Freshwater mussel (Family: Unionidae) data collection in the middle Trinity River

Final Report

Prepared by:

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TWDB Contract No. 1348311646

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Executive Summary

The overall goal of this project was to examine the effects of low and high flows on selected mussel populations within two Texas Instream Flow Program study reaches located in the middle Trinity River. Using quantile regression and habitat suitability curves, we found that low values of shear stress and relative substrate stability (RSS) were associated with high mussel species richness and abundance, corroborating studies in the Brazos and San Antonio drainages. We also found that riffle habitats had higher species richness and abundance than littoral habitats such as banks, which is atypical for large river systems in central and east Texas. High flow events like the ones sampled during this study (~ 400 m^3/s) may be partially responsible for differences we observed in mussel species richness and abundance between banks and riffles. Based on these findings we recommend that instream flow studies should continue to examine hydraulic variables like shear stress and RSS to better describe the hydraulic forces that are important to mussels.

Appendix A includes responses to Texas Water Development Board required changes.

Introduction

Unionid mussels play important roles in freshwater ecosystems through nutrient cycling, increasing habitat heterogeneity and as a food source for some fishes, mammals, and birds (Haag 2012). North America contains the highest diversity of mussels, with about 300 recognized species. However, many of these species are in decline or have become extinct due to habitat loss or elimination of host fish (Williams et al. 1993). In Texas, similar declines have occurred but until recently have gone largely unnoticed. Currently, there are 15 species listed as state threatened, of which 6 are candidates for protection under the Endangered Species Act (TPWD 2010; USFWS 2009, 2011). As a result of these listings, mussel conservation strategies are now beginning to emerge in Texas. One of these strategies includes the use of mussel-habitat data to predict quality and quantity of mussel habitat across a range of modeled stream flows.

Habitat alteration through modified flows is a major impact to mussel populations. Mussels are sedentary, have slow growth rates, long life spans and late maturity, and have a complex reproductive life history, involving fish for both dispersal and successful completion of reproduction (Haag 2012). As a result of these traits, mussels are generally unable to seek refuge during disruptive flow events or rapidly recolonize areas where they have been significantly reduced in numbers or completely extirpated. Recent studies have shown that extreme low and high flow events are important factors in regulating mussel distribution and abundance in lotic systems (Maloney et al. 2012; Gates et al. 2015). That is, mussels most often occur in patches that are stable during high flow events and remain wetted during periods of low flow. Unfortunately, these two conditions can be mutually exclusive, such that a habitat patch may be stable during high flows but become dry during subsistence flows, or vice versa. As a result, it is important that both low and high flow conditions are studied to develop a more comprehensive understanding of mussel habitat utilization.

To support Texas Instream Flow Program (TIFP) efforts in the Trinity River basin, the purpose of this study was to examine the effects of subsistence and high pulse flows on freshwater mussel populations located in the middle Trinity River. This information is important because research on mussel-habitat relationships in the Trinity River basin have been primarily descriptive or focused on measuring habitat only at low flows. Moreover, three Texas state-threatened species, *Fusconaia askewi* (Texas Pigtoe), *Pleurobema riddellii* (Louisiana Pigtoe), and *Potamilus amphichaenus* (Texas Heelsplitter), inhabit this portion of the Trinity (Randklev et al. 2017), and it is unknown to what extent low and high flow events impact these species. Emphasis in this study was to provide information that will inform instream flow analysis and recommendations.

Methods

Study Area

The Trinity River basin is located in the Southwestern United States and has an overall length of 579 km and encompasses approximately 46,539 km^2 making it one of the larger river basins in Texas. The human population in the basin was about 6.9 million people in 2010, a majority of which (~ 5.3 million) reside in the Dallas-Fort Worth metroplex (Perkin and Bonner 2016), located in the headwaters of the Trinity River. The Trinity River, which is formed by the Clear, West, Elm and East forks, flows from just west of Dallas, Texas, to ultimately the Gulf of Mexico (Kleinsasser and Linam 1990). This study was located in the middle Trinity River within two reaches selected *a priori* as part of the TIFP draft study design for the middle Trinity River (Figure 1).

Site Selection

Sample sites within the two TIFP study reaches were chosen using a stratified random sampling design. Specifically, bank and riffle habitat types were randomly selected for sampling using satellite imagery and subsequently monitored under low and high flow conditions. Bank habitats were defined as the zone from the bank to the point in the channel where the slope of the bank leveled out, which indicated the beginning of the mid-channel habitat. Riffle habitat was defined as shallow areas with moderate to fast flows, where small hydraulic jumps over rough bed material cause small ripples, waves and eddies.

Mussel Surveys

At each site, we randomly selected 30 0.25-m^2 quadrats within a 150-m^2 area and excavated sediment from quadrats to depth of 20 cm using a modified Surber sampler. Sediment excavated from quadrats was passed through a 0.25-inch sieve to separate mussels. For quadrats where sediment was difficult to excavate, we searched each quadrat for 15 minutes in lieu of excavation. We separated mussels from the sediment and stored them in mesh bags prior to identification. Data from the quantitative sampling were then used to calculate species richness and mussel density (mussels/0.25 m^2) for each quadrat and site.

Habitat Sampling

Within each quadrat, we recorded current velocity and water depth using an electromagnetic flow meter (OTT MF Pro). Substrate types were qualified using an index following Randklev et al. (2014a). Briefly, this index represents a number where higher values correspond to coarser substrate material (substrate type), which is classified based on a modified Wentworth Scale. Based on this assessment we then identified and measured the diameter of the median particle size (D_{50}) within each quadrat. The location of each quadrat was recorded using a Trimble GeoCollector so that each quadrat could be resampled during high flows. All habitat measurements were collected at approximately the center of each quadrat.

We returned to each site during periods of high flow and measured water depth and velocity using an Acoustic Doppler Current Profiler (ADCP). We did not reevaluate substrate type between low and high flow sampling events. Sampled high and low flow ranges during the study are provided in Table 1. The substrate and hydraulic variables used to describe mussel microhabitat were calculated using the formulae listed in Table 2.

Data Analyses

Quantile regressions were used to examine the relationship between mussel species richness and density and complex hydraulic variables. Quantile regression is a method used to investigate the relationships between variables for all portions of a probability distribution and has been used in ecological studies to estimate limiting factors (Cade and Noon 2003). Quantile regression is based on the least absolute deviation regression, which models the conditional median (50^{th} quantile), but the approach can be extended to any quantile. Unlike traditional least-square regressions, quantile regression can be applied to non-normal datasets or to those with heterogeneous variances, which is common in ecological studies (Cade and Noon 2003; Allen and Vaughn 2010).

