

# 1104831145 Final Report

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**Freshwater mussel (Family: Unionidae) data collection in the middle and lower  
Brazos River**

Submitted by:

Charles R. Randklev, Mark Cordova, Julie Groce, Eric Tsakiris, and Bryan Sowards

Institute of Renewable Natural Resources, Texas A&M University

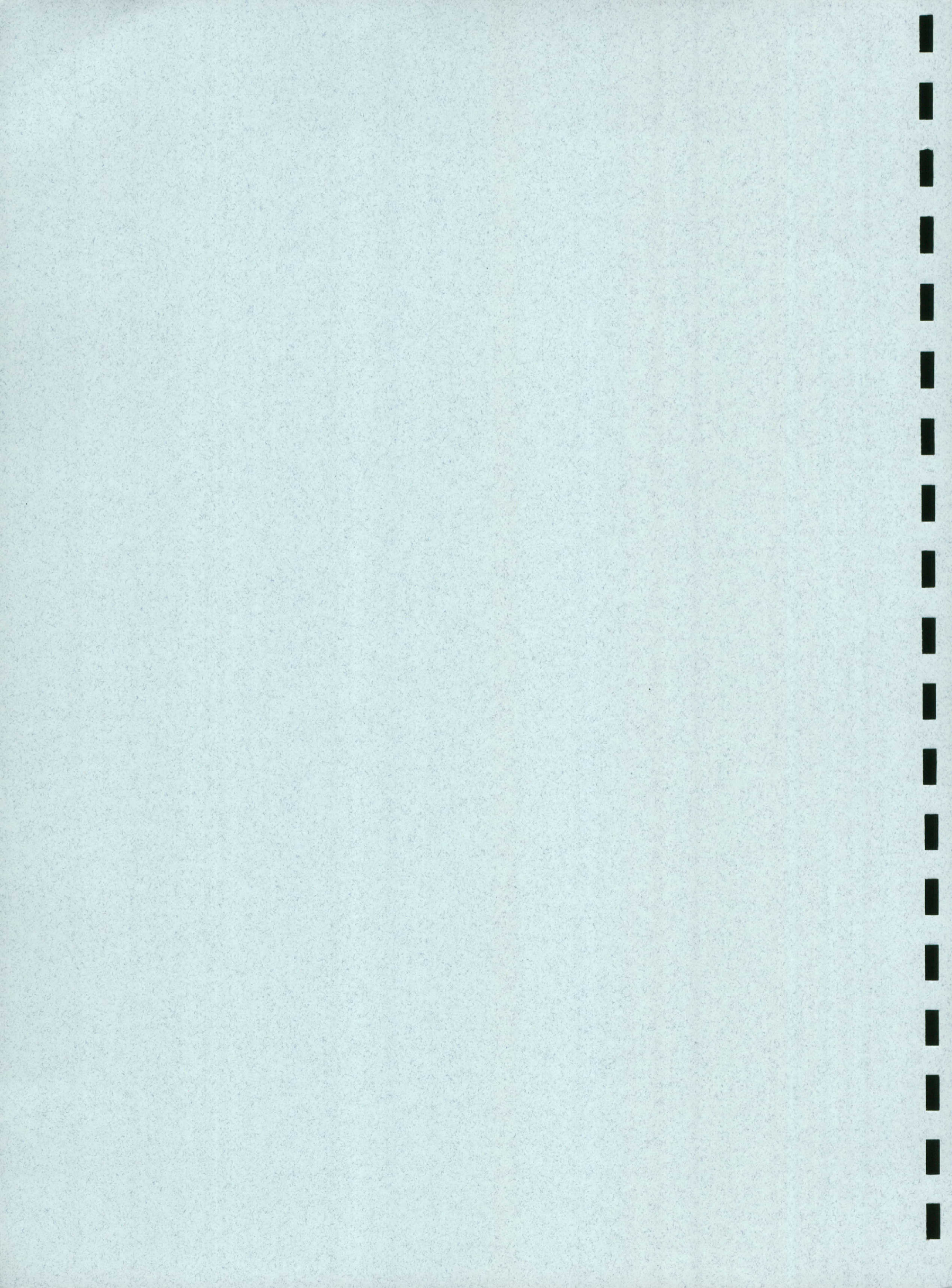
Initial Submission Date

March 2014

**Final Project Report Date July 2018**

**Texas Parks and Wildlife Department Contract No. 424520**

**Texas Water Development Board Contract No. 1104831145**



### Executive Summary

The overall goal of this project was to determine the current status and distribution of freshwater mussels in three Texas Instream Flow Program (TIFP) study reaches located on the Brazos River and to examine the effects of low and high flows on select mussel populations within each of the reaches. In total, we surveyed 15 sites, 5 per reach, each of which represented an unique mesohabitat type. We observed 2,135 live individuals of 12 species, including two candidates for listing under the Endangered Species Act (ESA), *Quadrula houstonensis* (smooth pimpleback) and *Truncilla macrodon* (Texas fawnsfoot). We also examined the meso- and microhabitat relationships of mussels during low and high flows. Mussel abundance and richness differed among mesohabitat types; bank, backwater and behind point bar habitats were more speciose and dense than front of point bar and midchannel habitats. The physical characteristics of these habitat types differed, particularly shear stress, which was higher in front of point bar and midchannel habitats during both low and high flows. These results support the hypothesis that substrate stability during high flows restricts mussel abundance and richness. Our mussel microhabitat association results mirror those from the mesohabitat analyses in that hydraulic variables related to substrate stability, particularly shear stress, were most limiting to mussel species richness and abundance. By contrast, hydraulic variables estimated during low flow were not limiting to mussel abundance or richness, which suggests that these attributes are more important to mussel habitat quality during higher flows.

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## Introduction

Spatiotemporal variability in flow determines the solid and fluid physical characteristics of aquatic habitat (Lancaster 1993). During low flows aquatic organisms can be exposed to reduced oxygen concentrations, increased water temperature, reduced habitat area and desiccation whereas, during high flows increased water velocity and hydraulic forces on the stream bed can be equally detrimental. These constraints create a mosaic of habitats that influence the distribution and abundance of aquatic biota (Southwood 1988).

For freshwater mussels (family: Unionidae), recent studies have suggested that high flow events regulate their distribution and abundance (Morales et al. 2006; Allen and Vaughn 2010). That is, mussel aggregations will only occur in areas that remain stable during high flow events. Other researchers have observed that mussel aggregations are restricted to areas within a stream that provide refugia during low flows (Johnson et al. 2001). Therefore, recognizing and conserving patches of habitat that are stable during high flows yet remain underwater during periods of drought is critical for the maintenance of viable unionid populations.

In Texas, few studies have examined the influence of habitat on the distribution of unionid mussels. Notable exceptions include Karatayev and Burlakova (2008), Randklev et al. (2010), and Hammontree et al. (2012) whose studies obtained insightful results regarding low flow thresholds but were somewhat limited in that they did not examine mussel habitat utilization during periods of high river discharge. High and low flow habitat patches can be mutually exclusive, that is, areas within a stream that are stable during high flows can become dry during periods of low rainfall or discharge and areas within a stream that remain wetted during low flow can become unstable during high flows. As a result, it is important that both conditions are studied to develop a more comprehensive understanding of mussel habitat utilization.

The purpose of this study is to examine the effects of subsistence and high pulse flows on freshwater mussel populations in the lower and middle Brazos River. This information is important because two Texas state threatened species, *Q. houstonensis* and *T. macrodon*, inhabit this portion of the Brazos River (Randklev et al. 2010), and both of these species are being considered for protection under the ESA (USFWS 2009). Additionally, there are several significant and unique aggregations of mussels in this basin (Randklev et al. unpublished data), which may be impacted by changes to the flow regime in the lower and middle Brazos River. Emphasis in this study will be placed on providing results that will inform instream flow analysis and habitat management.

## Study Area

The Brazos River originates in New Mexico and is considered the third longest river in Texas, traveling 1,516 km before emptying into the Gulf of Mexico near Freeport, TX (Huser 2000). Flow in the Brazos River basin is regulated by several flood control dams and reservoirs (Gelwick and Li 2002; Osting et al. 2004). In the lower and middle Brazos River, where this study is located, the nearest on-channel impoundment is Lake Brazos in Waco, TX located approximately 190 km upstream, although there are also

several tributary impoundments within our study area as well. Land use in the lower Brazos River basin is predominately agricultural and open rangeland.

## Site Selection

The reaches of the TIFP used in this study were selected based on the following criteria: 1) presence of live mussels, which was determined through reconnaissance surveys, 2) ease of access, and 3) diversity of instream habitat as it relates to mesohabitat type. Of the five reaches examined initially, three were identified as meeting these criteria (Table 1; Figures 1-4). Sample sites within each TIFP study reach were selected using a stratified random sampling design. Specifically, the following habitat types were randomly selected for sampling using satellite imagery and subsequently monitored under low and high flow conditions: 1) bank habitats (BH), 2) the front of point bars (FPB), 3) behind point bars (BPB), 4) backwaters (BW), and 5) midchannels (MC) (Figure 5). 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 midchannel habitat. The front-of-point bars and behind-point bars were located in the up- or downstream portions, respectively, of sand and gravel bars. Backwater habitats were areas with minimal velocities and variable water depths and were often located near obstructions. Midchannel habitats were located in the middle of the river channel.

## Methods

### Mussel Surveys

Qualitative surveys using the timed search method were performed in each randomly selected mesohabitat type. At each site (i.e., mesohabitat type), we confined the search boundaries to the specific habitat type, ensuring that the search area was 50 m in length and did not exceed 15 m in width. Each site was surveyed tactilely and visually for a minimum of 1 person-hour (p-h). Additional 1 p-h searches were added until no new species were recorded. Effort was made to examine all of the available microhabitats at each site. The resulting data were then used to calculate species richness, catch per unit effort (CPUE) and total mussel abundance per site and mesohabitat type.

In addition to timed searches, we also estimated mussel densities at each site using a simple random sampling methodology. Specifically, we partitioned each site into a grid of 0.25 m<sup>2</sup> quadrats and randomly selected 15 - 17 for mussel sampling and habitat assessment. We excavated each quadrat to a depth of 20 cm. 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<sup>2</sup>) for each site.

### Habitat Sampling

Prior to mussel sampling, microhabitats were characterized within 15 -17 randomly selected 0.25 m<sup>2</sup> quadrats. 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. Water velocity and depth were measured using an electromagnetic flow meter (OTT MF Pro); the former

was measured near the bed surface because flows in this portion of the water column have a greater effect on mussels than those from the middle of the water column. Fliesswasserstammtisch (FST) hemispheres were constructed according to Statzner and Muller (1989) and were used to determine near-bed shear stress. The FST hemisphere numbers only correspond to the density of a given hemisphere, so the near-bed shear stress values were calculated using the minimum bottom shear stress values ( $\text{dyn/cm}^2$ ) presented by Statzner et al. (1991). Substrate compaction was measured using a soil penetrometer (Humboldt Soil Penetrometer, H-4200). Substrate type was determined by taking one sediment core (1.5" diameter) to a depth of 15 cm per quadrat. Substrate samples were then taken back to the laboratory and dried for 24 hours at  $100^\circ\text{C}$  in a convection oven. Dried samples were then passed through a series of 5, 10, 18, 35, 60, 120 and 230 number sieves (4, 2, 1, 0.5, 0.25, 0.125 and 0.063 mm, respectively), and the sediment in each sieve was weighed. The resulting information was used to create cumulative frequency distribution curves that were then used to determine the D16, D50, and D84 quantiles.

