. 2016). Urban stream syndrome can be characterized by a flashier hydrograph, altered channel morphology (e.g., reduced complexity, increased channel width, pool depth, and scour), elevated concentrations of nutrients and contaminants, and reduced biotic richness with increased dominance of tolerant species (Walsh et al. 2005). These stressors can detrimentally affect biological communities, especially so for sensitive taxa such as benthic macroinvertebrates including native freshwater mussels (Gillis et al. 2017, Machado et al. 2014, Chiavacci et al. 2018, Cope et al. 2021). Understanding the effects of these anthropogenically altered streams on local biological communities can provide insight into the health of representative communities and their potential resiliency to environmental stressors.

Native freshwater mussels (family Unionidae) are indicators of stream health, biological integrity, and resilience and are impacted by the stress of anthropogenically induced flood, erosion, and/or water pollution (Grabarkiewicz and Davis 2008). They provide ecosystem services that improve water quality through filter feeding and removing particulates from both the water column and the interstitial spaces in sediment. Mussels stimulate food web production by redistributing nutrients from the water column to the substrate while filter-feeding and releasing nutrients from the sediment while burrowing (Strayer et al. 1994, Haag 2012). In addition, they stabilize streambeds, provide habitat for other benthic organisms, and they serve as a food source for certain mammals, birds, reptiles, and fish (Vaughn and Hakenkamp 2001, Vaughn et al. 2008, Vaughn 2018). Unionid mussel population declines, driven in part by anthropogenic stressors, are likely reducing the ecosystem services provided by these species (Strayer et al. 2004, Burlakova et al. 2011, Ford and Oliver 2015, Gillis et al. 2017).

Across Texas 18 of the approximately 52 recognized taxa are currently listed as either threatened or endangered at the state or federal level. As a result, conservation efforts for freshwater mussels have dramatically expanded among resource managers at the federal, state, and municipal levels. Water quality has been a priority for City of Austin since the environmental movement in the 1970s, manifesting itself in protective watershed protection regulations beginning in the late 1970s through the 1980s. Efforts to protect the natural environment within the jurisdictional limits of the City of Austin in central Texas have largely relied on code and criteria developed to regulate impervious cover, establish stream buffers, require stormwater controls that sequester pollutants, and reduce sediment from entering waterways. The overall goal of those rules is to protect the physical, chemical, and biological integrity of the City’s waterways.

Previous research into native freshwater mussels in the Austin region suggest the populations are experiencing numerous environmental stressors from both anthropogenic sources and climactic

variability (Perry et al. 2008, Perry et al. 2010, Duncan et al. 2011a, Riley et al. 2015, Johnston and Jackson 2017). Researchers at the City initiated the study of native freshwater mussels after observing *Pyganodon grandis* in a lagoon area of Lady Bird Lake during routine benthic macroinvertebrate monitoring (Perry et al. 2008). Subsequently, the City undertook an inventory of native freshwater mussels across 29 watersheds including the reservoirs of Lake Austin and Lady Bird Lake. Researchers collected specimens from 15 species with live specimens identified from nine species, and four species (*Lampsilis teres*, *P. grandis*, *Unio merus tetralasmus*, and *Utterbackia imbecillis*) constituting 82% of all specimens located (Perry et al. 2010, Duncan et al. 2011a). Duncan et al. (2011a) suggested a temporal shift in composition towards more tolerant, generalist species. Further research on mussels in Lake Austin's nearshore habitat indicated that a hiatus in lake drawdowns may have positively affected mussel abundance and diversity (Johnston and Jackson 2017). For example, Watershed Protection Department (WPD) biologists replicated a 2011 survey in 2017 which followed a 5-year period of no drawdowns and observed four additional mussel species, with mussels observed at all sites during the latter survey (Johnston and Jackson 2017).

Downstream of Lake Austin and Lady Bird Lake, investigations in the mainstem of the Colorado River below the Longhorn Dam (~29 km searched in total) yielded no indications of large mussel beds in the sections surveyed (Duncan et al. 2011b). Those results should be taken with the context that surveys were in search of large mussel beds, performed rapidly without the aid of underwater breathing apparatus (i.e., SCUBA), and covered a generous geographic area. The study results suggest that large mussel beds may not be present in shallow waters of the Colorado River despite a lack of major impoundments downstream reducing barriers to host fish. An absence of large mussel beds observed in this section of the Colorado River may also play a role in limiting mussel colonization or sustaining population in tributaries connected to this section of the river.

Although previous observations and in-situ studies have expanded our understanding of mussels in the Austin area, a comprehensive census of species occurrences and status, including the locations of potentially endangered species, had yet to be conducted. Additionally, past efforts did not attempt to relate site characteristics with mussel observations. This study therefore attempted to address these critical data gaps in the City's knowledge. Specifically, this study aimed to standardize search effort and collect environmental data likely to be influential to mussel presence. Our objectives therefore were to:

- (1) identify streams/watersheds where mussels were detected or not, determine species richness (i.e., number of detected species), catch per unit effort (CPUE), density, diversity indices, and size distribution of mussels detected (as a means to approximate potential recruitment of mussels into the population); and,
- (2) to identify potential relationships between environmental data and detected mussels. Study sites were distributed in major creeks downstream of the Edwards Aquifer recharge zone that are hydrologically connected to the Colorado River downstream of Longhorn Dam. We used this information to better understand spatial variations in mussel populations across the target area and to identify environmental variables correlated with mussel detection.

From this information, we aim to begin making inferences about the environmental factors that are either supporting or constraining mussel populations in the Austin area. This analysis will

help guide future research by identifying key environmental attributes that should be evaluated or possibly included in subsequent studies. Additionally, it will establish a baseline for understanding and contextualizing long-term population trends and variations, providing a foundation for monitoring changes over time and informing conservation strategies.

Methods

Survey Area and Site Selection

We selected study sites based on an absence of dams (that is, no physical barrier to host fish dispersion), drainage area size, underlying geology and urbanization gradients, and previously observed evidence of native freshwater mussels (Figure 1). Dams can act as barriers to movement of fish that the obligate ectoparasitic native freshwater mussel larvae rely upon for successful reproduction (Kat 1984, Barnhart et al. 2008, Pelicice et al. 2015, Marshall et al. 2018). As such, all sites were within tributaries with confluences to the Colorado River downstream of the Longhorn Dam which is the last significant inline dam of the Colorado River. We targeted major creeks (defined as having a drainage area ≥ 640 acres by City of Austin Land Development Code §25-8-91(B)(3)) for multiple purposes. Firstly, studies in the United States have documented an increase in species richness with increasing drainage area (Daniel and Brown 2013, Haag 2012). Given our goal of identifying species occupying the survey area, major creeks may provide a more comprehensive composition of mussel species diversity. Next, focusing on major creeks removes smaller watersheds located in the highly urbanized core of Austin and that tend to drain into the Colorado River above the Longhorn Dam. Additionally, mussels are known to generally reside within stream reaches with perennial flow and sufficient connectivity to major water bodies to allow movement of host fishes (Daniel and Brown 2014, Haag 2012), conditions which tend to be rarer in the western (i.e., Edwards Plateau) watersheds. And lastly, previous benthic macroinvertebrate monitoring programs at the city (Clamann et al. 2019) have found evidence of freshwater mussels in almost all targeted watersheds.