The 95^{th} quantile is often used to evaluate limiting factors, but we also modeled 90^{th} and 75^{th} quantiles since they are commonly used to construct habitat suitability tolerance limits. Following Allen and Vaughn (2010), we fit univariate models using linear, quadratic, Ricker, or exponential curves to the data (with and without *y*-intercepts) and chose the best-fitting model for each quantile based on the Akaike information criterion (AIC) provided it gave non-0 parameter estimates for the model coefficients. We calculated AIC as equal to n x ln (deviance of the model of interest/n) + 2*K*, where n is the total sample size, and *K* is the number of estimated variables + 2 (intercept and residual variance) (Vaz et al. 2008). The fit of each quantile regression model was evaluated with the AIC corrected for small sample size (AICc) (Burnham and Anderson 2002). In addition to the AICc, we calculated a pseudo-R^2 for each model, which provided an additional line of evidence for how well a particular function fit the data. Pseudo-R^2 was calculated as $1 – (1 – R)^2$, where R is 1 (deviance of the model of interest divided by the deviance of the intercept-only model) (Allen and Vaughn 2010). Quantile regression analyses were performed using the QUANTREG package in R version 3.02 (R Foundation for Statistical Computing, Vienna, Austria). Finally, we also developed habitat suitability criteria using non-parametric tolerance limits (Bovee 1986). Suitability values derived from this method range from 0 to 1 with 0 indicating unsuitable and 1 indicating optimal suitability. The bin widths of the suitability curves were determined using the Sturges (1926) equation.

Results and Discussion

Quantile Regression

Quantile regression analyses between mussel species richness and abundance and shear stress and relative substrate stability (RSS) exhibited limiting-factor relationships for at least two of the 95th, 90th and 75th quantiles (Figure 2). The response curves for both biotic responses, regardless of quantile, were best described by exponential functions, either Ricker or negative exponential (Table 3). For both functions, mussel species richness and abundance was maximized at low values of shear stress and RSS. Increases in either hydraulic variable resulted in decreases in mussel species richness and abundance. Pseudo-R^2 values were relatively high depending on quantile and hydraulic variable (Table 3). These results corroborate previous studies, which have shown that mussel abundance and richness decrease with higher levels of shear stress and RSS (Layzer and Madison 1995; Di Maio and Corkum 1995; Morales et al. 2006; Gangloff and Feminella 2006; Allen and Vaughn 2010; Randklev et al. 2014a).

The degree to which shear stress and RSS became limiting to mussel species richness and abundance appears to be partially attributable to mesohabitat type (i.e., banks and riffles). Our results show that the greatest increases in shear stress and RSS occurred in bank habitats (Figure 2), which on average had lower species richness and abundance compared to riffle habitats (Table 4). This is not unexpected as particle size is a primary determinant of when bed mobility is likely to occur for a given discharge. For bank habitats, the dominant substrate was sand, which likely becomes mobilized at a lower discharge compared to the riffles we sampled, which were mainly comprised of gravel and cobble. These findings indicate that channel form features can help minimize hydraulic stress to mussels (Morales et al. 2006; Steuer et al. 2008; Randklev et al. 2014a).

Suitability Curves

Suitability curves based on shear stress and RSS across low and high flows indicate that optimal habitat occurs at low values for both, which corroborates the results of our quantile regression analyses. Specifically, suitability curves based on shear stress indicate that optimal mussel habitat occurs in areas where shear stress ranges near or at 96 dyn/cm^2 (Figure 3). Suitability curves based on RSS indicate that mussels occur primarily in areas where shear stress is low and RSS values remain well below a value of 1, which is considered a threshold for substrate entrainment (Figure 4). Suitability curves by species (Figures 5 and 6) show similar results to those developed for all mussels (Figures 3 and 4). The exception is *Lampsilis teres*, yellow sandshell, which occupied areas with high entrainment potential (Figure 7), which were mainly bank habitats. This suggests that this species may tolerate entrainment or possess traits (morphological or behavioral) that help prevent dislodgment in habitats, like banks, that experience high scour.

Conclusions

Strayer (2009) hypothesized that mussel distribution is affected by large floods with return intervals of 3 to 30 years. During this study mussel habitat was assessed at high flows exceeding 400 m^3/s, which is much higher than when most mussel-habitat studies are performed. Given that a discharge of 400 m^3/s appears to be limiting to mussels at bank habitats (Figure 2), we performed a flood frequency analysis to determine the return interval of peak flood flows near our study sites. We found that peak floods of approximately400 m^3/s occur frequently, often with a return interval of a year or less (Figure 7). This could explain why mussel species richness and abundance are reduced in bank habitats, which is somewhat atypical for river systems in Central and East Texas. For example, Randklev et al (2014b, c) found that bank habitats in the Brazos and Sabine rivers often support high species richness and abundance, often comparable to riffle habitats. Thus, these results could indicate that water management practices upstream of our study sites are having a negative impact on the mussel fauna in the middle Trinity River.

Our finding that shear stress and RSS are limiting to mussel abundance and richness corroborates previous research in the San Antonio and Brazos River drainages. Thus, instream flow studies should continue to examine these hydraulic variables to better describe the hydraulic forces that are important to mussels. Continued assessment of shear stress and RSS at locations with and without mussels across different river drainages might eventually lead to the development of species-specific preference models that could be used to better estimate the impact of various flow scenarios on mussel communities. This has already been done for benthic macroinvertebrates (e.g., Doldec et al. 2007; Merigoux et al. 2009) but not for mussels, in part because the dataset required to do so does not yet exist.

Finally, because shear stress and RSS are not the sole determinants of mussel distribution as it relates to instream flows, further research is needed on other limiting factors such as extreme low flow events and how they regulate mussel distribution and community composition. This additional information when combined with shear stress and RSS data will allow managers to take a more holistic approach for determining the amount of water needed to protect ecosystem function and conserve existing mussel populations (Maloney et al. 2012; Gates et al. 2015).

Table 1. Sampled flows.

USGS gauging station	08065000 Oakwood	08065350 Crockett
TIFP Site #	**080295**	**080224**
High Flow – sampled (m^3/s; cms)	413.43	484.22
Date	4/17/2017	6/8/2017
Low Flow – sampled (m^3/s; cms)	31.43 – 35.11	31.15 – 34.55
Date	9/19/2016 – 9/22/2016	9/22/2017; 10/5/2016

Table 2. Summary of physical variables measured in the study. U = average velocity (0.6 x d or the mean of 0.2 and 0.8 x d), d = water depth (cm), g = acceleration of gravity (980 cm/s^2), v = kinematic viscosity of water (0.01 cm^2/s), ρ_s = density of substrate (2.65g /cm^3), ρ = density of water (0.998 g/cm^3), θ_c = Shield's parameter (0.065). Asterisks denote variables used in quantile regression and to construct suitability curves.