We returned to each site during periods of high flow and measured water depth, velocity, and shear stress from the center of each quadrat. We did not reevaluate substrate type or compactness because the duration between low and high flow sampling events was short, in some cases only a few months, so our working assumption was that substrate composition and type were unlikely to change. For low flows, discharge within each reach was estimated by measuring water velocity and depth at the center of 1-m cells along a single transect, reaching from bank to bank, at one site within each TIFP reach. During high flows, safety concerns prevented us from recording discharge, so we used nearby USGS gauging stations to estimate flows within each TIFP reach. Criteria for the high and low flow ranges were based on TPWD TIFP target base flow ranges for the Brazos River (Table 2). The substrate and hydraulic variables used to describe mussel microhabitat were calculated using the formulae listed in Table 3.

#### Benthic Macroinvertebrate Sampling

Benthic macroinvertebrates were sampled using a Hess sampler ( $0.25 \text{ m}^2$ ) at each of the 15  $0.25 \text{ m}^2$  quadrat mussel sampling locations within each sampling location and on the same dates listed in Table 1. These samples were collected in conjunction with the mussel samples so that habitat data collected from each quadrat (as described in the methods above) could be utilized for both mussel and benthic macroinvertebrate associations. Benthic macroinvertebrates were sampled prior to mussel sampling to avoid any disturbance associated with sediment excavation during mussel sampling. Each individual benthic macroinvertebrate sample was placed in a whirlpack and preserved with 95% ethanol and stored at the TPWD River Studies Lab in San Marcos, TX. As per the contract, samples are being stored for future processing when funds are available.

#### 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<sup>th</sup> 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).

In ecological studies, the 95<sup>th</sup> quantile is often used to evaluate limiting factors, but our sample size was small ( $n = 231$ ) so we modeled 3 extreme quantiles (95<sup>th</sup>, 90<sup>th</sup>, and 85<sup>th</sup>). 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 based on the Akaike information criterion (AIC) provided it gave non-0 parameter estimates for the model coefficients. We calculated AIC as equal to  $n \times \ln(\text{deviance of the model of interest}/n) + 2K$ , where  $n$  is the total sample size, and  $K$  is the number of estimated variables + 2 (intercept and residual variance) (Vaz et al. 2008). We then used the best-fitting functions to fit the following multiple quantile regression models: 1) Substrate model (Penetrometer + D50), 2) Low and High flow hydraulics models ( $Re + \tau + RSS$ ), 3) Low and High flow substrate stability models ( $\tau + RSS$ ), and 4) an intercept only model for comparison. Prior to fitting these models, all of the potential variables (see Table 3) were screened for collinearity, and redundant variables were excluded from further analyses. Pearson correlation was used to evaluate collinearity, and a correlation coefficient  $> 0.8$  for two variables was interpreted as being redundant (i.e., they conveyed the same information). 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  $1 - (\text{deviance of the model of interest divided by the deviance of the intercept-only model})$  (Allen and Vaughn 2010). For each quantile, we report AIC differences ( $\Delta_i$ ) and Akaike weights ( $w_i$ ) and the average pseudo- $R^2$  calculated from the best-performing models ( $\Delta_i < 2$ ) (Burnham and Anderson 2002). Differences in AIC provide a way to easily interpret the relative differences between the model of interest and the best-fitting model. For a given dataset and set of models, Akaike weights provide a means of interpreting the relative likelihood of a particular model through repeated sampling (Burnham and Anderson 2002). Quantile regression analyses were performed using the QUANTREG package in R version 3.02 (R Foundation for Statistical Computing, Vienna, Austria).

We also developed habitat suitability criteria based on Froude number, Reynolds number, Boundary Reynolds number, shear stress (inferred from FST hemispheres), and substrate type. For continuous variables, non-parametric tolerance limits (Bovee 1986) were used to construct suitability criteria for all mussel species. 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. For categorical variables, suitability curves were developed using the Strauss Linear Food Selection Index (Strauss 1979). The values of this index are based on the difference between the proportion of a particular habitat category utilized by a species and the proportion of that category that is available. The sampling variance in the linear index allows for a statistical comparison between the calculated value and the null-hypothesis value of zero (Strauss, 1979).  $P$ -values less than or equal to 0.05 were considered significant. Suitability values were assigned to each index value based on significance as follows: 1 = significant, positive values, 0.5 = non-significant, positive



values, 0.2 = non-significant, negative values and 0 = significant negative values (Persinger et al. 2011).

In addition to suitability criteria, we used Indicator Species Analysis (Dufrêne and Legendre 1997) to test the affinities of different mussel species to different habitat types. Indicator species analysis assigns an indicator value (IV) to each taxon by calculating the product of the relative frequency (percent occurrence of a taxon among sample units in each group) and relative average abundance (percent of the total abundance of a taxon within each group) of each species to a group. The probability of achieving an equal or larger IV value among groups ( $P$ ) was estimated based on 999 random permutations of the original data (Dufrêne and Legendre 1997). Indicator species analysis was performed with the INDICSPECIES package in R version 3.02 (R Foundation for Statistical Computing, Vienna, Austria).

Finally, a post-hoc  $X^2$  was performed to test whether the observed differences in mussel abundance and richness by habitat type could be explained by shear stress, RSS, Re, particle size, and substrate compactness. Following Gangloff and Feminella (2006), we drew horizontal and vertical lines connecting the maximum x and y values and then constructed a diagonal line connecting the maximum x and y values for mussel abundance or richness and an environmental variable of interest. All points falling above the diagonal line were given a score of 1 whereas those below the diagonal were given a score of -1. If the data on the scatter plot were random, the number of points above and below the diagonal line should have approximately a 50:50 distribution.

## Results

### Timed Searches

A total of 2,135 live individuals of 12 species were collected from the three study reaches. Live mussels were observed at all of the survey sites (Table 4). The number of species at each site ranged from 4 to 9 ( $\bar{x} \pm SE$ ;  $6.93 \pm 0.41$ ). Species richness across the three study reaches was generally the same and averaged 6.6 (0.67), 7.2 (0.80), and 7 (0.77) for Mussel Shoals (MS), Washington on the Brazos (WOB), and Wildcat Bend (WCB), respectively. *Cyrtoneias tampicoensis* (Tampico pearlymussel) and *Q. houstonensis* were the most ubiquitous species and occurred at all 15 sites. In the MS and WOB study reaches, prevalence was generally the same for *Amblema plicata* (three-ridge), *C. tampicoensis*, and *Q. houstonensis* while *T. macrodon* was the most common species. WOB was slightly different in that *Lampsilis teres* (yellow sandshell) was also widespread, occurring at all 5 sites compared to only 3 in the MS reach. For WCB, *C. tampicoensis*, *Leptodea fragilis* (fragile papershell), and *Q. houstonensis* were the most prevalent (Table 4). *Q. houstonensis* and *T. macrodon* are currently considered state-threatened and are candidates for listing under the ESA; the species occurred at 15 and 14 sites, respectively.

Overall, CPUE ranged from 7 to 211 mussels/p-h ( $63.68 \pm 14.69$ ), and total abundance ranged from 13 to 402 live individuals ( $119.00 \pm 30.73$ ) per site (Table 4). CPUE across the three study reaches was similar, averaging 68.95 ( $\pm 38.65$ ), 66.70 ( $\pm 22.46$ ), and 55.40 ( $\pm 15.55$ ) for MS, WOB, and WCB, respectively. *C. tampicoensis* (44% of all live individuals;  $n = 950$ ), *A. plicata* (18% of all live individuals;  $n = 381$ ), and *L. teres* (10% of all live individuals;  $n = 209$ ) were the most abundant species across all three reaches; no other species comprised more than 10% of the live individuals collected.

Within MS and WOB, *C. tampicoensis*, *A. plicata* and *L. fragilis* (only found in WOB) were the most abundant species whereas *C. tampicoensis*, *L. teres*, *Q. houstonensis*, and *T. macrodon* were dominant in WCB. The two threatened species, *Q. houstonensis* (8 % of all live individuals; n = 168) and *T. macrodon* (6% of all live individuals; n = 138), were the fifth and sixth most abundant species overall.

#### Quadrat sampling

A total of 524 live individuals representing 10 species were collected during quantitative sampling; 15 to 17 quadrats were sampled at each site for a total effort of 230 quadrat samples across all sites. Species richness ranged from 0 to 9 ( $2.69 \pm 0.70$ ) per site (Table 5) and was, on average, highest within the WOB ( $7 \pm 0.94$ ) and WCB ( $6 \pm 1.58$ ) study reaches and lowest at MS ( $3.8 \pm 0.58$ ). Densities ranged from 0.00 to 6.93 mussels/0.25 m<sup>2</sup> ( $2.27 \pm 0.51$ ) and were highest within WOB ( $3.83 \pm 1.06$ ) and WCB ( $2.15 \pm 0.69$ ) and lowest at MS ( $0.85 \pm 0.16$ ). *Q. houstonensis* and *T. macrodon* were observed in quadrat samples at densities (mussels/0.25 m<sup>2</sup>) ranging from 0.00 to 1.53 ( $0.38 \pm 0.12$ ) and 0.00 to 1.27 ( $0.29 \pm 0.09$ ), respectively.

#### Mussel Mesohabitat Associations

For timed searches, the overall mean CPUE by habitat type was, on average, highest for bank ( $95.56 \pm 24.36$ ), backwater ( $57.25 \pm 30.53$ ), and behind point bar ( $107.89 \pm 49.61$ ) mesohabitats and lowest for front of point bar ( $40 \pm 24.34$ ) and midchannel ( $16.72 \pm 7.50$ ) (Figure 6). Mean species richness was generally the same across all habitat types although the average number of species found in the midchannel habitats ( $5 \pm 0.58$ ) was lower compared to bank ( $8.33 \pm 0.33$ ), backwater ( $7 \pm 1.15$ ), behind point bar ( $7.67 \pm 0.33$ ), and front of point bar ( $6.67 \pm 0.88$ ) habitats (Figure 6). The CPUE by habitat type for *Q. houstonensis* was highest in bank ( $8.17 \pm 2.74$ ), backwater ( $6.00 \pm 3.18$ ), and to a lesser extent, front of point bar habitats ( $6.17 \pm 5.17$ ) and lowest for behind point bars ( $2.61 \pm 1.12$ ) and midchannel ( $2.11 \pm 1.06$ ) habitats. For *T. macrodon*, CPUE was greatest in bank ( $6.83 \pm 3.81$ ), behind point bar ( $4.78 \pm 2.79$ ), and front of point bar ( $6.00 \pm 3.33$ ) and lowest for backwater ( $1.42 \pm 0.65$ ) and midchannel ( $1.22 \pm 0.68$ ) habitats (Figure 7). For quantitative sampling, habitat usage across all mussel species, and for *Q. houstonensis* and *T. macrodon*, was similar to that of the timed searches (Figures 6 & 7).

The suitability curves constructed from the timed-search data for all species of each mesohabitat type indicate that mussels are primarily using bank and behind point bar habitats (Figures 8). However, suitability values for these habitat types were lower than expected (i.e., they did not exceed 0.5), which may be due to the fact that mean mussel abundance across both habitat types was generally similar or that our sample size per habitat type (n = 3) was too low to differentiate mesohabitat preferences. By contrast, the suitability value for backwater, front of point bar, and midchannel was 0.2, which indicates that these habitats were less suitable for mussels. Suitability values calculated from the quantitative sampling data for all mussels were generally the same, except that backwater habitats, not behind point bar, were as suitable as bank habitats (Figure 9). Suitability curves for *Q. houstonensis* show that this species prefers bank, backwater, and front of point bar habitats (Figure 10). Similarly, *T. macrodon* also prefers bank habitats but may utilize behind and front of point bar habitats as well (Figure 10).