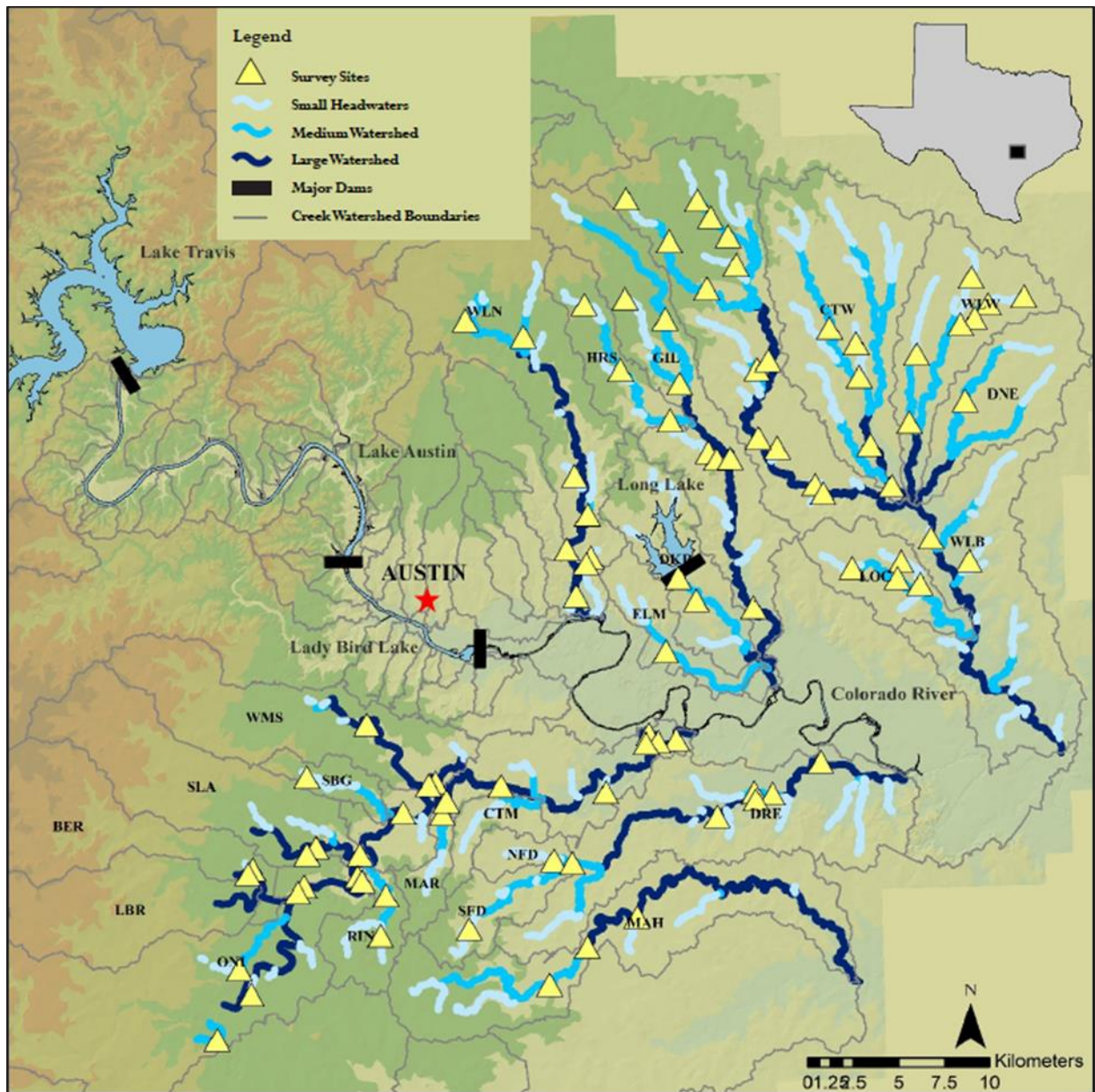


Figure 1 Survey area and potential survey sites. Creeks selected for this study are tributaries to the Colorado River downstream of Longhorn Dam with a drainage area >640 acres. The western extent of five of the southern watersheds (WMS, SLA, BER, LBR, ONI) were omitted from the survey due to lack of appropriate aquatic habitat in losing streams located over the Edwards Aquifer Recharge Zone.

With these qualifications, we selected 21 watersheds which included: Bear (BER), Cottonwood (CTW), Decker (DKR), Dry Creek East (DRE), Dry Creek Northeast (DNE), Elm (ELM), Gilleland (GIL), Harris Brach (HRS), Lockwood (LOC), Maha (MAH), Marble (MAR), North Fork Dry (NFD), Onion (ONI), Rinard (RIN), Slaughter (SLA), South Boggy (SBG), South Fork Dry (SFD), Walnut (WLN), Wilbarger (WLB), Williamson (WMS), and Willow (WLW) (Figure 1). Previous investigations have detected mussels (live or dead) in all targeted watersheds except for Bear, Walnut, and Williamson.

Due to variation in catchment size (640 to 220,734 acres) across the study area we conducted stratified random sampling to represent ecological variability and give equal representation of stream size classes within watersheds. First, a GIS point database was created to represent all reaches along creek mainstems and tributaries in targeted watersheds. This resulted in 230,509 creek-points with drainage areas that ranged from 640 to 220,734 acres. The creek-points were sorted into three groups according to the size of their respective catchment such that one third of the points were placed into each size class as follows: headwaters (640 – 2,346 acres), mid reaches (2,346 – 15,295 acres), and lower reaches (15,295 – 220,734 acres). From the creek-points, forty were selected randomly within each of the three size classes resulting in 120 potential sampling locations.

Field Surveys

The target number of completed surveys for this study was 90 sites from the list of 120. Sites spanned a maximum length of approximately 300 feet upstream and 300 feet downstream of the GIS-based sample point location. Habitat was considered suitable for mussels when inundated refugia and penetrable substrate were present in the form of bedrock with pockets of sediment, rooted macrophytes, tree roots, large woody debris, and/or other substrates such as cobble, gravel, sand, silt, or clay. Habitat was not considered suitable for mussels if inundated refugia were not present or if there was a lack of penetrable substrate such as only bedrock, boulder, or packed clay present. When a site could not be sampled due to lack of access or suitable habitat, sites were moved to the nearest suitable habitat within one mile upstream or downstream of the original site. In instances where there were no suitable options available within one mile of the randomly selected site, the location was skipped.

Environmental parameters characterizing the site and subsites were collected before searching for freshwater mussels. Sites were divided into subsites based on mesohabitat delineations (Table 1). Parameters collected for a site included conductivity, dissolved oxygen, flow severity, pH, site length, site width, and water temperature (Table 2). Flow severity code is a qualitative description (e.g., base, flood) of flow based on expected conditions at a given site (TCEQ 2007). Water quality parameters were collected using a Hydrolab® multi-sensor sonde. Parameters that were collected for each subsite include dominant substrate type (1st, 2nd, 3rd rank), whether macrophyte cover was present, mesohabitat type, search area length, search area width, time spent searching, the condition of any mussels found, number of individuals, length, and species (Table 2).