Variable	Formula	Description
Bed roughness (k_s, cm)	6.8 x D_{50}	Topographic variation of the stream bottom
*Shear stress (τ, dyn/cm^2)	$\rho(U*^2)$	Force of friction on substrate
Shear velocity (U_*, cm/s)	$U/5.75log_{10}(\frac{12d}{k_s})$	Friction velocity
Critical shear stress (τ_c dyn/cm^2)	$\theta_c g D_{50}(\rho_s - \rho)$	Shear stress required to initiate substrate motion for a typical sample substrate size (D_{50})
*Relative shear stress (RSS, dimensionless)	$\frac{\tau}{\tau_c}$	Ratio of observed to critical shear stress (values > 1 are thought to represent substrate movement for a typical sample substrate size [D_{50}])

Table 3. Summary of 95th, 90th, and 75th quantile regression models for mussel species richness and abundance.

		Shear stress				RSS		
Depth	Function	Model	Quantile	Pseudo-R2	Function	Model	Quantile	Pseudo-R2
0.6 x D	Ricker	Richness	95th	0.28	Exponential	Richness	95th	0.21
0.6 x D	Ricker	Richness	90th	0.45	Exponential	Richness	90th	0.20
0.6 x D	Ricker	Richness	75th	0.44	NS	Richness	75th	NS
0.6 x D	Ricker	Density	95th	0.55	Exponential	Density	95th	0.25
0.6 x D	Ricker	Density	90th	0.57	Exponential	Density	90th	0.20
0.6 x D	Ricker	Density	75th	0.37	Exponential	Density	75th	0.001

Table 4. Mussel species richness and mean density (± SE) and mean (± SE) shear stress and RSS by TIFP study reach, habitat and depth at which velocity was measured

TIFP	n	Habitat	Richness	Density	Shear stress	RSS
080295 (Oakwood)	30	Riffle1	9	2.47 (0.50)	694.01 (453.61)	0.61 (0.20)
080295 (Oakwood)	30	Riffle2	9	3.93 (0.66)	67.55 (3.10)	0.42 (0.03)
080295 (Oakwood)	30	Bank1	2	0.30 (0.10)	6.48 (0.94)	2.92 (0.43)
080295 (Oakwood)	30	Bank2	4	0.57 (0.12)	6.84 (0.94)	3.08 (0.42)
080224 (Crockett)	30	Riffle	1	0.03 (0.03)	42.67 (5.38)	0.98 (0.10)
080224 (Crockett)	30	Bank1	1	0.03 (0.03)	7.77 (1.05)	3.46 (0.48)
080224 (Crockett)	30	Bank2	0	0.00 (0.00)	5.71 (0.83)	2.57 (0.37)

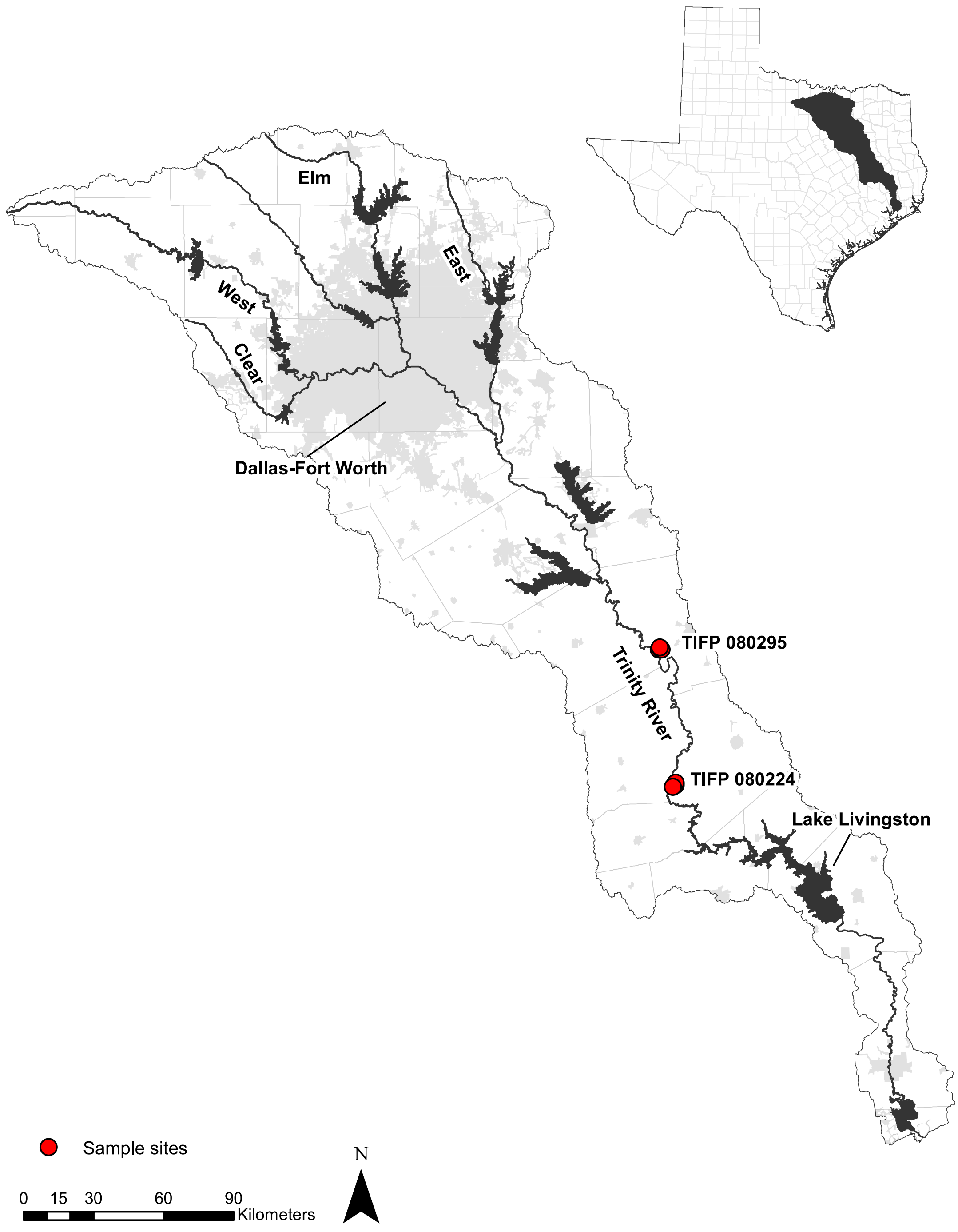


Figure 1. Sampled TIFP sites in the middle Trinity River.