Among the 12 species examined, we identified potential indicator species for 4 of the 5 habitat types. For example, *A. plicata*, *Q. houstonensis*, *Q. verrucosa*, and *T. macrodon* had high IVs for bank habitats, but the IVs for these species were not significant. *Quadrula apiculata* and *U. imbecillis* were indicators of backwater habitats, *P. grandis*, *C. tampicoensis*, *L. teres*, and *P. ohiensis* had high affinities for behind-point bar habitats, and *L. fragilis* was indicator of midchannel habitats (Table 6), but IVs for these species were also not significant. The lack of statistical significance for indicator species likely relates to small sample sizes per habitat type and that several species had high IVs for more than one habitat type (Table 6).

Post-hoc  $X^2$  analysis revealed that the distribution of sample sites by mussel abundance and species richness against shear stress was significantly non-random during both high and low flows (Table 7). Generally, mussel abundance and richness decreased with increasing values for shear stress, and protected habitats (i.e., backwater, behind point bar, and bank habitats) had lower values regardless of stage of flow (Figure 11). Similarly, sample points by Reynolds number were non-randomly distributed; abundance and richness were highest at low Reynolds values and then decreased as Reynolds values increased. Protected habitats, on average, had lower values for this measure during both high and low flows (Figure 12). The only exception was for sites where the relationship between richness and Reynolds number appeared to be random. For RSS, substrate compactness (i.e., penetrometer) and substrate type (i.e., D50), the distribution of mussel abundance and richness sample points by discharge was random for the most part (Table 6; Figures 13 & 14).

### Mussel Microhabitat Associations

#### *Suitability curves*

Suitability criteria based on Reynolds and Froude numbers during low flows indicate that optimal mussel habitat occurs in areas where flow is subcritical ( $Fr < 0.05$ ), and its structure ranges from laminar to turbulent ( $0.01 < Re < 72,000$ ) (Figure 15). Criteria for Boundary Reynolds number corroborate these observations as optimal values for this variable ranged from 0 to 20, indicating smooth to transitional flows (Figure 15). During high flows, values for all three variables increased as expected, but optimal habitat continued to be defined by areas along the stream bottom where values for  $Fr < 0.05$  and Boundary Reynolds number remained below 20. Values for Reynolds number continued to correspond to laminar to turbulent flows, but the upper limit for suitable habitat increased from 72,000 to 120,000 (Figure 15). Suitability curves, based on FST hemispheres for both low and high flows, 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 16). Suitability criteria based on D50 particle size indicate that coarser substrates, such as gravel and pebbles, are considered optimal whereas finer particle sizes are considered usable and coarser sands are unsuitable (Figure 17). Criteria for substrate compactness indicate that mussels prefer substrates that are relatively firm (Figure 17). Suitability curves for *Q. houstonensis* and *T. macrodon* showed similar results to those developed for all mussels (Figures 18-22).

### Quantile regression

Pearson correlation coefficients between all hydraulic variables indicate that shear stress and Froude number were highly correlated during low and high flows ( $r = 0.81$  and  $0.86$ , respectively). Similarly, shear stress ( $\tau$ ) and shear velocity ( $U^*$ ) were strongly correlated at low and high flows ( $r = 0.98$  and  $0.98$ , respectively), and Boundary Reynolds number ( $Re^*$ ) was correlated with D50 particle size at low and high flows ( $r = 0.87, 0.87$ ). To ensure that our results were comparable to other studies, we chose to retain shear stress and D50 particle size and ignore shear velocity, Froude number and Boundary Reynolds number.

Hydraulic variables estimated at low and high flows appeared to be limiting factors for mussel species richness and abundance (Figures 23 - 26). The relationships between shear stress and mussel species richness were best described by exponential and quadratic functions for all quantiles (Figure 23 & 24). The relationships between RSS and species richness and abundance were linear, but the rate of change (i.e., the slope) across all quantiles was greater for higher flows (Figures 23 & 24). Similarly, linear functions best described the limiting relationship between  $Re$  and mussel abundance and richness (Figure 25). For D50 particle size categories and substrate compactness (i.e., penetrometer measurements), quadratic functions best described the relationships, across all quantiles, between these variables and mussel abundance and richness (Figure 26).

Substrate stability models (shear stress + RSS) performed best, across all quantiles, for both species richness and mussel abundance (Tables 7 & 8). Overall, HF shear stress appeared to be the most important variable because it was included in all of the best performing models (Tables 8 & 9). By contrast, HF RSS appeared to be less important as it was only in models that also included shear stress and  $Re$ . HF  $Re$  also appeared to be important, but low AIC difference values ( $\Delta_i$ ), combined with very low AIC weights ( $w_i$ ), indicate that there is very little evidence that this factor explains substantial variation in mussel abundance or richness. Similarly, several LF hydraulic models appeared to be important, but low AIC differences and AIC weights indicate that these models are not plausible (Tables 8 & 9). All models with substrate variables performed poorly; neither univariate nor multivariate substrate models were among the best performing models (Tables 8 & 9).

### Discussion

The three TIFP reaches surveyed during this study contain a diverse and abundant mussel fauna that includes two species currently being considered for protection under the ESA. Mussel abundance and species richness was generally the same across all three reaches, and the dominant species within each were also similar. We found that abundance and, to a lesser extent, species richness (for both qualitative and quantitative sampling) were generally higher in bank, backwater, and behind point bar habitats. Based on the habitat suitability curves, these same habitats were also determined to be more suitable for mussels than front of point bar or midchannel habitats. For the two threatened species encountered during this study, *Q. houstonensis* appeared to prefer bank, backwater, and front of point bar habitats whereas bank, behind point bar and front of point bar habitats appeared more suitable for *T. macrodon*.

The differences in abundance and richness by habitat type are at least partially attributable to differences in physical habitat, particularly shear stress. We found that, on

average, mussel abundance and richness decreased with higher levels of shear stress and that bank, backwater, and behind of point bar habitat types had relatively low shear stress values during both low and high flows. By contrast, front of point bar and midchannel habitats had fewer mussels, were less speciose and generally had higher shear stress values, particularly during higher flows. These results support those of other studies, which suggest that substrate stability during high flows restricts mussel abundance and richness (Layzer and Madison 1995; Di Maio and Corkum 1995; Morales et al. 2006; Gangloff and Feminella 2006; Allen and Vaughn 2010) and that channel form features that minimize hydraulic stress are optimal mussel habitats (Morales et al. 2006; Steuer et al. 2008). However, our results differ from those of more recent studies (e.g., Morales et al. 2006; Allen and Vaughn 2010) that examine mussel-substrate stability relationships in that RSS was not a significant determinant of mussel abundance or richness. However, both population parameters did appear to decline with higher RSS levels.

Host fish availability and infection strategies may also explain the observed mesohabitat preferences. Freshwater mussels rely on certain fish species for part of their reproductive cycle, and the absence of such fish during critical periods could result in the absence of mussels even if all other habitat characteristics are ideal (Haag and Warren; 1998). It is well known that fish are able to move out of harm's way during periods of high river discharge to areas that provide protection from scouring flows. Fish encysted with juvenile mussels (i.e., glochidia) during such times could conceivably seed those locations with mussels. If particular refugia were visited by a large number of fish bearing glochidia, there is a greater likelihood of those mussel populations taking hold. These questions were outside of the scope of this project, but future studies, particularly those that evaluate habitat availability during low and high flow events, should consider measuring fish community composition within these habitat types.

Our mussel microhabitat association results mirror those from the mesohabitat analyses in that hydraulic variables related to substrate stability, particularly shear stress, were the most limiting to mussel species richness and abundance. These results corroborate those of Allen and Vaughn (2010), who found that RSS and shear stress were important determinants of mussel abundance and richness for hydraulic models in the 95<sup>th</sup>, 90<sup>th</sup>, and 85<sup>th</sup> quantiles. However, our results differ from that study in that we found that RSS was not predictive by itself, which indicates that it was less important than shear stress in determining mussel abundance and richness. There were several models that included Reynolds number which were identified as best-performing, but the AIC differences and weights for those models were low, indicating very little empirical support. Thus, our results suggest that Reynolds number may not always be a useful determinant of mussel abundance and richness. Moreover, it is unclear what the values of Re mean in relation to population parameters, such as mussel abundance and richness. Stone et al. (2004) speculated that high Re values may prohibit settlement of glochidia or result in substrate instability, but neither of these claims has been tested. Similarly, models that included substrate variables performed poorly, which supports the premise that these variables are not important determinants of mussel abundance or richness by themselves (Strayer and Ralley 1993).

Models including hydraulic variables estimated at low flows performed poorly, and therefore these factors appear to have little influence on mussel abundance and richness. These results support those of other studies that have shown that hydraulic characteristics

are more important to mussel habitat at high flows than at low flows (Gangloff and Feminella 2006; Allen and Vaughn 2010). For studies that plan to utilize mussels to determine instream flow criteria for benthic organisms, this means that these variables must be measured during high flow conditions. During this study, we found that divers could safely measure flow, depth and shear stress in flows near 42 m<sup>3</sup>/s. However, sampling at higher discharge rates became too dangerous due to floating debris and the inability to maintain position along the stream bottom. In situations where it is desirable to measure shear stress in flows that exceed 42 m<sup>3</sup>/s, we recommend that an Acoustic Doppler Current Profiler (ADCP) be used. When using an ADCP, shear stress will be enumerated using formulae that integrate depth and slope, so careful attention should be given to how those values compare with those derived from FST Hemispheres.

Although our results indicate that hydraulic variables estimated at low flows are not useful for determining mussel-limiting factors, they do provide a quantitative description of habitat that is not conditional on flow. Moreover, there is some evidence that these variables do in fact constrain mussel abundance and richness. Steuer et al. (2008) found that estimates for several hydraulic variables during low flows were predictive for mussels, and the authors subsequently hypothesized that minimum threshold values for these variables likely exist in conditions where flow becomes too stagnant to deliver food or transport waste. Thus, the reason low flow models were not limiting in our study could be that we did not measure habitat under low flow conditions that were truly limiting. However, it is unlikely this was the case as fresh-dead shell material was observed during low flow sampling at most of our locations. Thus, it could be that our measurement of these variables was too coarse to derive meaningful results, or there are other variables that are better surrogates for habitat suitability during low flow conditions. For example, recent studies have indicated that water temperature may be a limiting factor during periods of low flow, which is logical because mussels are poikilotherms (Pandolfo et al. 2010). Unfortunately, the thermal tolerances of most mussel species that occur in Texas are unknown or are based solely on laboratory studies that do not assess the temperatures characteristic of Texas streams during warm weather months when flows are at their lowest and the environment is most limiting. Therefore, further studies are needed if temperature is to be assessed as an instream flow criterion for mussels.

Our finding that shear stress is the main limiting factor at both the mesohabitat and microhabitat scales indicates that this variable is a useful predictor of mussel abundance and richness. Thus, instream flow studies should continue to examine this variable to better describe the near-bed hydraulic forces that are important to mussels. Continued assessment of this variable at locations with and without mussels across different river drainages might eventually lead to the development of species-specific microhabitat 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. Thus, these studies could serve as guides for developing meaningful suitability criteria based on shear stress.

Table 1. Location, survey date (high and low flows), and presence of live mussels for surveys sites within the following Texas Instream Flow Program (TIFP) study reaches: Mussel shoals (MS); Washington on the Brazos (WOB); and Wildcat Bend (WCB). Site codes denote the following habitat types: BH = bank habitat; BPB = immediately downstream of point bar; FPB = immediately upstream of point bar; BW = backwater; and MC = mid-channel Coordinates are NAD83, UTM Zone 14R.