Table 1 Mesohabitat descriptions.

Riffle ^{1,2}	Shallow reach with turbulent water, moderate to quick current velocity
Run ^{1,2}	Contained between thalweg and stream edge in straight reaches of shallow to moderate depth, and moderate current velocity
Pool ^{1,2}	Deep water, slow current velocity
Backwater ^{1,2}	Shallow to deep water, very slow to nonexistent current velocity, typically stray away from mainstream channel and change the shape of the river, are a result of eddies formed downstream of obstructions
¹ adapted from Parasiewicz 2007; ² adapted from Bisson and Montgomery 1996.	

Table 2 Parameters recorded in this study.

Parameter Type	Parameter	Collected
Solids/ Conductivity	Conductivity*	Site
Oxygen	Dissolved Oxygen*	Site
Habitat Evaluation	Dominant Substrate type	Subsite
Habitat Evaluation	Dominant Substrate type 2nd Rank	Subsite
Habitat Evaluation	Dominant Substrate type 3rd Rank	Subsite
Sample Collection Methods	Field Instrument WRM ID	Site
Surface Water	Flow Severity Code*	Site
Grain Size/Substrate Characterization	Macrophyte Cover*	Subsite
Benthic Macroinvertebrate	Mussel Fate Condition*	Subsite
Habitat Evaluation	Mussel Mesohabitat Type*	Subsite
pH	pH	Site
Sample Descriptors	Sampling Method Code	Site
Channel Assessment	Search Area Length	Subsite
Channel Assessment	Search Area Width	Subsite
Channel Assessment	Site Length*	Site
Channel Assessment	Site Width	Site
Temporal	Total Time Spent*	Subsite
Conventional	Water Temperature*	Site
Bivalve	Species	Subsite
Valve length	Length	Subsite
Detected mussels	count	Subsite

* Denotes parameters tested as predictors of mussel presence in GLM analysis.

We carried out timed searches for mussels which consisted of up to 4.5 person-hours at each site (Metcalf-Smith et al. 2000, Burlakova et al. 2011). We used visual search methods (e.g., unassisted view, snorkeling) when water clarity was sufficient to see substrate and semi-burrowed mussels. Tactile search methods were used when water was too turbid to see the substrate without the aid of a mask. During timed searches, we made an effort to search all available mesohabitats for mussels. We collected mussels into separate dive bags for each mesohabitat which remained submerged until mussel processing. When the maximum search area was surveyed prior to the end of the sampling period, either the survey was ceased or the area was searched again until time ran out, depending on the complexity of the habitat. For example, bedrock pools with little substrate are more easily searched to exhaustion than a pool with deep sediment. Sampling was delayed for three days following rain events to increase water clarity.

Any mussels found were visually identified to species, enumerated, and measured for valve length on the anterior to posterior axis. Nomenclature in this study follows Williams et al. (2017). Mussel lengths were measured to assess community structure and recruitment at sites. Juvenile mussels were assumed to be any individual less than 40 mm in length apart from Lilliputs (*Toxolasma* spp.) which may only grow to be 50 mm as adults although data for length

at maturity was not found (Haag and Rypel 2011, Haag 2012). The shell condition of all individuals found were recorded according to the Texas Commission on Environmental Quality Surface Water Quality Monitoring Procedures Volume 2, Chapter 5 (TCEQ 2007). This study focused on locating and identifying live individuals; however, relic shell data were also recorded. Following identification and enumeration, all live mussels were returned to the same mesohabitat from where they were collected. DNA was collected from the single live *Lampsilis bracteata* observed to verify identification, and the sample was processed by the Texas A&M Natural Resources Institute. These survey methods comply with Texas Parks and Wildlife Department Scientific Research Permit No. SPR-0113-006. Recently dead mussel shells were collected for the City's reference collection.

Statistical Analyses

Metric Calculation

We grouped mussels into the categories and counted the number of representatives as live (LV), very recently dead (VRD), recently dead (RD), relatively recently dead (RRD), long dead (LD), and very long dead (VLD) (TCEQ 2007). However, we only used the LV and VRD mussels to calculate catch per unit effort (CPUE; # person-hr⁻¹), density (# ft⁻²), species richness, diversity, evenness, and dominance indices were assessed. All metrics were calculated on a watershed basis due to low numbers of individuals across sites. Separation of LV/VRD vs. long dead was meant to distinguish between occupation at time of survey from historic occupation.

Indices of diversity, evenness, and dominance were estimated using the Brillouin Diversity Index (HB), Brillouin Evenness Index (E), and Berger-Parker Dominance Index (d) to provide a numerical means of comparing mussel community structure among drainages.

Brillouin's diversity index (HB) measures the diversity of a collection, and this index can be used when data are not random, and species differ in their capture rates (Bollarapu and Ramarao 2021). Brillouin's evenness index (E) is sensitive to the abundance of rare species and can be used when the randomness of a sample cannot be guaranteed (Brillouin 1956). Berger-Parker's dominance index (d) is independent of species richness and can therefore be used when the community is dominated by a few species (high evenness).

Population Recruitment Evidence

Evidence of recruitment was investigated by determining whether juvenile individuals (less than 40 mm in length) were located for a given species across watersheds. We considered sites within the same watershed as interconnected for the purpose of population exchange and therefore aggregated count data within watersheds for each species and to offset low detection sample sizes at some sites. Only data from live and very recently dead individuals were used to represent population structure during the time of survey. However, we did not assess recruitment potential for *Toxolasma parvum* and *T. texasiense* due to their small size at maturity (approximately 40 mm and 50 mm, respectively), which, in the absence of much smaller individuals, can lead to erroneous assumptions about recruitment.

Predictors of mussel detection/ non detection at sites

The second objective of this study was to understand the effect of environmental variables on detection of live and/or VRD mussels. Due to potential differences in response to environmental variables between species, we assessed predictors of mussel detection both on a species-by-species basis and on data aggregated without regard to species. For both of these variables, we aggregated mussel detection data at the site level by summing the number of individuals found at all subsites within a site and grouped by species when required.

The following parameters were explored as predictors of mussel detection: conductivity, dissolved oxygen, water temperature, site length, flow presence or absence, macrophyte presence, riffle/run presence, and total time spent searching. We did not use dominant substrate type in this analysis due to the qualitative nature of this parameter and being unable to find a reliable way to convert information from the subsite to the site level. Data on parameters that were collected at the subsite (mesohabitat) level were adjusted to convey information at the site level for the following parameters: macrophyte cover, time spent searching, and mussel mesohabitat type.

We conducted data exploration of environmental parameters in R (R Core Team 2020) prior to running any models (Zuur et al. 2010). Continuous variables (i.e., conductivity, water temperature, pH, dissolved oxygen, site length, total time spent searching) were visualized using the function `ggpairs` in the `GGally` package. Categorical variables (flow, macrophyte presence, riffle/run presence) were visualized using the function `mosaic` in the `vcd` package. We visually assessed for outliers in the continuous data and examined the effect of outlier removal on model performance with outliers removed prior to running models. Any parameters that were significantly correlated ($\alpha < 0.05$) and strongly related ($r^2 > 0.5$) were not used as covariates within the same model.