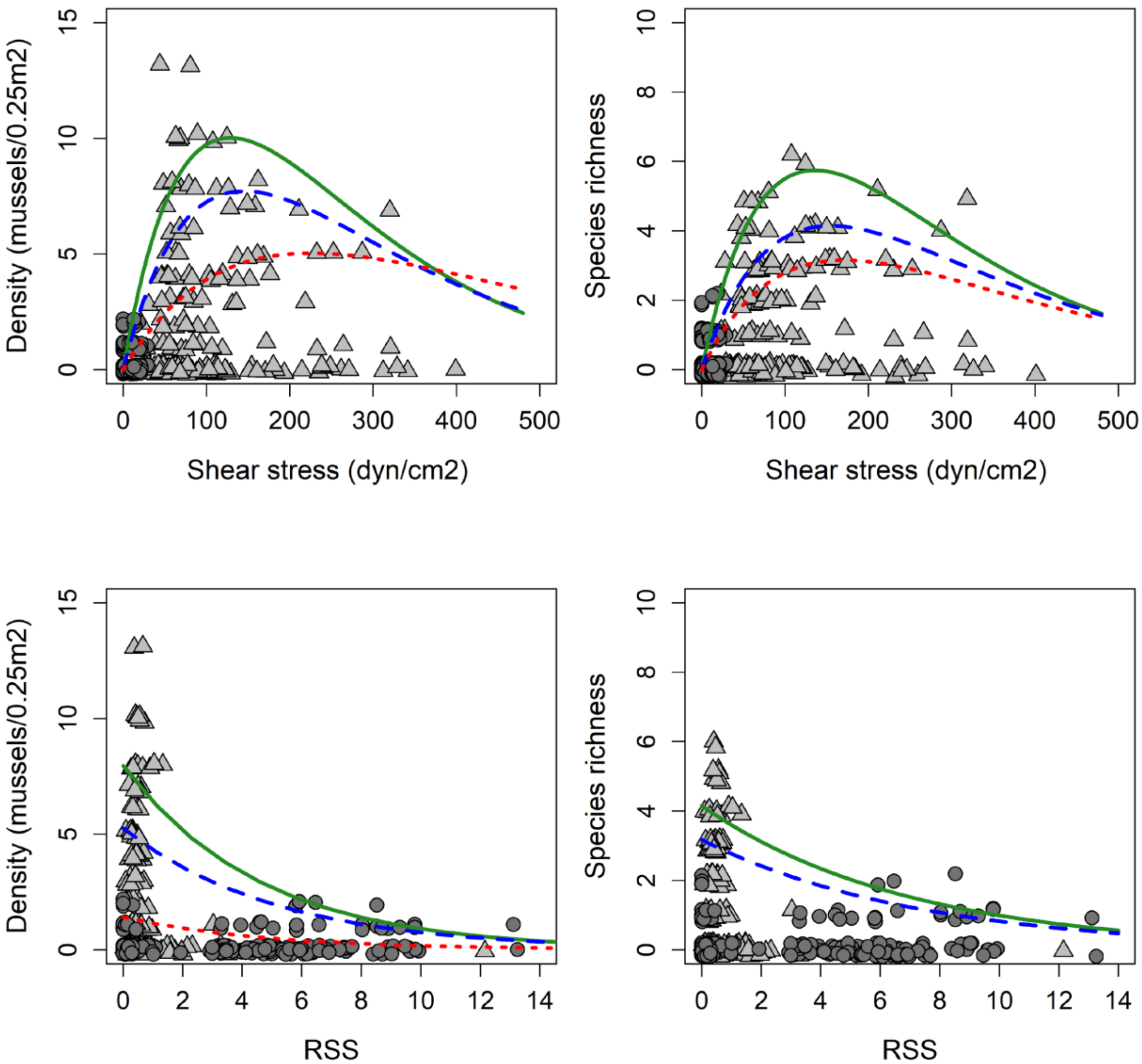


Figure 2. Quantile regression models for mussel density and species richness for shear stress (τ, dyn/cm^2) and relative shear stress (RSS) across high and low flows. Green solid, blue dashed, and red dotted lines represent 95th, 90th, and 75th quantile regression lines, respectively. Circles denote 0.25 m^2 quadrats from bank habitats and triangles denote quadrats from riffles. Only significant quantiles are shown.

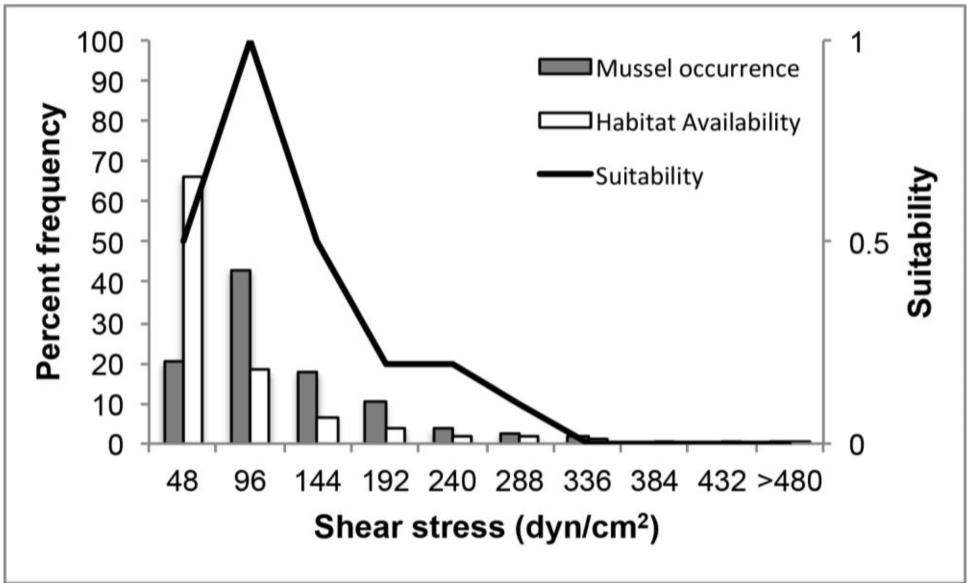


Figure 3. Percent frequency of occurrence (grey bars), habitat availability (white bars), and NPTL values (solid line; 95% confidence level) for shear stress across low and high flows. Suitability criteria are shown across all mussels and are based on abundance data from quantitative sampling. The number of observations used were: N = 420.