TIFP Site #	TIFP Site Code	Site	Coordinates		Low Flow: Date of collection	High Flow: Date of collection	Live mussels
			x	y			
12050	MS	BH	745350	3385973	7-Aug-13	1-Oct-2013	Y
12050	MS	BW	744568	3387406	9-Aug-13	1-Oct-2013	Y
12050	MS	BPB	744611	3386750	6-Aug-13	15-Oct-2013	Y
12050	MS	FPB	743992	3387736	5-Aug-13	15-Oct-2013	Y
12050	MS	MC	745098	3386334	9-Aug-13	17-Oct-2013	Y
12030	WOB	BH	771465	3364317	19-Dec-2012	25-Sept-2013	Y
12030	WOB	BW	771665	3363565	10-Sept-13	24-Sept-2013	Y
12030	WOB	BPB	773732	3363422	17-Dec-2012	3-Oct-2013	Y
12030	WOB	FPB	772804	3364195	18-Dec-2012	25-Sept-2013	Y
12030	WOB	MC	773250	3363937	11-Sept-2013	3-Oct-2013	Y
12020	WCB	BH	779622	3316032	13-Nov-2012	1-Oct-2013	Y
12020	WCB	BW	777827	3318615	12-Sept-2013	2-Oct-2013	Y
12020	WCB	BPB	778708	3318176	17-Nov-2012	2-Oct-2013	Y
12020	WCB	FPB	779223	3317295	15-Nov-2012	2-Oct-2013	Y
12020	WCB	MC	777703	3317471	11-Sept-2013	2-Oct-2013	Y

Table 2. Target flows and sampled flows.

USGS gauging station	08108700 Bryan		08111500 Hempstead
TIFP Site	MS	WOB	WCB
TIFP Site #	12050	12030	12020
High - Target flows (m <sup>3</sup> /s)	29.02 – 41.77	29.02 – 41.77	43.89 – 70.79
Sampled (m <sup>3</sup> /s)	56.92 – 64.85	28.31 – 44.17	54.93 – 60.31
Mean increase in water depth (m)	0.67 – 0.87	0.69 – 0.90	0.75 – 0.90
Low - Target flows (m <sup>3</sup> /s)	7.79 – 15.57	7.79 – 15.57	12.74 – 24.07
Sampled (m <sup>3</sup> /s)	12.37	12.58	10.39

Table 3. Summary of physical variables measured in the study.  $U$  = mean bottom velocity (cm/s); except for calculating Reynolds and Froude number, in which case mean column velocity was used,  $d$  = water depth (cm),  $g$  = acceleration of gravity (980 cm/s<sup>2</sup>),  $\nu$  = kinematic viscosity of water (0.01 cm<sup>2</sup>/s),  $\rho_s$  = density of substrate (2.65 g/cm<sup>3</sup>),  $\rho$  = density of water (0.998 g/cm<sup>3</sup>),  $\theta_c$  = 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)	$3.5 \times D_{84}$	Topographic variation of the stream bottom
*Froude number (Fr, dimensionless)	$\sqrt{\frac{U^2}{gd}}$	Ratio of inertial to gravitational forces
*Reynolds number (Re, dimensionless)	$\frac{Ud}{\nu}$	Ratio of inertial to viscous forces
*Boundary Reynolds number (Re*, dimensionless)	$\frac{U_*k_s}{\nu}$	Roughness of flow near substrate
*Shear stress ( $\tau$ , dyn/cm <sup>2</sup> )	Derived from FST hemispheres; see Statzner et al. (1991)	Force of friction on substrate
Shear velocity ( $U_*$ , cm/s)	$\sqrt{\frac{\tau}{\rho}}$	Friction velocity
Critical shear stress ( $\tau_c$ , dyn/cm <sup>2</sup> )	$\theta_c g D_{50} (\rho_s - \rho)$	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 4. Mussel data for sites (Table 1) qualitatively sampled on the Brazos River. Numbers in columns are the total number of live individuals collected during timed-searches. TIFP Codes denote the following: MS = Mussel Shoals; WOB = Washington on the Brazos; and WCB = Wildcat Bend. Site codes denote the following habitat types: BH = bank habitat; BPB = immediately downstream of point bar; FPB = immediately upstream of point bar; BW = backwater; and MC = mid-channel.

Species	Common name	TIFP Code/Site Code														
		MS BH	MS BW	MS BPB	MS FPB	MS MC	WOB BH	WOB BW	WOB BPB	WOB FPB	WOB MC	WCB BH	WCB BW	WCB BPB	WCB FPB	WCB MC
Subfamily Anodontinae																
<i>Pyganodon grandis</i>	Giant floater	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
<i>Utterbackia imbecillis</i>	Paper pondshell	-	-	-	-	-	-	15	-	-	-	1	-	1	-	-
Subfamily Ambleminae																
<i>Amblema plicata</i>	Threeridge	94	7	6	3	2	98	110	8	30	8	-	4	8	2	1
<i>Quadrula apiculata</i>	Southern mapleleaf	-	-	-	-	-	3	17	-	-	-	4	2	-	4	-
<i>Quadrula houstonensis</i>	Smooth pimpleback	24	2	1	4	1	7	12	13	2	6	26	23	6	33	8
<i>Quadrula verrucosa</i>	Pistolgrip	2	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Subfamily Lampsilinae																
<i>Cyrtonaias tampicoensis</i>	Tampico pearlymussel	173	38	347	4	26	107	37	27	11	3	9	29	59	79	1
<i>Lampsilis teres</i>	Yellow sandshell	15	1	10	-	-	20	22	10	1	4	17	26	74	9	-
<i>Leptodea fragilis</i>	Fragile papershell	18	-	28	7	1	4	12	2	-	69	8	1	8	20	3
<i>Potamilus ohioensis</i>	Pink papershell	12	-	4	1	-	4	-	4	-	-	4	-	21	3	-
<i>Toxolasma parvum</i>	Lilliput	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-
<i>Truncilla macrodon</i>	Texas fawnsfoot	3	1	3	2	7	11	5	31	11	4	28	3	5	24	0
Total individuals		341	49	402	22	37	255	231	95	55	94	97	88	182	174	13
Time (p-h)		3	4	2	4	3	2	2	3	2	3	2	2	2	2	2
CPUE		113.7	12.3	201	5.5	12.3	127.5	115.5	31.7	27.5	31.3	48.5	44	91	87	6.5
Species richness		8	5	8	7	5	9	9	7	5	6	8	7	8	8	4
Length (m)		50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Width (m)		10	10	10	15	15	6.5	7	8	15	15	6.8	15	15	15	15

Table 5. Mussel data for sites (Table 1) quantitatively sampled on the Brazos River. Numbers in columns are the total number of live individuals collected during timed-searches. TIFP Codes denote the following: MS = Mussel Shoals; WOB = Washington on the Brazos; and WCB = Wildcat Bend. Site codes denote the following habitat types: BH = bank habitat; BPB = immediately downstream of point bar; FPB = immediately upstream of point bar; BW = backwater; and MC = mid-channel.

Species	Common name	TIFP Code/Site Code														
		MS BH	MS BW	MS BPB	MS FPB	MS MC	WOB BH	WOB BW	WOB BPB	WOB FPB	WOB MC	WCB BH	WCB BW	WCB BPB	WCB FPB	WCB MC
Subfamily Anodontinae																
<i>Pyganodon grandis</i>	Giant floater	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Utterbackia imbecillis</i>	Paper pondshell	-	-	-	-	-	2	21	-	-	-	-	-	-	-	-
Subfamily Ambleminae																
<i>Amblema plicata</i>	Threeridge	6	2	-	1	-	30	33	5	23	2	1	-	1	1	-
<i>Quadrula apiculata</i>	Southern mapleleaf	-	-	-	-	-	1	7	-	-	-	2	5	-	-	-
<i>Quadrula houstonensis</i>	Smooth pimpleback	1	2	-	2	1	3	6	11	8	-	23	11	1	20	-
<i>Quadrula verrucosa</i>	Pistolgrip	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Subfamily Lampsilinae																
<i>Cyrtonaias tampicoensis</i>	Tampico pearlymussel	9	4	18	1	6	21	3	4	1	-	4	8	7	4	-
<i>Lampsilis teres</i>	Yellow sandshell	-	-	-	-	-	16	14	9	1	2	11	5	18	1	-
<i>Leptodea fragilis</i>	Fragile papershell	-	-	1	2	1	-	9	1	1	13	3	1	2	5	-
<i>Potamilus ohioensis</i>	Pink papershell	1	-	-	-	-	1	-	-	-	-	1	-	2	1	-
<i>Toxolasma parvum</i>	Lilliput	1	-	-	-	-	7	2	-	1	-	1	-	2	-	-
<i>Truncilla macrodon</i>	Texas fawnsfoot	-	-	-	2	3	4	9	8	9	2	19	1	3	6	-
Total		18	8	19	8	11	85	104	38	44	19	65	31	36	38	0
# of Quadrats		15	15	15	15	15	15	15	15	16	15	15	15	17	17	15
Density (mussels/0.25m <sup>2</sup> )		1.2	0.5	1.3	0.5	0.7	5.7	6.9	2.5	2.8	1.3	4.3	2.1	2.1	2.2	0.0
Species richness		5	3	2	5	4	9	9	6	7	4	9	6	8	7	0
Length (m)		50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Width (m)		10	10	10	15	15	6.5	7	8	15	15	6.8	15	15	15	15

Table 6. Indicator values derived from qualitative sampling for all mussel species based on relative abundance and frequency of occurrence across all mesohabitat types. *P* is the probability of exceeding the observed indicator value and was calculated using 999 permutations of the original data. Bold numbers indicate the value that is highest for each species.

Species	Common name	Habitat type					P-value
		BH	BPB	BW	FPB	MC	
Subfamily Ambleminae							
<i>Amblema plicata</i>	Threeridge	<b>0.58</b>	0.24	0.56	0.30	0.17	0.64
<i>Quadrula apiculata</i>	Southern mapleleaf	0.39	0.00	<b>0.65</b>	0.21	0.00	0.51
<i>Quadrula houstonensis</i>	Smooth pimpleback	<b>0.58</b>	0.35	0.47	0.48	0.30	0.50
<i>Quadrula verrucosa</i>	Pistolgrip	<b>0.47</b>	0.00	0.00	0.33	0.00	1.00
Subfamily: Anodontinae							
<i>Pyganodon grandis</i>	Giant floater	0.00	<b>0.58</b>	0.00	0.00	0.00	1.00
<i>Utterbackia imbecillis</i>	Paper pondshell	0.14	0.14	<b>0.54</b>	0.00	0.00	1.00
Subfamily: Lampsilinae							
<i>Cyrtonaias tampicoensis</i>	Tampico pearlymussel	0.55	<b>0.68</b>	0.33	0.31	0.18	0.40
<i>Lampsilis teres</i>	Yellow sandshell	0.50	<b>0.67</b>	0.48	0.18	0.08	0.39
<i>Leptodea fragilis</i>	Fragile papershell	0.41	0.46	0.22	0.32	<b>0.64</b>	0.69
<i>Potamilus ohioensis</i>	Pink papershell	0.61	<b>0.74</b>	0.00	0.24	0.00	0.13
<i>Toxolasma parvum</i>	Lilliput	<b>0.41</b>	0.00	<b>0.41</b>	0.00	0.00	1.00
<i>Truncilla macrodon</i>	Texas fawnsfoot	<b>0.55</b>	0.53	0.26	0.52	0.23	0.71

Table 7. Summary of post-hoc  $X^2$  analyses for mussel abundance and species richness for Shear stress, RSS, Reynolds number, Penetrometer, and D50 during low and high discharge. P-values are provide only for sites that were found to be non-randomly distributed.