After covariate selection, we assessed relationships between environmental variables and all mussel species, and species specific responses with generalized linear models (GLMs) using a binomial distribution. Final reported models included all selected environmental variables as predictors of mussel detection for a given species and significant variables were determined at an $\alpha < 0.05$.

Results

Field Survey Summary

Our field efforts resulted in completion of 89 site visits out of the 120 randomly generated potential sites. Twenty-three of the 89 sites surveyed did not contain inundated refugia and therefore environmental parameters and mussel data were not collected as the sites were determined not suitable for mussels. Of the 66 sites with inundated refugia, we used over 200 person hours to search over 400,00 ft² of mesohabitat with an average of 107 person-minutes (SE ± 7.84) per mesohabitat. Site reaches ranged from 10 ft to 479 ft in length (mean = 100 ft) and between 2 ft to 250 ft width (mean = 21 ft). We sampled a total of 142 discrete mesohabitats with

runs being the most prevalent mesohabitat surveyed (64%; Figure 2). Only 25% of the mesohabitats had macrophyte cover present.

Average water temperatures across sites were generally warm (> 25°C), with moderate DO (i.e., 6 mg/L), basic pH, and elevated specific conductivity (i.e., nearly 1,000 µS/cm) (Table 3).

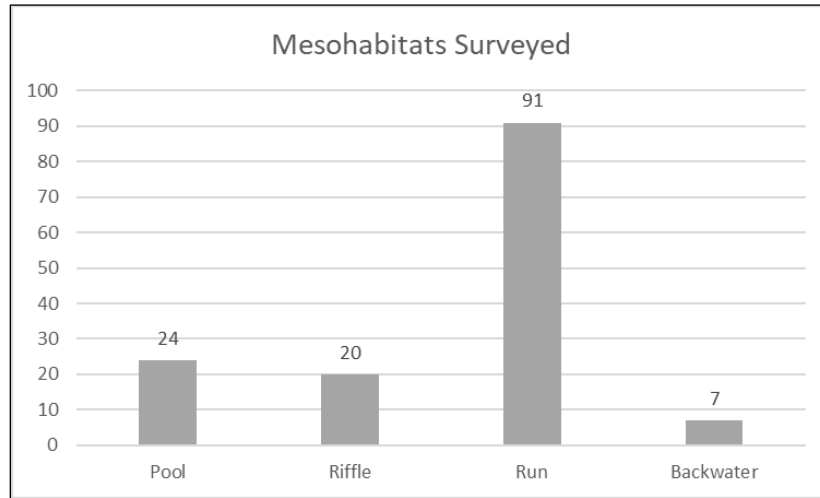


Figure 2 Mesohabitats surveyed ($n = 142$) across all sites sampled ($n = 66$).

Table 3 Physicochemical parameters across all sites sampled ($n = 66$).

Parameter	Range	Average	Std. dev.
Water Temperature (°C)	18.2 – 32.9	26.1	3.2
Dissolved Oxygen (mg/L)	0.5 – 14.3	6.0	2.4
pH (standard units)	7.1 – 8.9	7.6	0.3
Specific Conductivity (µS/cm)	233.7 – 9,975.0	949.7	1443.0

Mussel Observation Summary

Descriptive Statistics

We documented 535 individual mussels during this study: 262 LV, 4 VRD, 247 RD, 32 RRD, 9 LD, and 5 VLD (Table 4). Evidence of mussel presence (all shell conditions) was documented at 44 of the 89 sites (49%) representing 17 of the 21 surveyed watersheds. However, LV and VRD individuals were only documented at 30 sites (34%) from 13 watersheds (Figure 3; Table 4).

We documented thirteen species of Unionid mussels including *Amblema plicata*, *Cyclonaias pustulosa*, *Cyrtanaias tampicoensis*, *Lampsilis bracteata*, *Lampsilis teres*, *Potamilus fragilis*, *Pyganodon grandis*, *Quadrula quadrula*, *Toxolasma parvum*, *Toxolasma texasiense*, *Unio merus declivis*, *Unio merus tetralasmus*, and *Utterbackia imbecillis*. However, most mussels found in this study (92%) belonged to only five species: *L. teres*, *P. grandis*, *T. texasiense*, *U. tetralasmus*, and *U. imbecillis*. Four species were only represented by relic shells: *A. plicata*, *C. pustulosa*, *C. tampicoensis*, and *U. declivis*. The greatest count of live mussels was detected in the Wilbarger Creek watershed ($n = 74$), followed by Cottonwood Creek ($n = 51$), and Decker

Creek watersheds (n = 46; Table 4). Of note, a live *Lampsilis bracteata* (Texas Fatmucket), a State threatened and Federally endangered species under the U.S. Fish and Wildlife Service, was located on Onion Creek (Figure 4).

Table 4 Counts of all species located in this study by watershed and shell condition.

Watershed, Species	Live	Very Recently Dead	Recently Dead	Grand Total
Cottonwood Creek	51	0	49	100
LAMPSILIS TERES	0	0	2	2
TOXOLASMA TEXASIENSE	37	0	32	69
UNIOMERUS TETRALASMUS	13	0	14	27
UTTERBACKIA IMBECILLIS	2	0	0	2
Decker Creek	46	0	15	61
LAMPSILIS TERES	35	0	12	47
PYGANODON GRANDIS	11	0	3	14
Dry Creek East	24	0	5	29
UNIOMERUS TETRALASMUS	4	0	4	8
UTTERBACKIA IMBECILLIS	20	0	1	21
Dry Creek NE	0	0	1	1
TOXOLASMA TEXASIENSE	0	0	1	1
Elm Creek	0	1	3	4
UNIOMERUS TETRALASMUS	0	1	3	4
Gilleland Creek	4	0	1	5
UNIOMERUS TETRALASMUS	3	0	1	4
UTTERBACKIA IMBECILLIS	1	0	0	1
Maha Creek	15	0	34	49
PYGANODON GRANDIS	0	0	28	28
TOXOLASMA PARVUM	0	0	1	1
TOXOLASMA TEXASIENSE	0	0	1	1
UNIOMERUS TETRALASMUS	15	0	4	19
Marble Creek	23	0	0	23
UTTERBACKIA IMBECILLIS	23	0	0	23
North Fork Dry Creek	4	0	6	10
UNIOMERUS TETRALASMUS	4	0	6	10
Onion Creek	10	1	1	12
LAMPSILIS BRACTEATA (TEXAS FATMUCKET)	1	0	0	1
LAMPSILIS TERES	1	0	0	1
LEPTODEA FRAGILIS	1	0	0	1
PYGANODON GRANDIS	1	0	1	2
UTTERBACKIA IMBECILLIS	6	1	0	7
Rinard Creek	2	0	0	2
UNIOMERUS TETRALASMUS	1	0	0	1
UTTERBACKIA IMBECILLIS	1	0	0	1
Slaughter Creek	3	0	7	10
TOXOLASMA TEXASIENSE		0	1	1
UTTERBACKIA IMBECILLIS	3	0	6	9
Wilbarger Creek	72	2	107	181
LAMPSILIS TERES	3	0	2	5
PYGANODON GRANDIS	1	0	0	1
QUADRULA APICULATA	4	0	2	6
TOXOLASMA PARVUM	3	0	3	6
TOXOLASMA TEXASIENSE	27	1	66	94
UNIOMERUS TETRALASMUS	29	1	31	61
UTTERBACKIA IMBECILLIS	5	0	3	8
Willow Creek	4	0	14	18
TOXOLASMA PARVUM	0	0	8	8
TOXOLASMA TEXASIENSE	1	0	4	5
UNIOMERUS TETRALASMUS	3	0	2	5
Grand Total	259	4	242	505