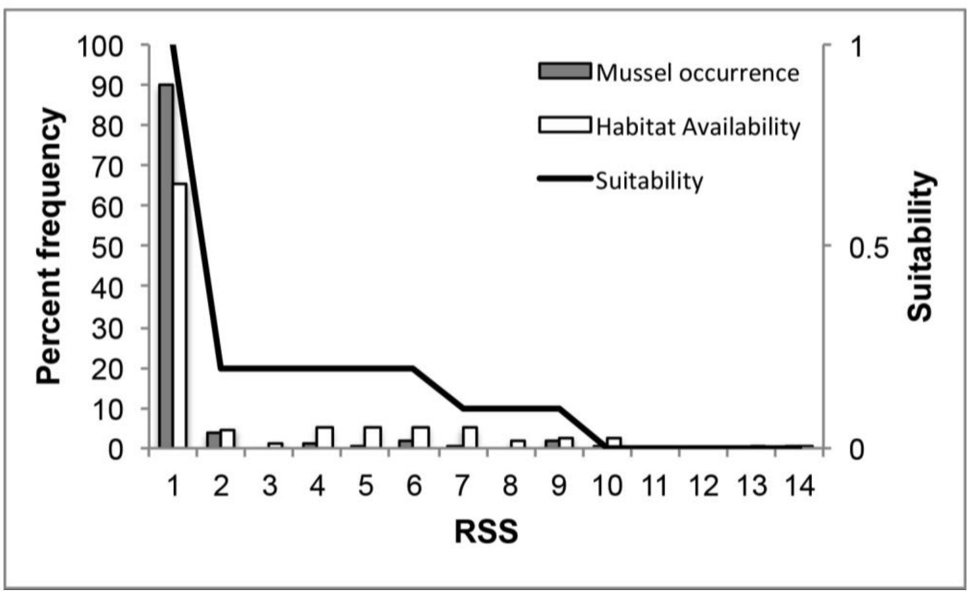


Figure 4. Percent frequency of occurrence (grey bars), habitat availability (white bars), and NPTL values (solid line; 95% confidence level) for RSS across low and high flows. Suitability criteria are shown across all mussel species and are based on abundance data from quantitative sampling. The number of observations used were: N = 420.

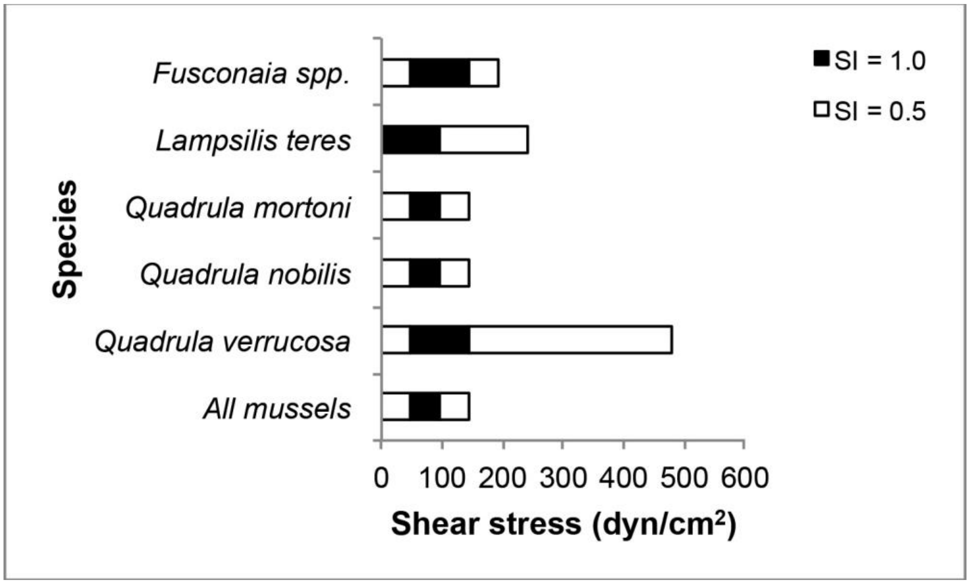


Figure 5. Suitability based on NPTL values (95% confidence level) for shear stress across low and high flows. Suitability criteria are shown only for species with 25 or more observations. *Fusconaia* spp. includes both *Fusconaia chunii* (Trinity Pigtoe) and *Fusconaia flava* (Wabash Pigtoe), which co-occur and cannot be distinguished using external morphology (Pieri et al. 2018).

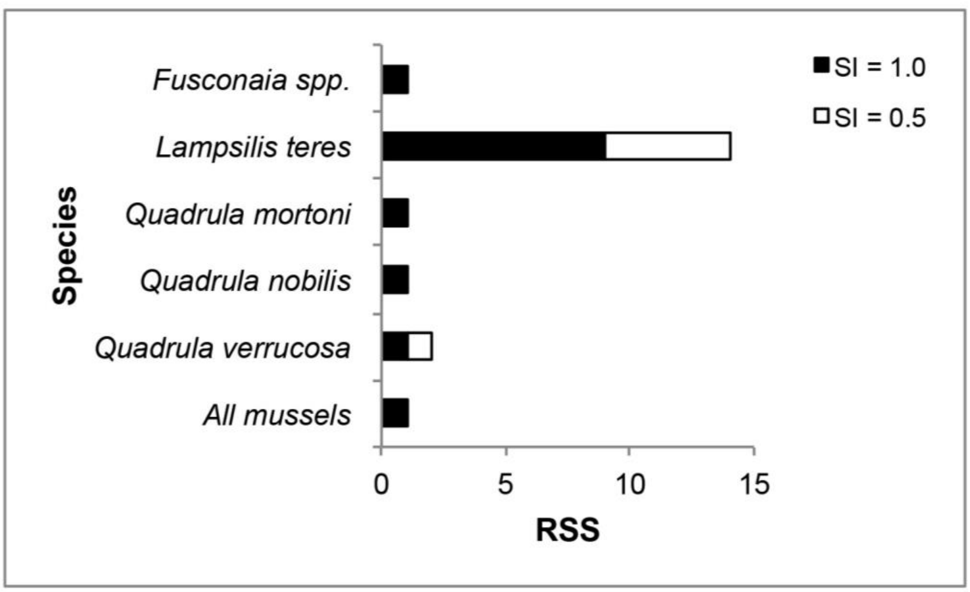
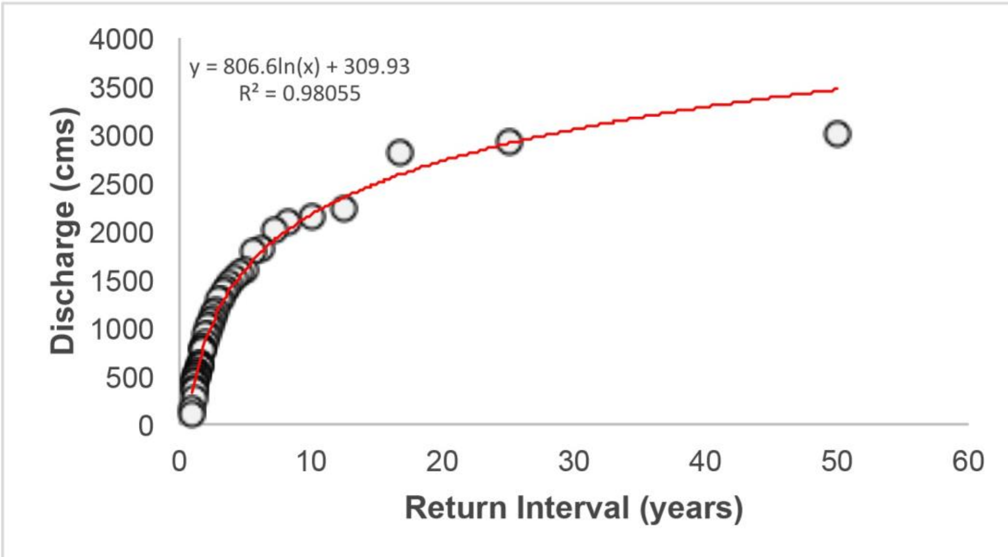