Parameter	Discharge	Covariate	$X^2$	d.f.	<i>p</i> -value
Abundance	Low	Shear stress	11.29	1	$p < 0.001$
Abundance	High	Shear stress	8.07	1	$p < 0.001$
Richness	Low	Shear stress	11.29	1	$p < 0.001$
Richness	High	Shear stress	8.07	1	$p < 0.001$
Abundance	Low	RSS	1.67	1	n.s.
Abundance	High	RSS	4.57	1	$p < 0.05$
Richness	Low	RSS	1.67	1	n.s.
Richness	High	RSS	0.60	1	n.s.
Abundance	Low	Reynolds	5.40	1	$p < 0.05$
Abundance	High	Reynolds	3.00	1	$p < 0.10$
Richness	Low	Reynolds	4.57	1	$p < 0.05$
Richness	High	Reynolds	0.69	1	n.s.
Abundance	-	Penetromer	1.67	1	n.s.
Richness	-	Penetromer	0.60	1	n.s.
Abundance	-	Substrate type	5.40	1	$p < 0.05$
Richness	-	Substrate type	0.60	1	n.s.

Table 8. Summary of small-sample Akaike information criterion ( $AIC_c$ ) selection of univariate and multiple 95<sup>th</sup>, 90<sup>th</sup>, and 85<sup>th</sup> quantile regression models for mussel abundance. LF and HF designate that the model used hydraulic variables estimated at low or high flows.  $K$  = number of parameters in the model = 2,  $\Delta_i$  =  $AIC_c$  of the model relative to lowest  $AIC_c$ ,  $w_i$  = Akaike weight,  $R^2$  = pseudo- $R^2$  of an averaged model using the best performing models ( $\Delta_i < 2$ ). Only the 5 best-performing models are shown. Abbreviations for variables are shown in Table 1.

95 <sup>th</sup> quantile ( $R^2 = 0.38$ )					90 <sup>th</sup> quantile ( $R^2 = 0.33$ )					85 <sup>th</sup> quantile ( $R^2 = 0.32$ )				
Rank	Model	$K$	$\Delta_i$	$w_i$	Rank	Model	$K$	$\Delta_i$	$w_i$	Rank	Model	$K$	$\Delta_i$	$w_i$
1	HF $\tau$ + RSS	4	0.00	1.00	1	HF $\tau$ + RSS	4	0.00	0.71	1	HF $\tau$ + RSS	4	0.00	0.90
2	HF Re + $\tau$ + RSS	5	16.00	0.00	2	HF Re + $\tau$ + RSS	5	1.82	0.29	2	HF Re + $\tau$ + RSS	5	4.53	0.09
3	HF $\tau$	3	23.14	0.00	3	HF $\tau$	3	13.34	0.00	3	LF Re + $\tau$ + RSS	5	10.80	0.00
4	HF Re	3	25.56	0.00	4	HF Re	3	14.77	0.00	4	HF $\tau$	3	13.11	0.00
5	LF $\tau$ + RSS	4	26.38	0.00	5	LF $\tau$	3	15.57	0.00	5	HF Re	3	14.42	0.00

Table 9. Summary of small-sample Akaike information criterion ( $AIC_c$ ) selection of univariate and multiple 95<sup>th</sup>, 90<sup>th</sup>, and 85<sup>th</sup> quantile regression models for mussel species richness. LF and HF designate that the model used hydraulic variables estimated at low or high flows.  $K$  = number of parameters in the model = 2,  $\Delta_i$  =  $AIC_c$  of the model relative to lowest  $AIC_c$ ,  $w_i$  = Akaike weight,  $R^2$  = pseudo- $R^2$  of an averaged model using the best performing models ( $\Delta_i < 2$ ). Only the 5 best-performing models are shown. Abbreviations for variables are shown in Table 2.

95 <sup>th</sup> quantile ( $R^2 = 0.41$ )					90 <sup>th</sup> quantile ( $R^2 = 0.36$ )					85 <sup>th</sup> quantile ( $R^2 = 0.33$ )				
Rank	Model	$K$	$\Delta_i$	$w_i$	Rank	Model	$K$	$\Delta_i$	$w_i$	Rank	Model	$K$	$\Delta_i$	$w_i$
1	HF $\tau$ + RSS	5	0.00	1.00	1	HF $\tau$ + RSS	5	0.00	1.00	1	HF $\tau$ + RSS	5	0.00	0.99
2	HF Re + $\tau$ + RSS	5	10.40	0.00	2	HF Re + $\tau$ + RSS	5	13.21	0.00	2	HF Re + $\tau$ + RSS	5	13.21	0.01
3	HF $\tau$	4	18.40	0.00	3	HF $\tau$	4	17.89	0.00	3	LF Re + $\tau$ + RSS	5	17.89	0.00
4	LF $\tau$ + RSS	4	25.49	0.00	4	LF $\tau$ + RSS	4	18.36	0.00	4	LF $\tau$ + RSS	4	18.36	0.00
5	HF Re	3	25.84	0.00	5	LF Re + $\tau$ + RSS	5	18.89	0.00	5	HF $\tau$	4	18.89	0.00

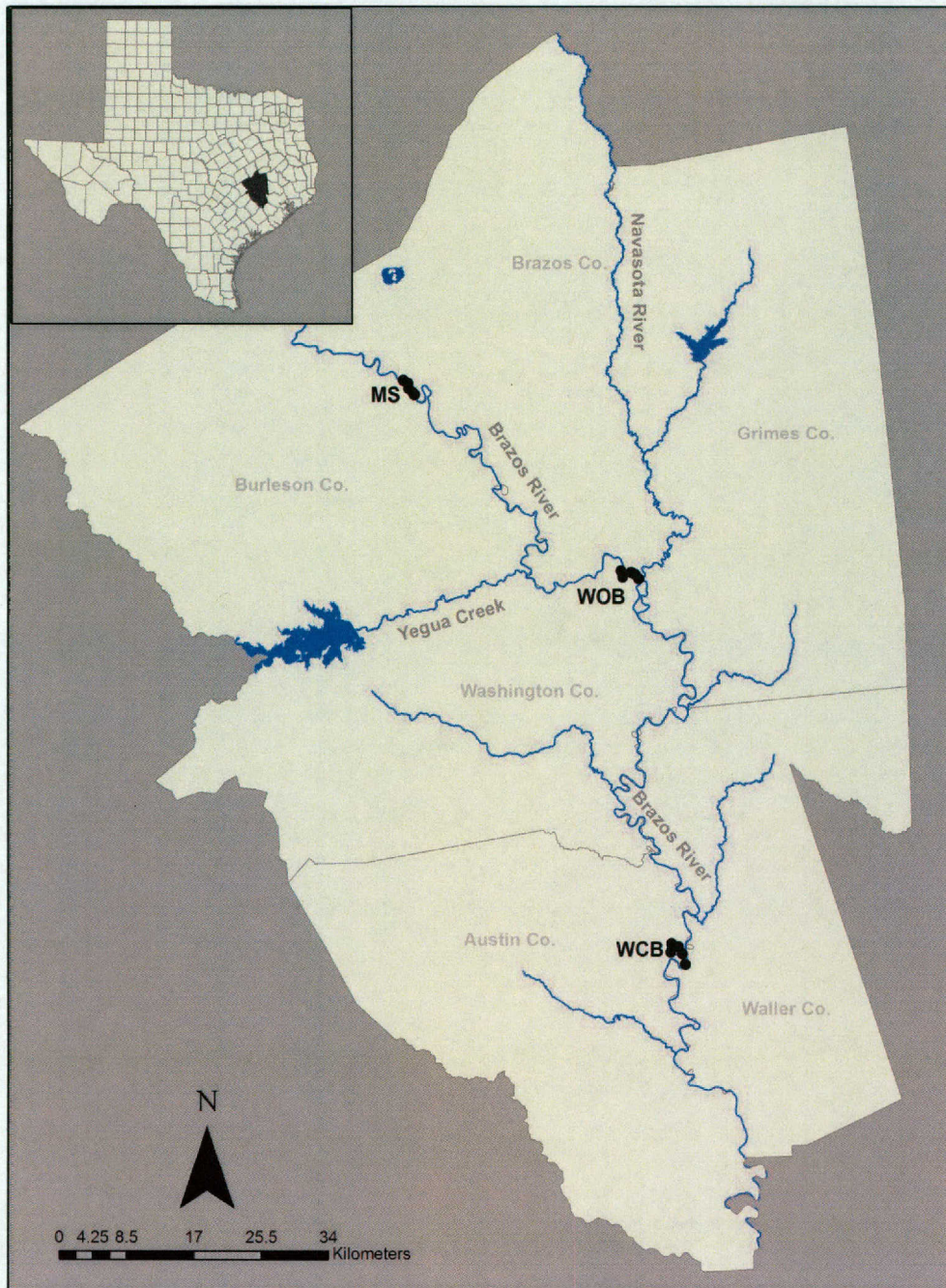


Figure 1. TIFP sites sampled in the middle and lower Brazos River. TIFP Codes denote the following: MS = Mussel Shoals; WOB = Washington on the Brazos; and WCB = Wildcat Bend.

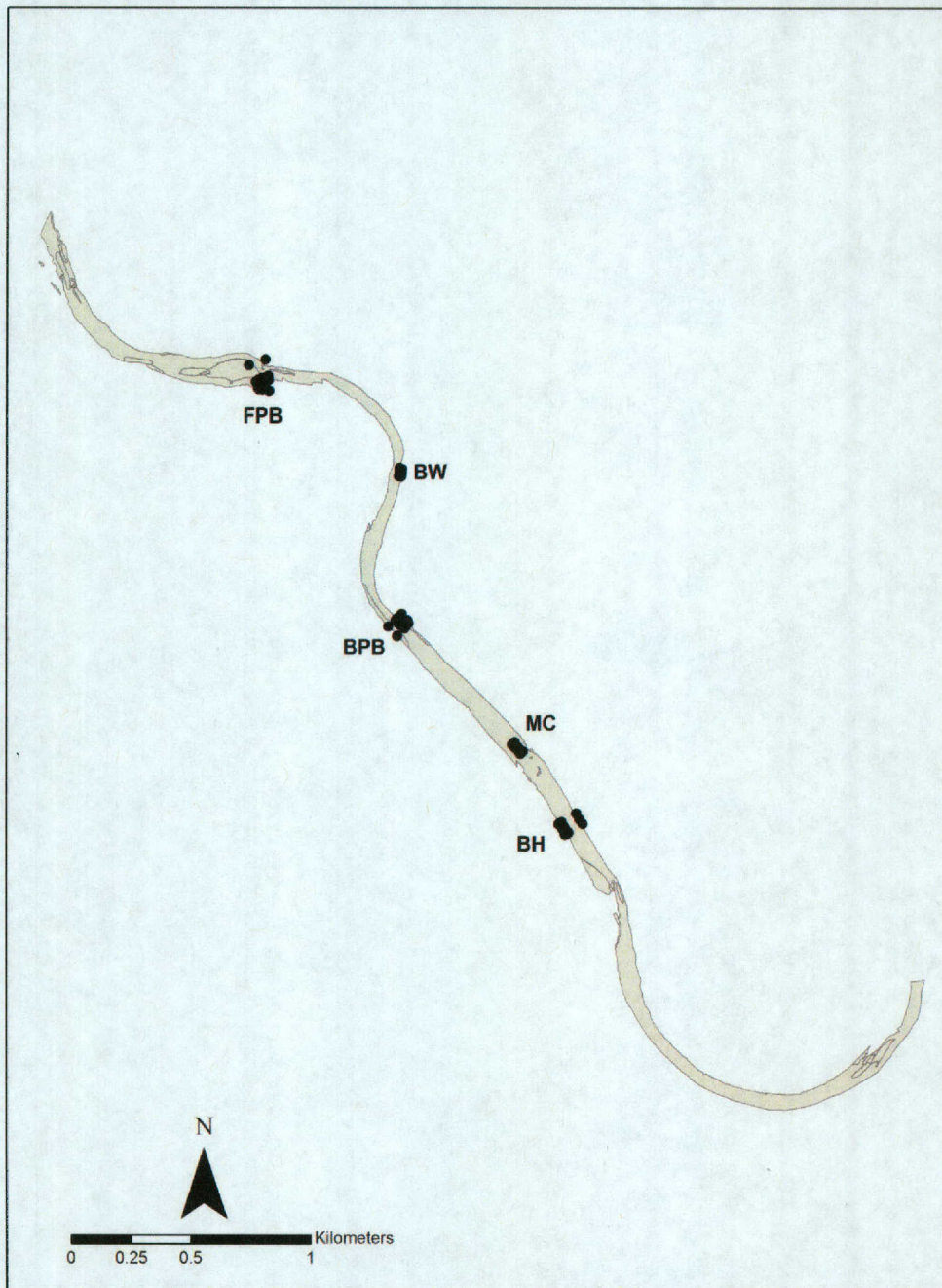


Figure 2. Mussel sampling locations in Mussel Shoals (MS). Site codes denote the following habitat types: BH = bank habitat; BPB = immediately downstream of point bar; FPB = immediately upstream of point bar; BW = backwater; and MC = mid-channel.