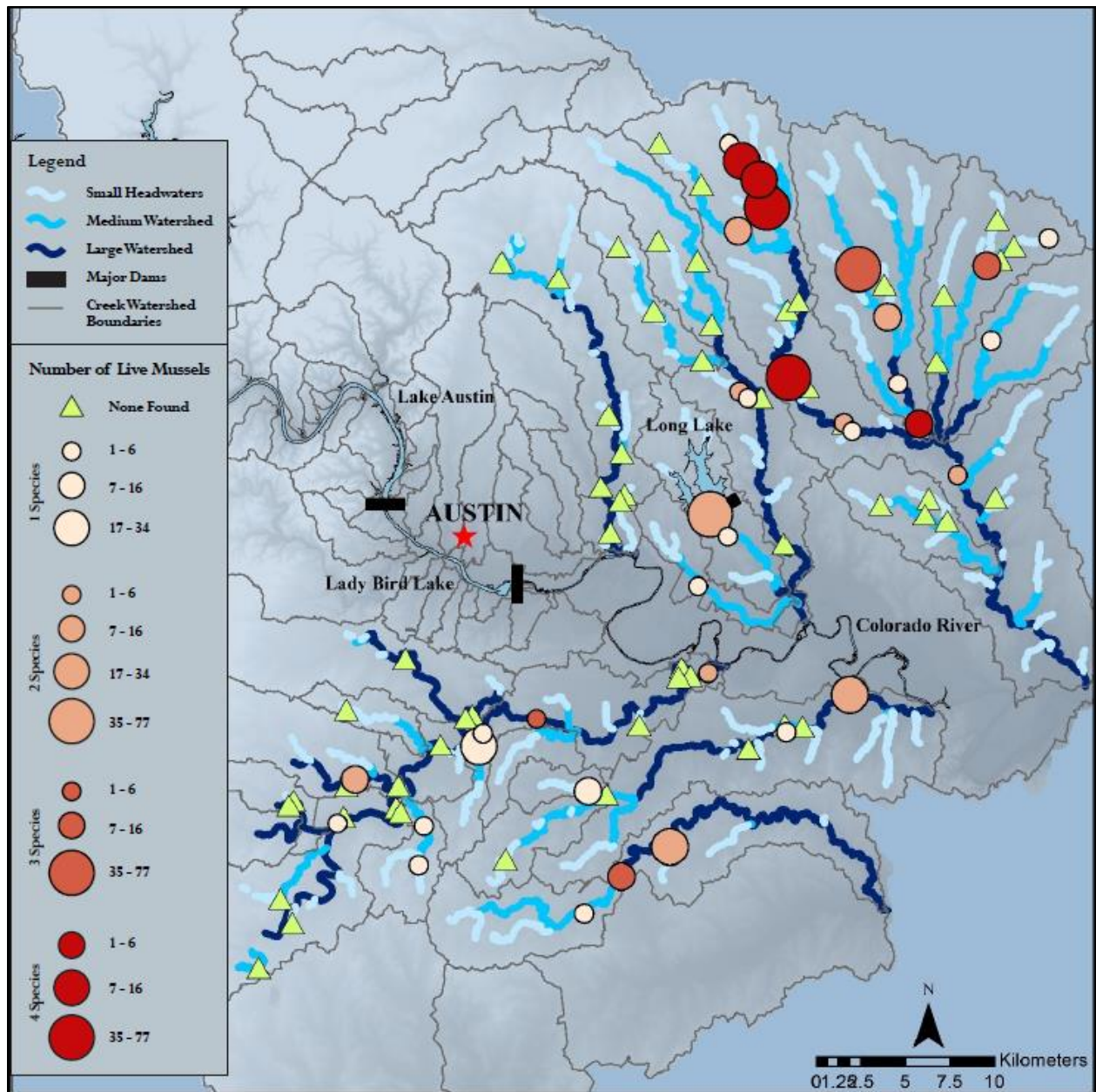


Figure 3 Observations of live and very recently dead mussels (circles) and relative abundances (circle size). Absence of live or very recently dead mussels indicated by triangle.



Figure 4 *Lampsilis bracteata* is a federally endangered and state threatened species. This female specimen was found in lower Onion creek and confirmed with DNA swab analysis before being released back into the wild.

Population Metrics

Watersheds with the highest species richness included Wilbarger Creek (n = 7) and Onion Creek (n = 5) (Figure 3; Table 4). Mussel CPUE was highest in the Marble Creek and Decker Creek watersheds with approximately 5 mussels located per person hour of searching (Figure 5), and densities were also highest in the Decker Creek watershed followed by Cottonwood and Dry Creek East (Figure 6).

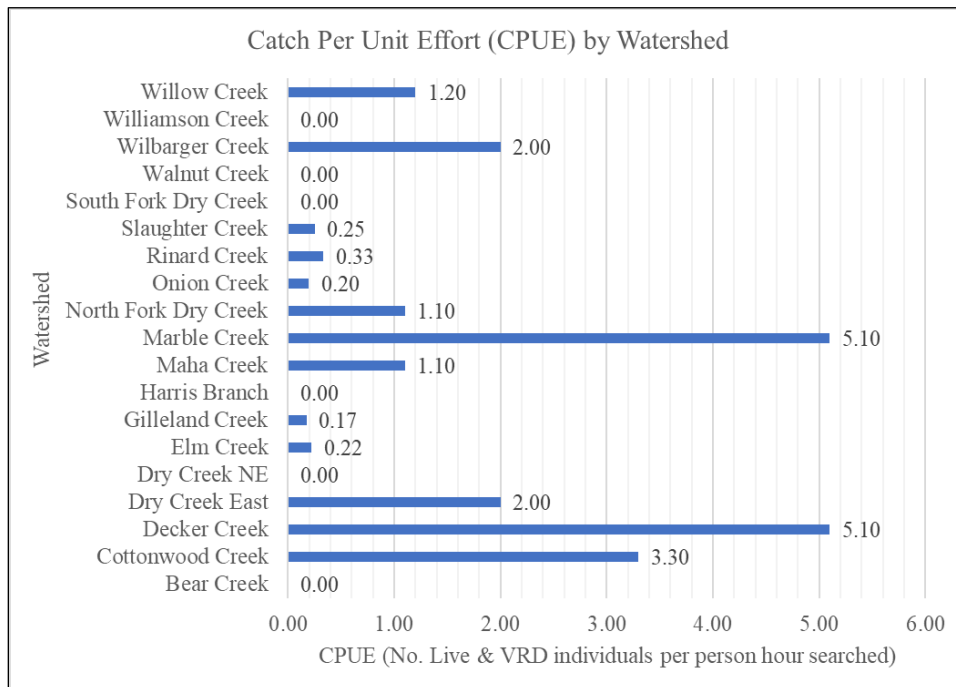


Figure 5 Catch per unit effort (CPUE) of live and VRD individuals by watershed.