Figure 6. Suitability based on NPTL values (95% confidence level) for RSS across low and high flows. Suitability criteria are shown only for species with 25 or more observations. *Fusconaia* spp. includes both *Fusconaia chunii* (Trinity Pigtoe) and *Fusconaia flava* (Wabash Pigtoe), which co-occur and cannot be distinguished using external morphology (Pieri et al. 2018).



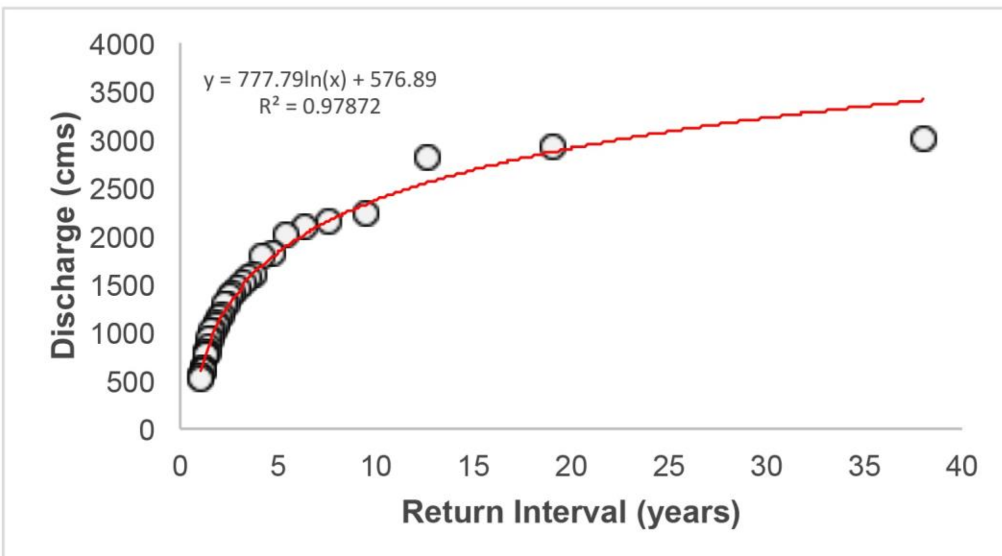


Figure 7. Flood-frequency curves for USGS gage 08065000 (Oakwood; 49 years of data), which is located near TIFP site 080295, and gage 08065350 (Crockett; 37 years of data), which is near TIFP site 080224. Annual peak discharge was determined using Indicators of Hydrologic Alteration (Richter et al. 1996) and recurrence intervals were determined using the Weibull formula using the same data. Discharge during high flow sampling at TIFP site 080295 (Oakwood) was approximately 413 m^3/s (cms), which has a return interval of 1.34 years. At TIFP site 080224 (Crockett) discharge during high flow sampling was approximately 484 m^3/s (cms), which has a return interval of less than 1 year.