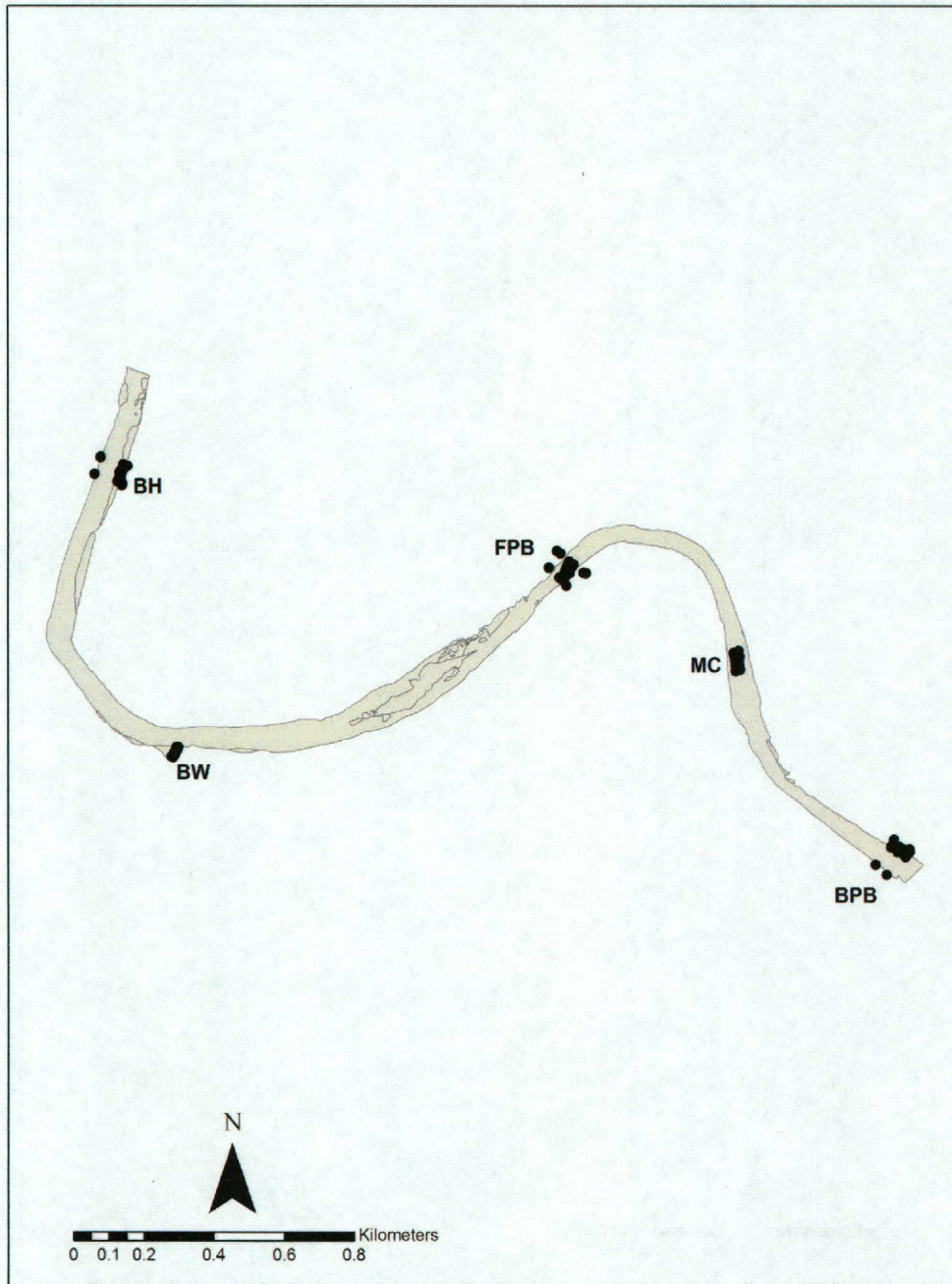


Figure 3. Mussel sampling locations in Washington on the Brazos (WOB). Site codes denote the following habitat types: BH = bank habitat; BPB = immediately downstream of point bar; FPB = immediately upstream of point bar; BW = backwater; and MC = mid-channel.

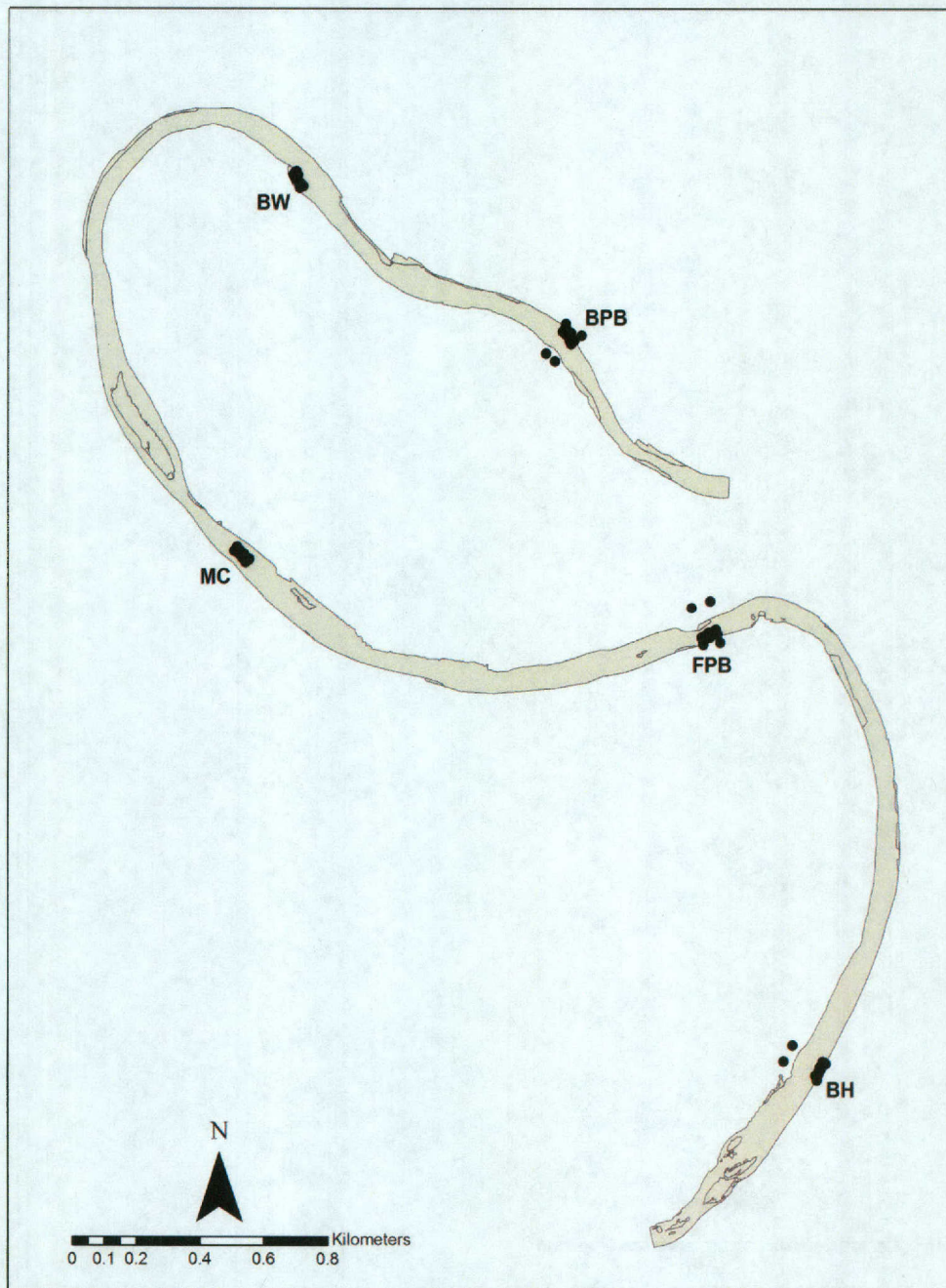


Figure 4. Mussel sampling locations in Wildcat Bend (WCB). Site codes denote the following habitat types: BH = bank habitat; BPB = immediately downstream of point bar; FPB = immediately upstream of point bar; BW = backwater; and MC = mid-channel.



Figure 5. Mesohabitat types sampled within each TIFP study reach: A) bank habitats, B) behind point bars (BPB); C) backwater (BW); D) front of point bars (FPB); and midchannel (MC).

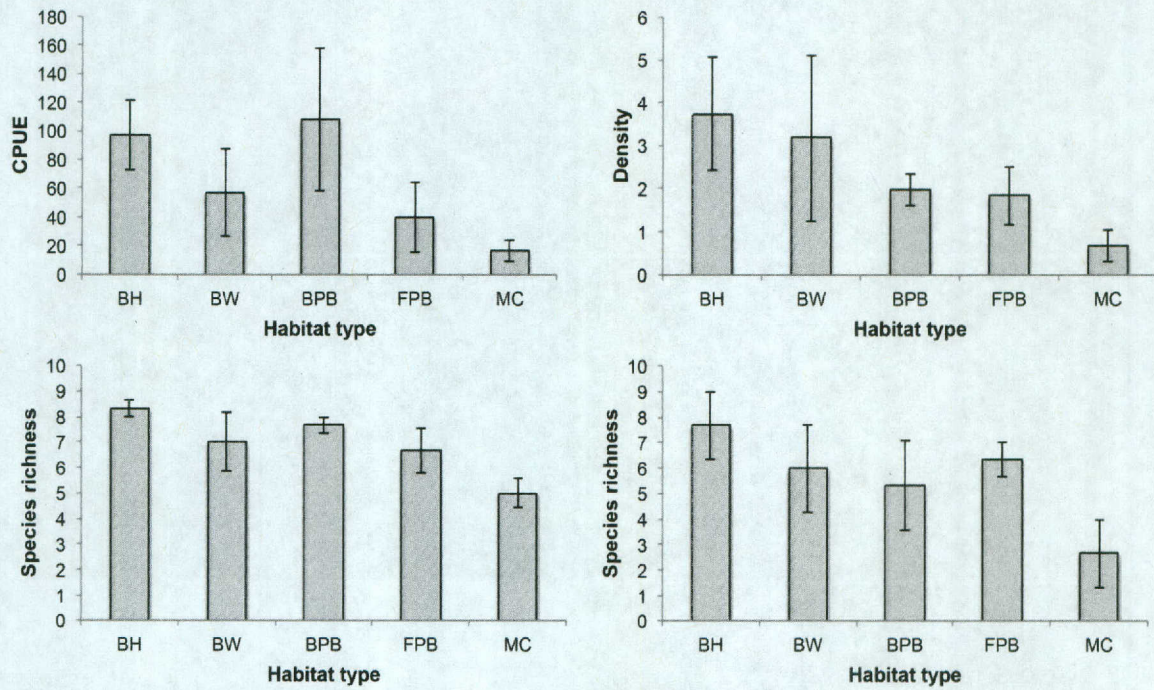


Figure 6. Mean CPUE (mussels/p-h) and species richness (left) and mussel densities (mussels/0.25m<sup>2</sup>) and richness (right) from timed-searches and quantitative sampling, respectively, by mesohabitat type. Error bars = ± 1 SE and acronyms for each habitat type correspond to the following: (BH) bank habitat; (BPB) behind point bar; (BW) backwater; (FPB) front of point bar (FPB); and (MC) midchannel.