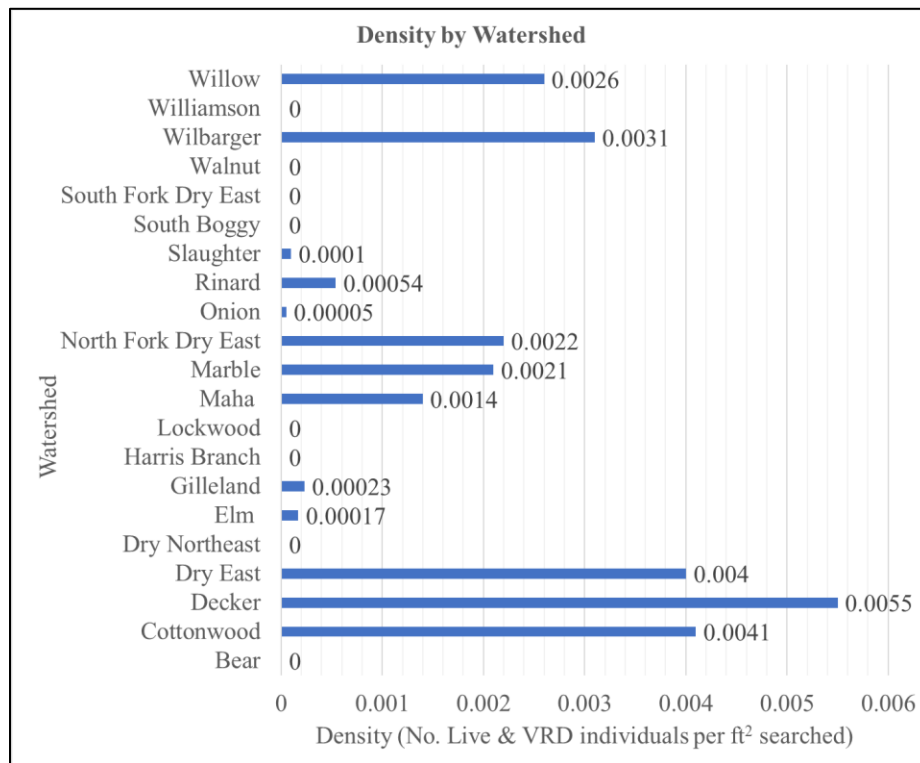


Figure 6 Mussel density of live and VRD individuals by watershed.

The Brillouin Diversity index (HB) was highest in Wilbarger Creek watershed at 1.25, whereas the Brillouin Evenness Index (E) was highest in Rinard Creek watershed at 1.00 (Table 7). The dominance index (d) was lowest in Wilbarger Creek watershed at 0.43. Watersheds with no metric calculations was due to no LV or VRD individuals located during time of survey and included Bear, Dry Northeast, Harris Branch, Lockwood, South Boggy, South Fork Dry East, Walnut, and Williamson (Table 7).

Table 7 Brillouin Diversity (HB) and Evenness Index (E), and Berger-Parker Dominance (d) of live and very recently dead individuals by watershed.

Watershed	Sites Surveyed	Total Individuals	No. Species	Diversity (HB)	Evenness (E)	Dominance (d)
Bear Creek	2	0	0	NA	NA	NA
Cottonwood Creek	4	52	3	0.165	0.15	0.73
Decker Creek	2	46	2	0.167	0.241	0.76
Dry Creek East	3	24	2	0.139	0.2	0.83
Dry Creek Northeast	1	1	0	NA	NA	NA
Elm Creek	1	0	1	0.00	NA	1.00
Gilleland Creek	6	4	2	0.188	0.271	0.75
Harris Branch	3	0	0	NA	NA	NA
Maha Creek	3	15	1	0.00	NA	1.00
Marble Creek	1	23	1	0.00	NA	1.00
North Fork Dry Creek	1	4	1	0.00	NA	1.00
Onion Creek	13	11	5	0.106	0.066	0.636
Rinard Creek	2	2	2	0.125	0.180	0.50
Slaughter Creek	3	3	1	0.00	0.00	1.00
South Fork Dry Creek	1	0	0	NA	NA	NA
Walnut Creek	5	0	0	NA	NA	NA
Wilbarger Creek	10	74	7	0.289	0.148	0.405
Williamson Creek	2	0	0	NA	NA	NA
Willow Creek	3	4	2	0.186	0.270	0.75
	66	263				

Recruitment Evidence

Based on the shell lengths of seven of the LV and VRD species, *Lampsilis teres*, *Pyganodon grandis*, *Quadrula quadrula*, *Utterbackia imbecillis*, *Toxolasma parvum*, *Toxolasma texasiense*, and *Unio merus tetralasmus*, we posit that *P. fragilis*, *Unio merus tetralasmus*, and *Utterbackia imbecillis* have active recruitment (Table 8). However, all individual *T. parvum* and a majority of *T. texasiense* were below 40 mm, which may be a consequence of naturally short adults with *T. parvum* reaching approximately 40mm in length (Williams et al. 2014). *T. texasiense* has been described as larger in length than *T. parvum* (Randklev et al. 2023), but maximum length could not be determined from the literature.

Table 8 Estimates of recruitment as Yes (Y; <40 mm) or No (N; >40 mm) for all live and very recently dead individuals collected within a watershed. Numbers in parenthesis refer to the number of individuals below 40mm in length out of the total number of live and very recently dead individuals detected. *Toxolasma* spp. are not included due to their small size at maturity.

Watershed	<i>Lampsilis bracteata</i>	<i>Lampsilis teres</i>	<i>Potamilus fragilis</i>	<i>Pyganodon grandis</i>	<i>Quadrula apiculata</i>	<i>Unio merus tetralasmus</i>	<i>Utterbackia imbecillis</i>
Cottonwood Creek	---	---	---	---	---	Y (5/13)	N (0/1)
Decker Creek	---	N (0/35)	---	N (0/11)	---	---	---
Dry Creek East	---	---	---	---	---	Y (1/4)	Y (7/20)
Elm Creek	---	---	---	---	---	Y (1/1)	---
Gilleland Creek	---	---	---	---	---	N (0/3)	N (0/1)
Maha Creek	---	---	---	---	---	N (0/15)	---
Marble Creek	---	---	---	---	---	---	N (0/23)
North Fork Dry Creek	---	---	---	---	---	N (0/4)	---
Onion Creek	N (0/1)	N (0/1)	Y (1/1)	N (0/1)	---	---	N (0/7)
Rinard Creek	---	---	---	---	---	N (0/1)	N (0/1)
Slaughter Creek	---	---	---	---	---	---	N (0/3)
Wilbarger Creek	---	---	---	N (0/1)	N (0/4)	Y (1/30)	Y (4/5)
Willow Creek	---	---	---	---	---	Y (2/3)	---

Predictors of mussel detection/ non detection at sites

When we modeled the influence of site-level environmental attributes on the likelihood of detection of mussel of any species, we found no significant predictors (Table 9). However, when we modeled site variables associated with detection of a single species, we identified that *Toxolasma texasiense* was negatively correlated to DO (i.e., more likely to be found at sites with lower oxygen concentrations) and *Unio merus tetralasmus* was positively correlated with conductivity (Table 10).