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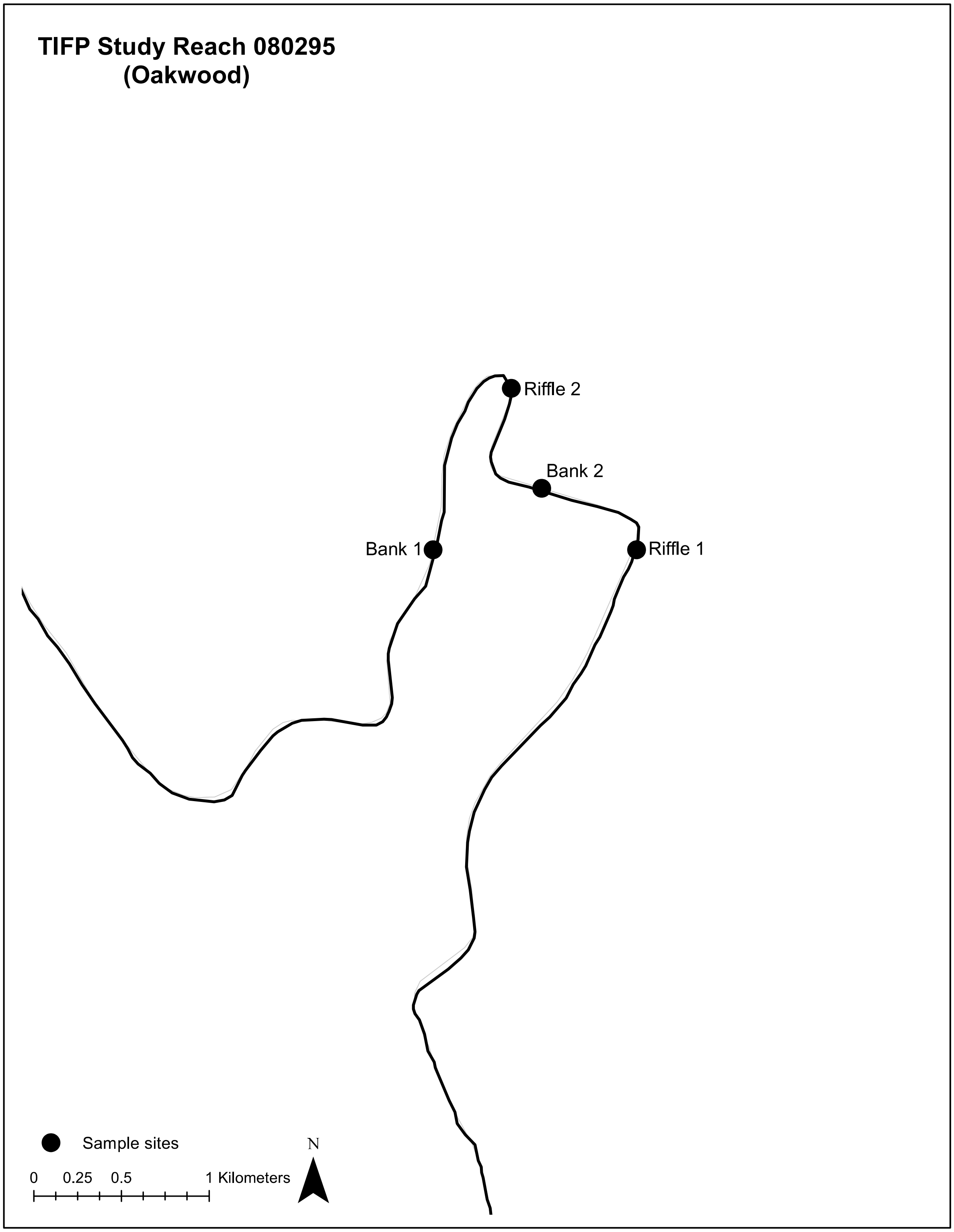
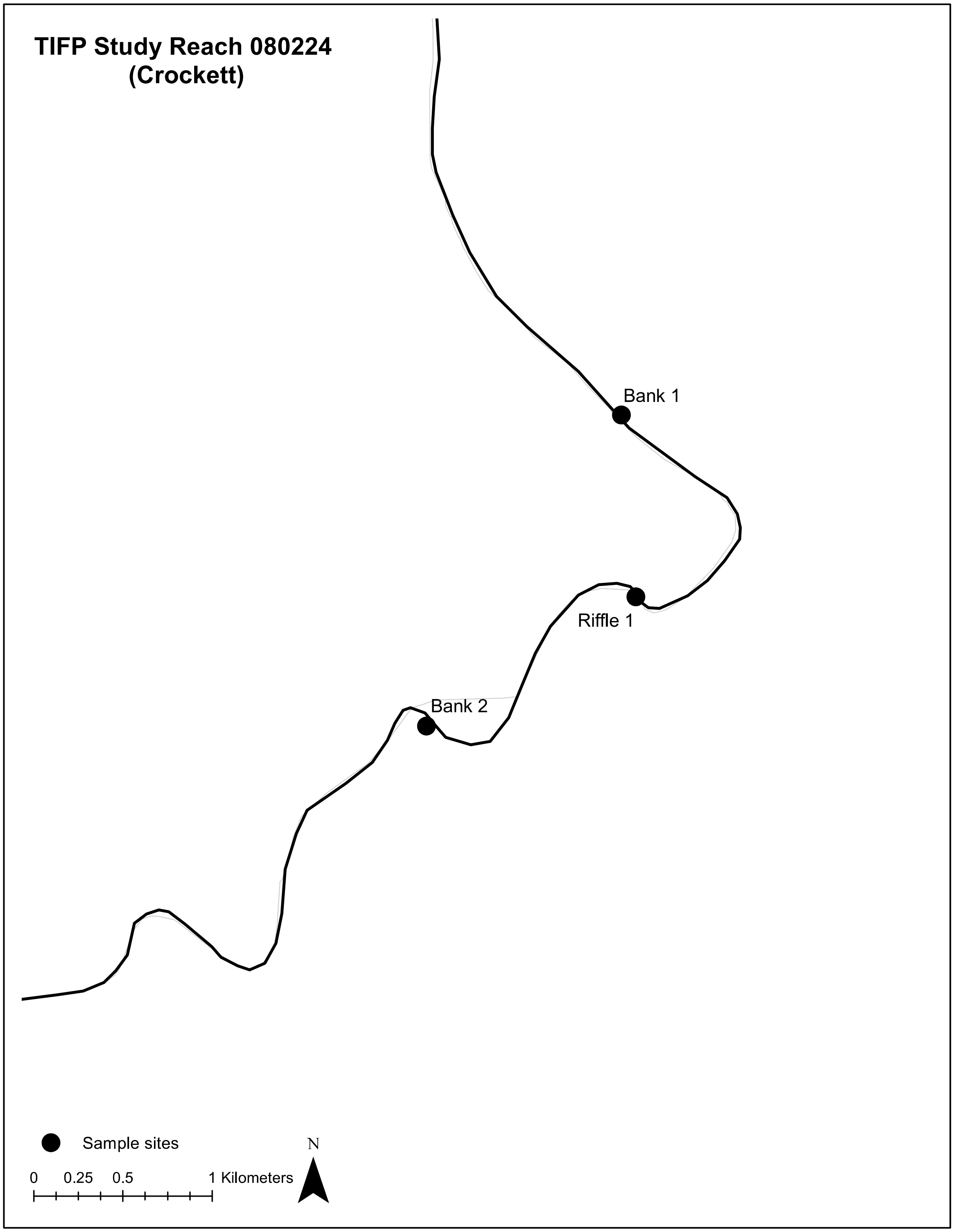
Supplemental Table 1. Total number of individuals by species by site collected from randomly selected 30 0.25-m^2 quadrats within a 150-m^2 area. *Fusconaia* spp. includes both *Fusconaia chunii* (Trinity Pigtoe) and *Fusconaia flava* (Wabash Pigtoe), which co-occur and cannot be distinguished using external morphology (Pieri et al. 2018).

	080295 (Oakwood)				080224 (Crockett)		
Species	Riffle 1	Riffle 2	Bank 1	Bank 2	Riffle 1	Bank 1	Bank 2
Fusconaia spp.	11	17	0	0	0	0	0
Lampsilis teres	1	1	4	8	0	1	0
Leptodea fragilis	1	2	6	0	0	0	0
Megalonaias nervosa	10	1	0	0	0	0	0
Plectomerus dombeyanus	10	0	0	0	0	0	0
Potamilus purpuratus	2	0	1	0	0	0	0
Quadrula mortoni	14	48	6	0	0	0	0
Quadrula nobilis	22	28	0	0	1	0	0
Quadrula verrucosa	3	10	0	0	0	0	0
Obliquaria reflexa	0	7	0	1	0	0	0
Truncilla macrodon	0	4	0	0	0	0	0



Supplemental Figure 1. Map of TIFP Study Reach 080295 (Oakwood) showing mussel sampling locations.



Supplemental Figure 2. Map of TIFP Study Reach 080224 (Crockett) showing mussel sampling locations.

Appendix A. Responses to Texas Water Development Board required changes.

REQUIRED CHANGES TO TASK 2 REPORT

1. Please reference “TWDB Contract No. 1348311646” on the cover of the report.

   Response: Done

2. Please check the report for typos such as the following and correct as necessary:
   a. Page 3, 2nd paragraph, 3rd sentence, “as a result of the traits” should be “as a result of these traits.”
   b. Page 4, 1st paragraph, 3rd sentence, “flows just west of Dallas, TX,” should be “flows just west of Dallas, Texas.”
   c. Page 4, 4th paragraph, 4th sentence, “median particle size (D50)” should be “median particle size (D_{50}).”