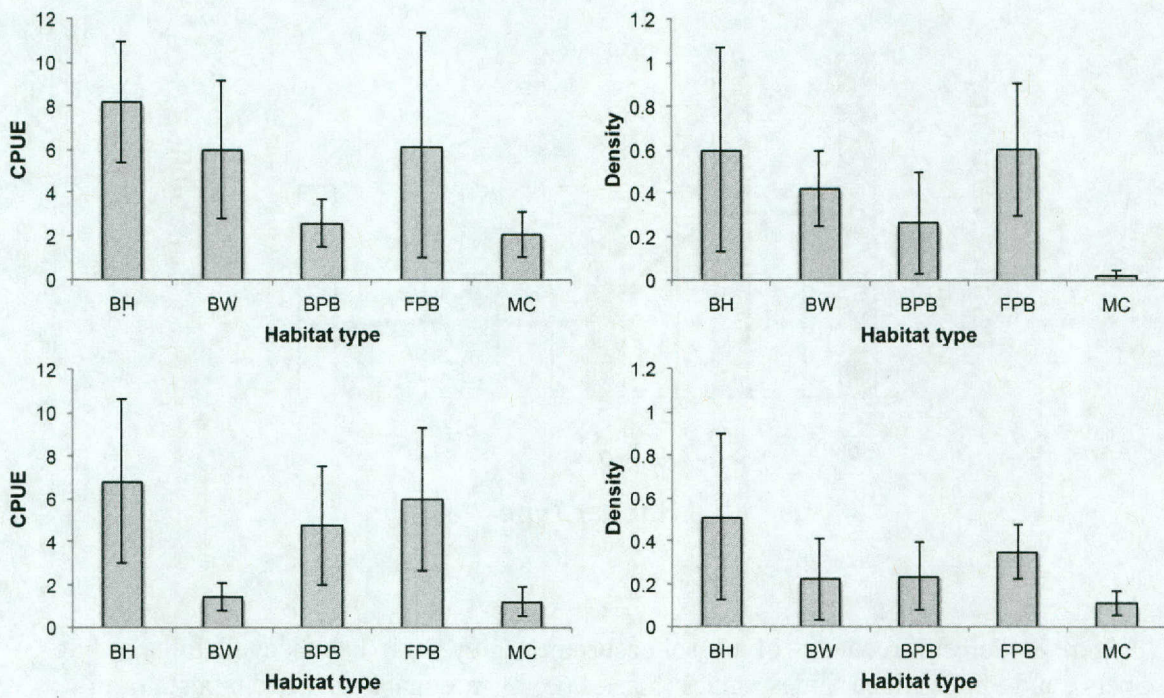


Figure 7. Mean CPUE (mussels/p-h) and mussel densities (mussels/0.25m<sup>2</sup>) from timed-searches and quantitative sampling, respectively, by mesohabitat type for *Q. houstonensis* (top) and *T. macrodon* (bottom). Error bars = ± 1 SE and acronyms for each habitat type correspond to the following: (BH) bank habitat; (BPB) behind point bar; (BW) backwater; (FPB) front of point bar (FPB); and (MC) midchannel.

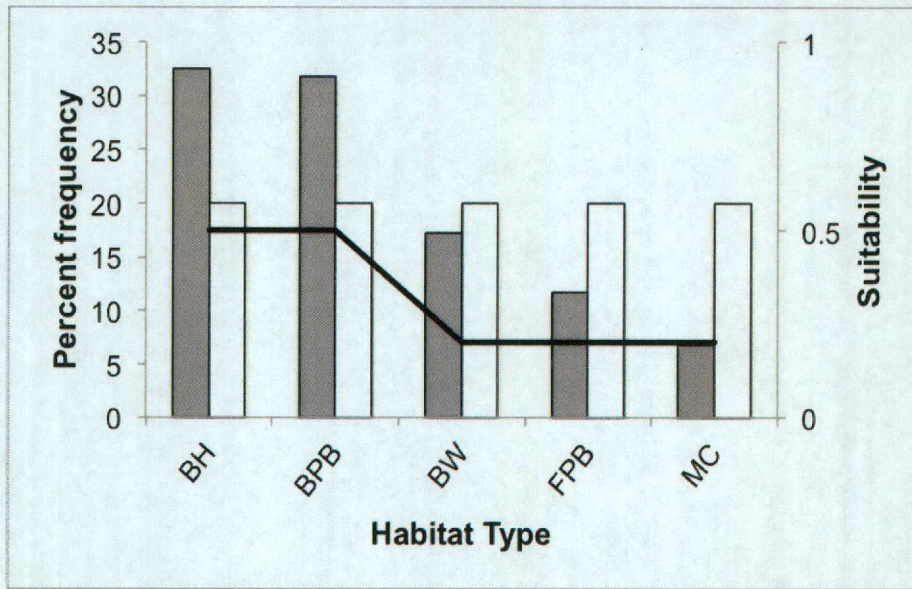


Figure 8. Percent frequency of mussel occurrence (grey bars), habitat availability (white bars), and Strauss linear index values (black line) by mesohabitats based on data from qualitative sampling. The number of observations used were: N = 2,135. Acronyms for each habitat type correspond to the following: (BH) bank habitat; (BPB) behind point bar; (BW) backwater; (FPB) front of point bar; and (MC) midchannel.

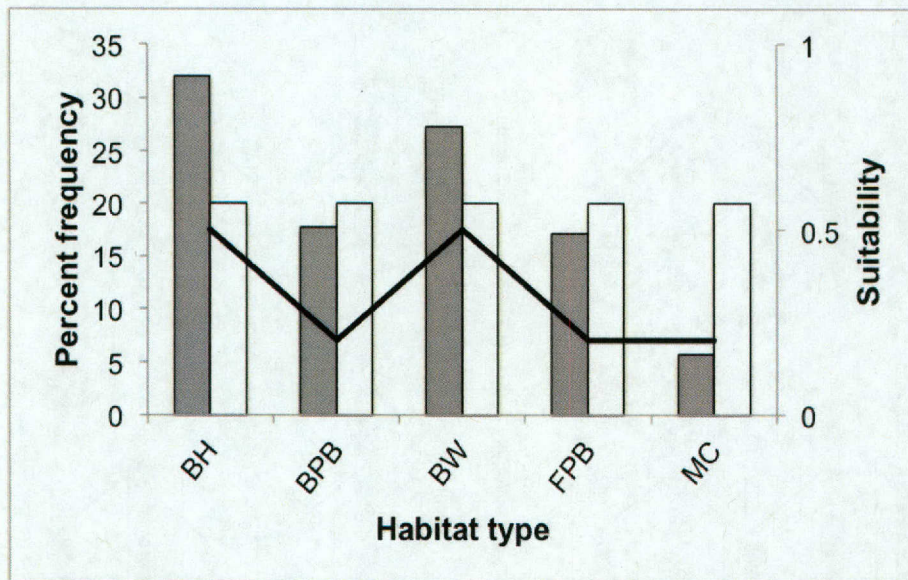


Figure 9. Percent frequency of mussel occurrence (grey bars), habitat availability (white bars), and Strauss linear index values (black line) by mesohabitats based on data from quantitative sampling. The number of observations used were: N = 524. Acronyms for each habitat type correspond to the following: (BH) bank habitat; (BPB) behind point bar; (BW) backwater; (FPB) front of point bar; and (MC) midchannel.

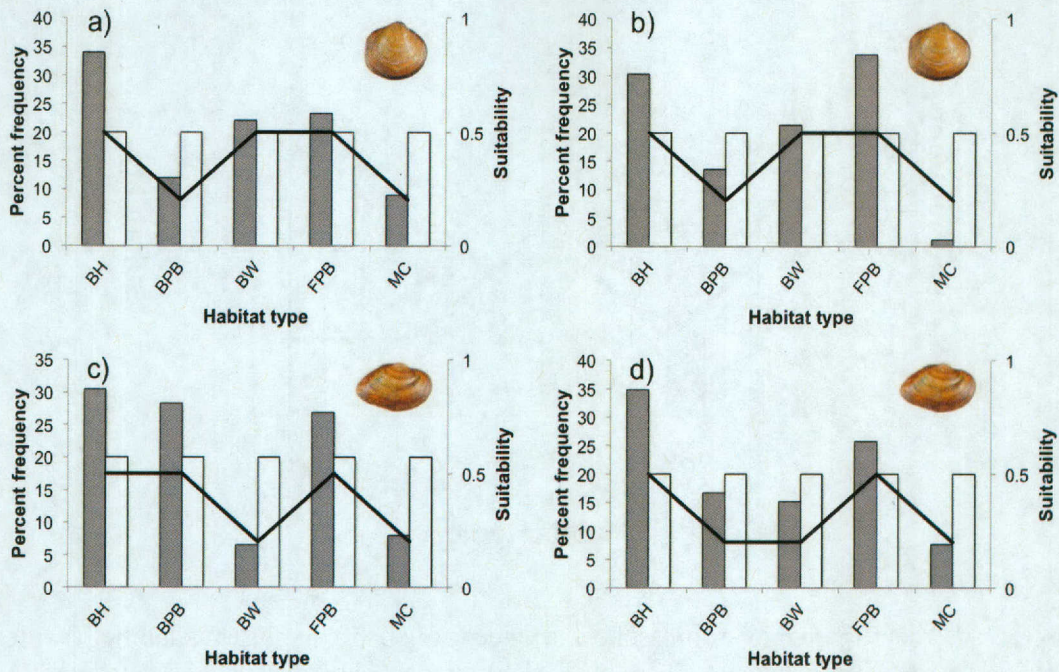


Figure 10. Percent frequency of occurrence (grey bars), habitat availability (white bars), and Strauss linear index values (black line) for mesohabitats. Suitability criteria are shown for *Q. houstonensis* (top; a, b) and *T. macrodon* (bottom; c, d). Panels (a) and (c) are based on abundance data from the timed-searches and panels (b) and (d) are based on data from quantitative sampling. The number of observations used were: N = 168 (a), N = 89 (b), N = 138 (c), and N = 66 (d). Acronyms for each habitat type correspond to the following: (BH) bank habitat; (BPB) behind point bar; (BW) backwater; (FPB) front of point bar; and (MC) midchannel.