Table 9 Generalized linear model results of the influence of site-level environmental attributes associated with the detection of mussels (all species combined).

Environmental Parameter	Estimate	Std. error	P-value
Intercept	-0.85	2.7	0.75
Conductivity	0.00093	0.0010	0.36
Water temperature	0.040	0.083	0.63
Dissolved oxygen	-0.11	0.13	0.43
Site length	-0.0041	0.0029	0.16
Flow	-0.56	0.84	0.51
Total time spent	0.0063	0.0057	0.27
Macrophyte presence	0.43	0.59	0.46
Riffle/run presence	-0.79	1.1	0.46

Table 10 Generalized linear model analysis results of the influence of site-level environmental attributes associated with the detection of live and very recently dead individuals by species. *n* denotes the number of sites a species was detected. Significant predictors ($P < 0.05$) are in bold.

Species	<i>n</i>	α	$\beta_{\text{Conductivity}}$	$\beta_{\text{Water temp}}$	$\beta_{\text{Dissolved oxygen}}$	$\beta_{\text{Site length}}$	$\beta_{\text{Total time spent}}$	$B_{\text{Mac presence}}$	$\beta_{\text{Riffle/run presence}}$	β_{Flow}
<i>Lampsilis teres</i>	7	-5.8 (4.5)	-0.000038 (0.0013)	0.032 (0.11)	0.18 (0.20)	-0.0024 (0.0039)	0.011 (0.012)	-0.49 (0.93)	-0.32 (1.3)	NA
<i>Pyganodon grandis</i>	6	-18 (9.7)	-0.00034 (0.0012)	0.65 (0.34)	-0.051 (0.29)	-0.01 (0.0054)	NA	0.35 (1.2)	-2.3 (1.4)	3.2 (1.6)
<i>Quadrula quadrula</i>	4	-4.6 (3.7)	0.0018 (0.0012)	-0.10 (0.13)	0.0061 (0.22)	0.011 (0.0063)	NA	0.42 (1.2)	NA	NA
<i>Toxolasma texasiense</i>	13	1.5 (3.3)	0.000096 (0.00079)	-0.024 (0.11)	-0.48 (0.20)	-0.0081 (0.0043)	0.0012 (0.0071)	0.13 (0.75)	0.16 (1.6)	1.8 (1.2)
<i>Toxolasma parvum</i>	5	9.9 (6.3)	-0.00095 (0.0013)	-0.28 (0.19)	-0.51 (0.35)	-0.013 (0.0086)	0.010 (0.011)	NA	-4.2 (2.3)	1.4 (2.2)
<i>Unio merus tetralasmus</i>	27	1.5 (2.5)	0.0024 (0.0012)	-0.11 (0.079)	0.0083 (0.12)	-0.0017 (0.0027)	-0.0019 (0.0049)	0.20 (0.60)	0.24 (1.1)	-0.25 (0.86)
<i>Utterbackia imbecillis</i>	13	-1.4 (4.0)	0.000048 (0.00084)	0.0093 (0.13)	-0.30 (0.18)	-0.0030 (0.0042)	0.016 (0.0095)	-0.38 (0.84)	-0.64 (1.2)	-1.7 (0.91)

Discussion

There are 23 species of native freshwater mussels known to the Colorado River Basin, four of which (i.e., *Cyclonaias petrina*, *Fusconaia iheringi*, *Lampsilis bracteata*, *Truncilla macrodon*) have been recently listed as threatened or endangered at the federal level (Randklev et al. 2023; USFWS 2024). In this study, we observed 13 species across 17 of the 21 watersheds surveyed, and importantly we also found a live individual of one endangered species (*L. bracteata*). Multiple species in our study were represented by only a few live individuals within a watershed highlighting the vulnerability of these populations. This work helps contribute to the growing body of literature of native mussels in urbanizing areas and provides documentation of the presence of a recently listed endangered species in the Austin area. We acknowledge that the unique and complex setting (geography, climate, historic land use, urbanization) and study design makes it difficult to disentangle natural and human-induced drivers of population variation.

The finding of Texas Fatmucket (*L. bracteata*) in lower Onion Creek provides critical information on the presence of at least one endangered freshwater mussel in the Austin area. *Lampsilis bracteata* only occur in the Colorado river basin, as previously synonymous individuals found in the Guadalupe River basin have been found to be a distinct species (Inoue et al. 2019). With a yellowish green to brown shell with prominent broken rays that widen towards the margin, a detailed morphological description of the Texas Fatmucket can be found in Howells et al. (1996) and Howells (2014). This species typically occurs in upper reaches of major tributaries (Lower Elm Creek, Upper/ Middle San Saba River, Llano River, Pedernales River) but has been found in shallow slower waters such as upstream of low head dams (USFWS 2024, BIO-WEST Inc. 2018). Preferred habitat for the Texas Fatmucket includes firm mud, stable sand, and gravel bottoms in shallow waters, and sometimes in bedrock fissures or among roots of aquatic vegetation such as bald cypress (*Taxodium distichum*) (Howells 2014). Like other Unionids, the Texas Fatmucket is an obligate ectoparasite which releases larvae called

glochidia for infestation on aquatic vertebrate hosts prior to transforming into juvenile mussels that will then colonize sediment substrate (Bauer 1994, Haag and Staton 2003, Modesto et al. 2018). Members of the *Lampsilis* genus expel packets of glochidia known as conglomerates and frequently utilize visual mantle lures to attract host fish for infestation. Host species for *L. bracteata* are members of the Centrarchidae family (sunfishes) including bluegill (*Lepomis macrochirus*), green sunfish (*L. cyanellus*), Guadalupe bass (*Micropterus treculii*), and largemouth bass (*M. salmoides*) (Howells 1997, Johnson et al. 2012, Howells 2014, Ford and Oliver 2015, Bonner et al. 2018). Related species are long-term brooders (bradytic) with timing of reproduction occurring in the fall and release of glochidia occurring in the spring (Barnhart et al. 2008). Recent observations of *L. bracteata* in the Austin area include three individuals found in 2010, one individual found in 2018 (this study), and five individuals found in 2021 (USFWS 2024). With the observation of *L. bracteata* in lower Onion Creek from the confluence with the Colorado River upstream to IH-35, U.S. Fish & Wildlife Service has designated this segment as critical habitat for the endangered species (USFWS 2024).