   Response: Done

3. On page 7, in the first paragraph, the observation is made that “mussel species richness and abundance are reduced in bank habitats, which is somewhat atypical for river systems in Central and East Texas. For example, Randklev et al (2014b, c) found that bank habitats in the Brazos and Sabine rivers often support high species richness and abundance, often comparable to riffle habitats.” These statements may need further explanation or clarification based on several factors. First, it is not clear whether bank habitats were defined similarly in this study and in the Brazos or Sabine studies. Bank habitats may have different shear stress characteristics, depending on whether they are located on straight sections of channel or on the outer or inner banks of curved sections of channel. Secondary flow patterns cause increased shear stress on the outer bank and decreased shear stress on the inner bank of a curved channel. In Randklev *et al.* (2014c), bank habitat was located on relatively straight sections of channel (see Figures 2, 3, and 4 in that report). It is unclear whether bank habitats described in this report were oriented similarly. Please provide figures similar to Figures 2, 3, and 4 in Randklev *et al.* (2014c) to show how bank and riffle habitats were located relative to channel pattern. Second, the number of stream segments where bank habitats were sampled in the Brazos, Sabine, and in this study on the Trinity River are quite small (3 on the Brazos, 14 on the Sabine, and 2 on the Trinity). Third, there seems to be significant variation in mussel richness and abundance among the bank habitats sampled on the Brazos and Sabine rivers. On the Brazos, one out of three bank habitat areas had reduced catch per unit effort (CPUE) (Table 4 of Randklev *et al.* 2014c), and one out of three had reduced density (Table 5 of Randklev *et al.* 2014c). On the Sabine, 6 out of 16 bank habitats had reduced CPUE and species richness (Table 2 of Randklev *et al.* 2014c). Fourth, neither the study on the Brazos nor the Sabine included “riffle habitat” as a habitat type, making the reference to “riffle habitats” unclear. In light of these factors, please provide additional explanation/clarification and/or revise the statements in the report as appropriate.

Response to first point by reviewer: Bank habitats in this study were oriented similarly to those of previous studies (see supplemental maps now included in the revised report).

Response to second and third point by the reviewer: Sample sizes may be low but relative to the data we have, which was collected in a similar manner across multiple basins, densities at bank habitats in the Trinity, even within the best reach (i.e., Oakwood), are low compared to other study reaches, and range from 0.0 to 0.3 mussels/0.25m^2 (species richness is between 0 and 1). To put these numbers into context, within the Brazos, where a similar study designed was used, mussel densities at bank habitats were 1.2 mussels/0.25m^2 at Mussel Shoals (TIFP# 12050), 5.7 mussels/0.25m^2 at Washington on the Brazos (TIFP# 12030), and 4.3 mussels /0.25m^2 at Wild Cat Bend (TIFP# 12020), which is ~ 4 to 19 times greater than what we observed in the Trinity. Similarly, in the lower Guadalupe River, mussel densities at bank habitats ranged from 5 mussels/0.25m^2 to 31 mussels/0.25m^2, which is ~17 to 103 times greater than the Trinity. Species richness in these other basins follows a similar pattern relative to the Trinity.

Variation within habitat type in these other basins is really a non-issue because densities, regardless of the specific bank habitat (see above examples for the Brazos and Guadalupe), were much higher than the Trinity. Moreover, low abundance at bank habitats in the Trinity was observed across both TIFP reaches, indicating this issue is systemic and not an artifact of poor site selection or idiosyncrasies in a given sample site or channel/reach morphology. For the other basins, we've yet to observe this pattern. Finally, the reviewer's own examples underscore our point; 2/3 (67%) of the bank habitats in the Brazos had high abundance while 10/16 (62%) of the bank habitats in the Sabine had high abundance and species richness. Compare this to the Trinity, in which 0% percent of the sites had high abundance or species richness.

Response to fourth point by the reviewer: The reviewer is correct that riffles were not sampled in those other basins, but other habitat types were such as bank or point bar habitats, and these habitats were often productive for mussels, although not always the "best habitat type." In the Trinity, we suspected, based on sampling the Guadalupe, that riffles would be one of the most productive habitats, but we did not anticipate that it would be the ***only*** productive habitat type. The difference in densities between the two is striking. We suspect this difference stems from the fact that the substrates within riffles in the Trinity are probably not being mobilized as often as those in bank habitats. This has to do, in part, with grain size and the flow regime. Substrates at riffles in the Trinity are comprised mainly of gravel and cobble whereas sand/silt are the dominant substrate type at bank habitats. At ~16,000 cfs, which is the highest flood pulse we assessed, bank habitats appeared to undergo significant entrainment relative to the riffles we were monitoring; our assessment of flood frequency in the basin indicates a flood pulse of this magnitude occurs often (less than a year in frequency). Finally, TRA in their 2013 long-term monitoring report documented significant bank erosion based on data from studies using erosion pins in reaches upstream of our study sites, in some cases up to 8 inches (and deposition up to 3 inches in depositional areas). In general, mussels live at the substrate/water interface and so loss of sediment or deposition can result in mortality, especially if the amounts reported from TRA are typical throughout the Trinity. Thus, TRA's findings along with our observations of reduced abundance at bank habitats, relative to riffles, density data for bank habitats from other TISF mussel studies, and the mussel-shear stress and RSS relationships modeled in this report indicate that persistent high flows in the Trinity may be an issue.

Trinity River Long-term Study. Master Report- Objectives, Progress and Summary through November 2013, Revision 03b. Prepared by RPS Espey in collaboration with TRA.

4. On page 9, caption for Table 2, there appears to be some confusion regarding the correct formula for calculating shear stress (average column velocity should be used in the calculation). Please insure shear stress is calculated correctly throughout the report.

   Response: Done

5. It is unclear how many individuals and species of mussels were identified as part of this study. Please provide this information in the report. A table similar to Table 4 in Randklev *et al.* (2018c) would be helpful for this purpose.

   Response: Table added as supplemental information.

6. Further information is required in order to understand the data shown in figures 6 and 7 on pages 20 and 21. Please provide an explanation in the text or in the title to describe which species (singular) or species (plural) are indicated by "Fusconaia sp" on the y-axis of both figures. If this is a grouping, please provide some explanation for this grouping (*e.g*. these species are known to have similar habitat preferences) and comment whether there were or were not other groupings of species that would be appropriate in order to achieve more than 25 observations.

   Response: *Fusconaia* spp. includes both *Fusconaia chunii* (Trinity Pigtoe) and *Fusconaia flava* (Wabash Pigtoe), which co-occur and cannot be distinguished using external morphology (Pieri et al. 2018). This is now stated in the figure captions for both.

Pieri, A., K. Inoue, N.A. Johnson, C.H. Smith, J.L. Harrish, C.R. Robertson, and C.R. Randklev. 2018. Molecular and morphometric analyses reveal cryptic diversity within freshwater mussels (Bivalvia: Unionidae) of the western Gulf coastal drainages of the USA. Biological Journal of the Linnean Society 124:261-277.