Exploring site and subsite attributes as predictors of mussel detection, only two parameters found to be significantly related to detection of mussels of individual species. Freshwater mussels have been shown to have species-specific autecologies and tolerances to environmental variables (Haag 2012). The variables selected from this study are common indicators of water quality conditions, but it could be that temporal (e.g., diel changes, minimum/maximum) variability of some parameters (e.g., temperature, dissolved oxygen) is more important in mussel survival than reflected by point-in-time measurements. Additionally, sediment grain size characteristics have been found to affect mussel burrowing ability (Hernández et al. 2021), but the way this variable was measured did not lend itself to inclusion in our modeling efforts. If future efforts are to be made to protect existing mussel populations, notably the endangered and threatened species, and expand the spatial extent of all species to the maximum extent possible, a better understanding of characteristics limiting mussel populations in Austin is needed.

Other studies have found that landscape-scale factors, such as agriculture and urbanization, can influence freshwater mussel populations. Agricultural land use still occurs in portions of the study area (Olinde et al. 2021) and frequently leads to nutrient pollution in receiving creeks (Weijters et al. 2009). In watersheds where agriculture is or was common, mussel species losses have been commonly observed (Krebs et al. 2010). For example, in Iowa watersheds converted from prairies and riparian woodlands to intensive agriculture, average species richness decreased, maximum species richness was reduced, and 47% of reaches surveyed had extirpation of all species (Poole and Downing 2004). Mussels in Minnesota watersheds where agriculture is prevalent were also shown to have reduced abundances and species richness (Hornbach et al. 2019). Elevated nutrient concentrations can also be associated with wastewater treatment plants (WWTPs) and similarly have been observed to have negative effects on freshwater mussels. In previous research of in-situ exposure of native *Amblema plicata* and non-native *Corbicula fluminea* to treated wastewater in Wilbarger Creek, each species experienced significantly lower mass and condition indices below the discharge compared to the upstream reference site after 72 days exposure (Nobles and Zhang 2015). Treatment plants influence the baseflow water quality and flow permanence of Gilleland Creek, the headwaters and mainstem of Wilbarger Creek, and the most downstream reaches of Walnut and Onion creeks near the confluence with the Colorado River. Interestingly, the largest number of observed species in this study was in the Wilbarger

and Onion Creek watersheds. Besides nutrients, agriculture and WWTPs can also lead to elevated salts in the water. We observed that *Unio merus tetralasmus* was significantly positively associated with elevated conductivity. However, salinization (sensu elevated specific conductivity) is more frequently associated with runoff due to urbanization (Kaushal et al. 2017).

Our study area is partially urbanized and undergoing rapid land-use conversion from agriculture to urban/sub-urban development. Evaluating common environmental attributes associated with urbanization should also be considered in future studies. For example, percent impervious cover within the drainage area of a site has been shown to negatively impact aquatic invertebrates and vertebrates (Alberti et al. 2007, Ourso and Frenzel 2003, Schiff and Benoit 2007, Walsh 2004). Altered environmental attributes associated with urbanization not considered here include increased turbidity and elevated nutrients. Elevated nutrients can lead to higher algal productivity and eutrophication which can lower dissolved oxygen concentrations after algae decay. Some species such as *Toxolasma texasiense* were found to be present in environments with lower oxygen concentration. In general, improving overall water quality conditions is likely to promote greater density and diversity of species than was observed in this study. Evidence of the influence of land-use is reflected in the diversity, evenness, and recruitment metrics estimated here. For example, reduced catch per unit effort, abundances, survivorship, body condition and overall diversity of mussels has been observed downstream of urban areas (Gillis 2012; Machado et al. 2014; Gillis et al. 2017). The low densities we observed suggests that either adults are having a difficult time producing enough viable offspring or that juveniles are not surviving after settlement. Together, reduced recruitment will be reflected in lower diversity, evenness, and density estimate.

These initial surveys have improved our understanding of mussel population distributions and overall resilience, while also underscoring the need for a deeper understanding of habitat preferences for all species, including endangered ones, to better protect and restore native freshwater mussel populations. For instance, future studies should expand their focus beyond in-stream habitat characteristics to include surrounding land use and its influence on population dynamics. By identifying and analyzing previously under-studied species and stream sections in the Austin area, this study provides crucial information to support conservation efforts for all native mussels. However, it also highlights the need to better understand the key factors driving mussel success and decline, particularly for the now federally listed endangered Texas Fatmucket (*Lampsilis bracteata*) in the Austin area, and to inform strategies for mitigating the impacts of urbanization on these vulnerable species more effectively.

Conclusions

This study documented evidence of 13 unionid species in tributaries of the Colorado River of the 23 species known to the Colorado River Basin, and importantly we observed a live specimen of a recently listed endangered species the Texas Fatmucket (*Lampsilis bracteata*). Further, we found evidence of mussel presence, whether current or historic, in over 80% of the watersheds surveyed. Despite attempts to model site and subsite parameters influence on mussel detection, we found few recorded parameters to be influential of mussel detection. Without a more comprehensive understanding of the inter-relatedness of mussel species in particular stream reaches with their immediate as well as the broader watershed attributes influencing successes or

failures, effective management recommendations in terms of water quality enhancements, instream habitat improvements and protections, and riparian land-use protections cannot be put forth. As such, further research should continue to build off of the initial findings of this research while recognizing that many of the selected attributes were not well correlated with mussel detection. Continuation of this research aligns with the Watershed Protection's core principals of improving stream conditions to protect and enhance native species, and especially endangered species that serve as key indicators of overall ecosystem health and integrity.

Recommendations

While this study enhanced our understanding of species occurrence, status, and distribution, it offers only a snapshot in time. We believe this should be expanded to allow for the effective tracking of population dynamics over time and the environmental factors influencing mussel success. Without ongoing data, we will be inadequately informed to recommend necessary mitigation strategies to protect critical habitats, develop water quality or local habitat improvement projects, or collaborate effectively with federal agencies dedicated to preserving native mussel populations. Below, we outline recommendations for future study goals and design improvements to deepen our understanding of mussel ecology, population dynamics, and potential recovery efforts.

1. Utilize best practices to correct for detection/observation error of individuals and/or species in future studies.
2. Continue to identify locations of mussel beds with an emphasis on recently listed federally endangered species and/or sufficiently abundant or diverse populations to warrant protection and/or long-term quantitative monitoring.
3. Begin routine monitoring of mussel abundances to track survival and recruitment.
4. Better standardize search effort and area for different substrate and mesohabitat types.
5. Better quantify in situ environmental variables that may contribute to unionid mussel survival or population declines including substrate and microhabitat type, and water quality data. This should include diel documentation of variables known to fluctuate throughout the day for which only a single point was collected in this study (e.g., dissolved oxygen, pH).
6. Investigate fish diversity in streams to determine if host species availability limits freshwater mussel dispersion. Fish are a mussel species-specific hosts early in their life cycle and therefore low abundances or absence of certain fish species may limit the ability of mussels to sustain a population within a waterway.
7. Document interactions between zebra mussels (*Dreissena polymorpha*) and native unionid populations.
8. Adapt additional monitoring techniques and technologies, such as eDNA, for more rapid assessment of mussel presence and abundances.

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