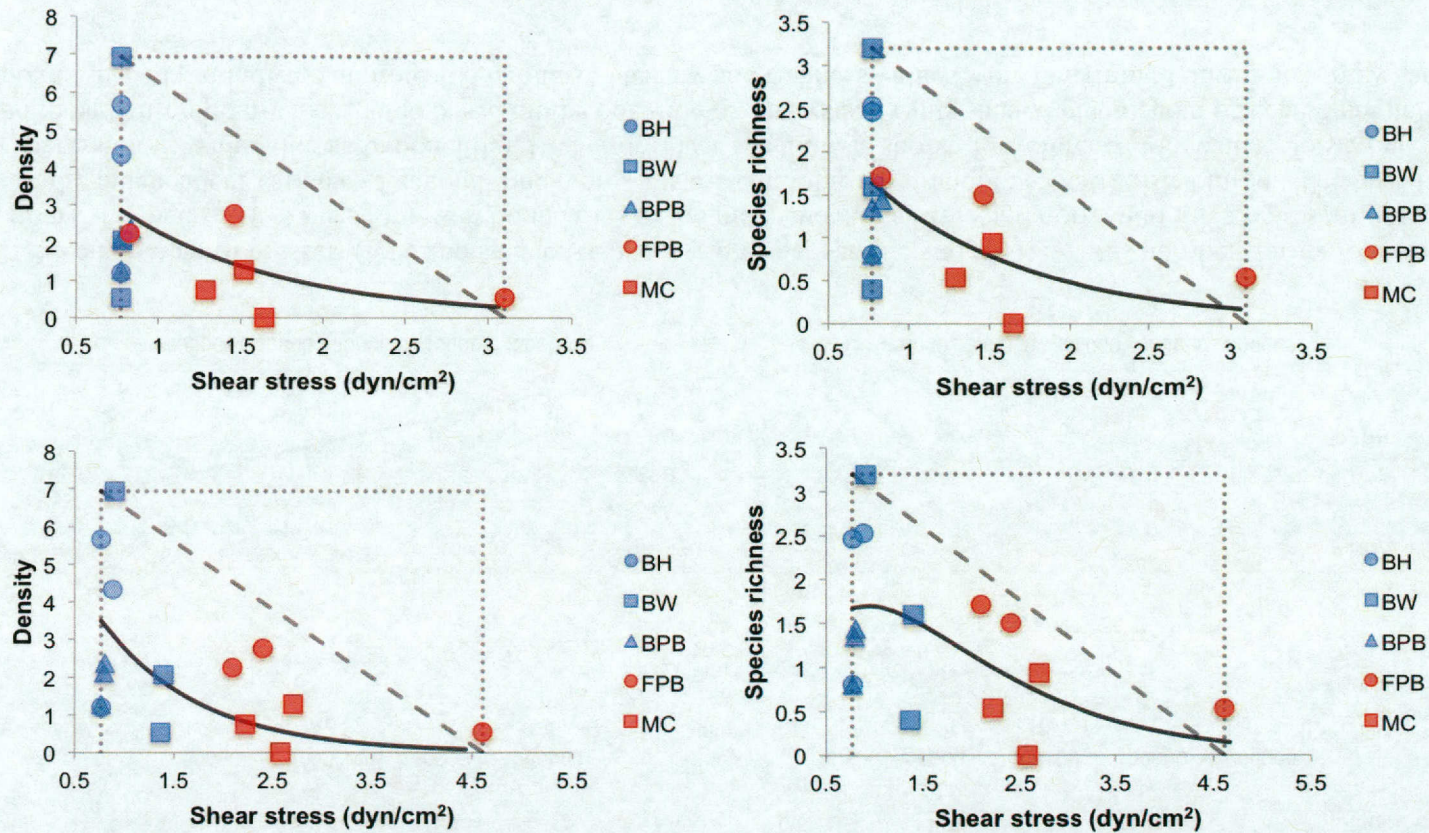


Figure 11. Mean number of mussels (left) and species richness (right) per sample site for Shear stress during low (top) and high flows (bottom). Dashed vertical lines represent boundaries of the independent variable and horizontal lines represent the upper boundary of the independent variable. Diagonal line represents the cut point for a random (50:50) distribution. The best-fit line, using nonlinear regression fit with either an exponential, Richer or linear function, is shown for reference for scatter plots where sites were determined to be non-randomly distributed based on the post-hoc  $X^2$  analysis. Points shaded blue denote protected habitat types, whereas points shaded red indicate unprotected habitats.

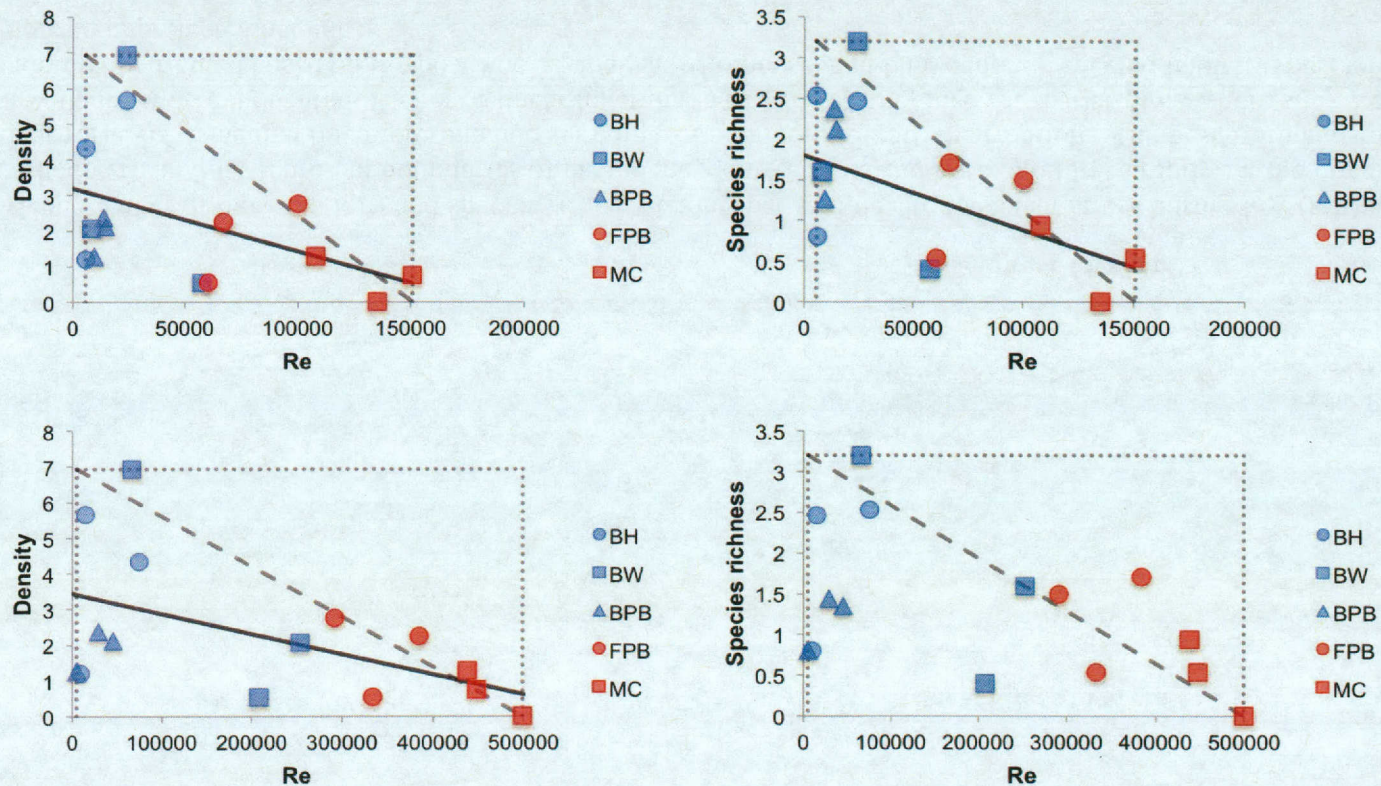


Figure 12. Mean number of mussels (left) and species richness (right) per sample site for Reynolds number during low (top) and high flows (bottom). Dashed vertical lines represent boundaries of the independent variable and horizontal lines represent the upper boundary of the independent variable. Diagonal line represents the cut point for a random (50:50) distribution. The best-fit line, using nonlinear regression fit with either an exponential, Richer or linear function, is shown for reference for scatter plots where sites were determined to be non-randomly distributed based on the post-hoc  $X^2$  analysis. Points shaded blue denote protected habitat types, whereas points shaded red indicate unprotected habitats. Density and richness values were determined during low flow sampling.

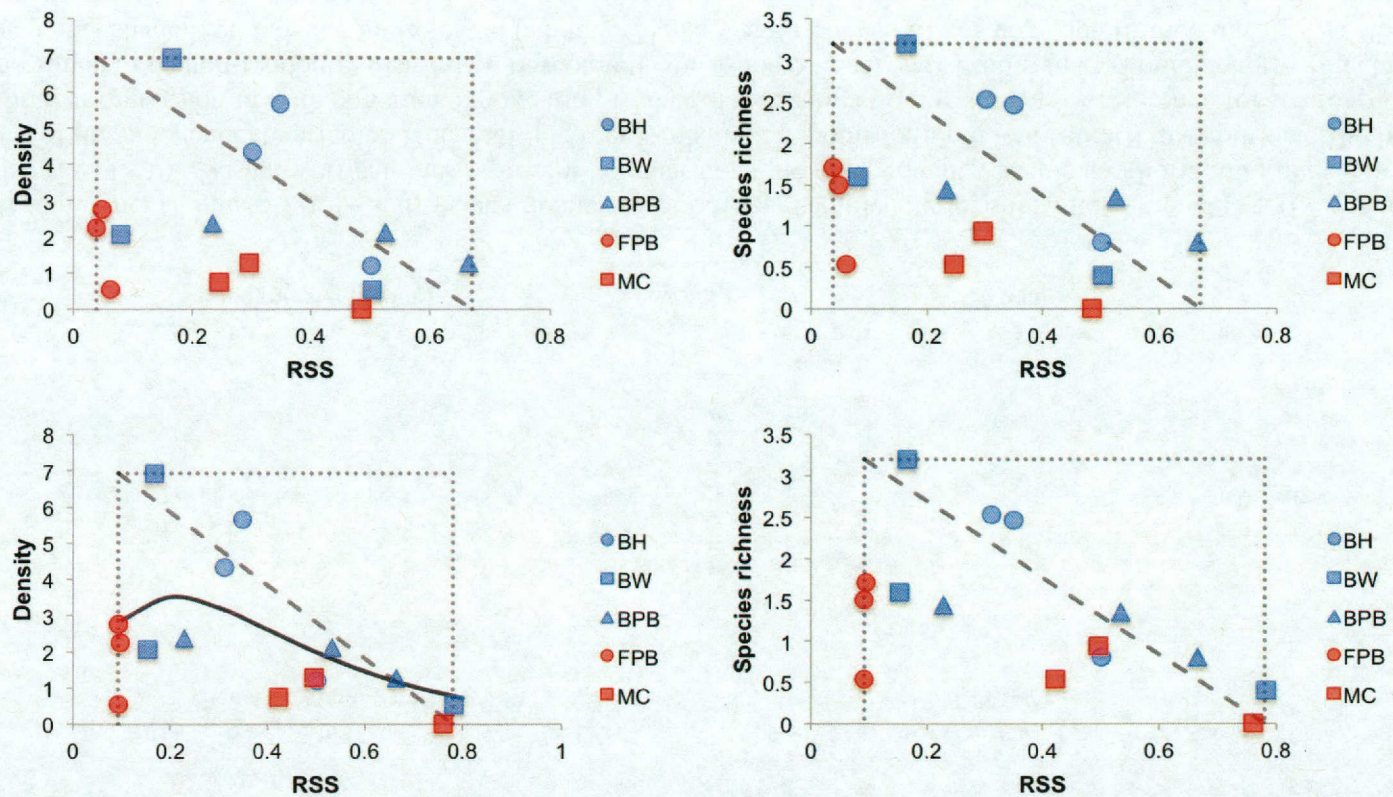


Figure 13. Mean number of mussels (left) and species richness (right) per sample site for RSS during low (top) and high flows (bottom). Dashed vertical lines represent boundaries of the independent variable and horizontal lines represent the upper boundary of the independent variable. Diagonal line represents the cut point for a random (50:50) distribution. The best-fit line, using nonlinear regression fit with either an exponential, Richer or linear function, is shown for reference for scatter plots where sites were determined to be non-randomly distributed based on the post-hoc  $X^2$  analysis. Points shaded blue denote protected habitat types, whereas points shaded red indicate unprotected habitats. Density and richness values were determined during low flow sampling.

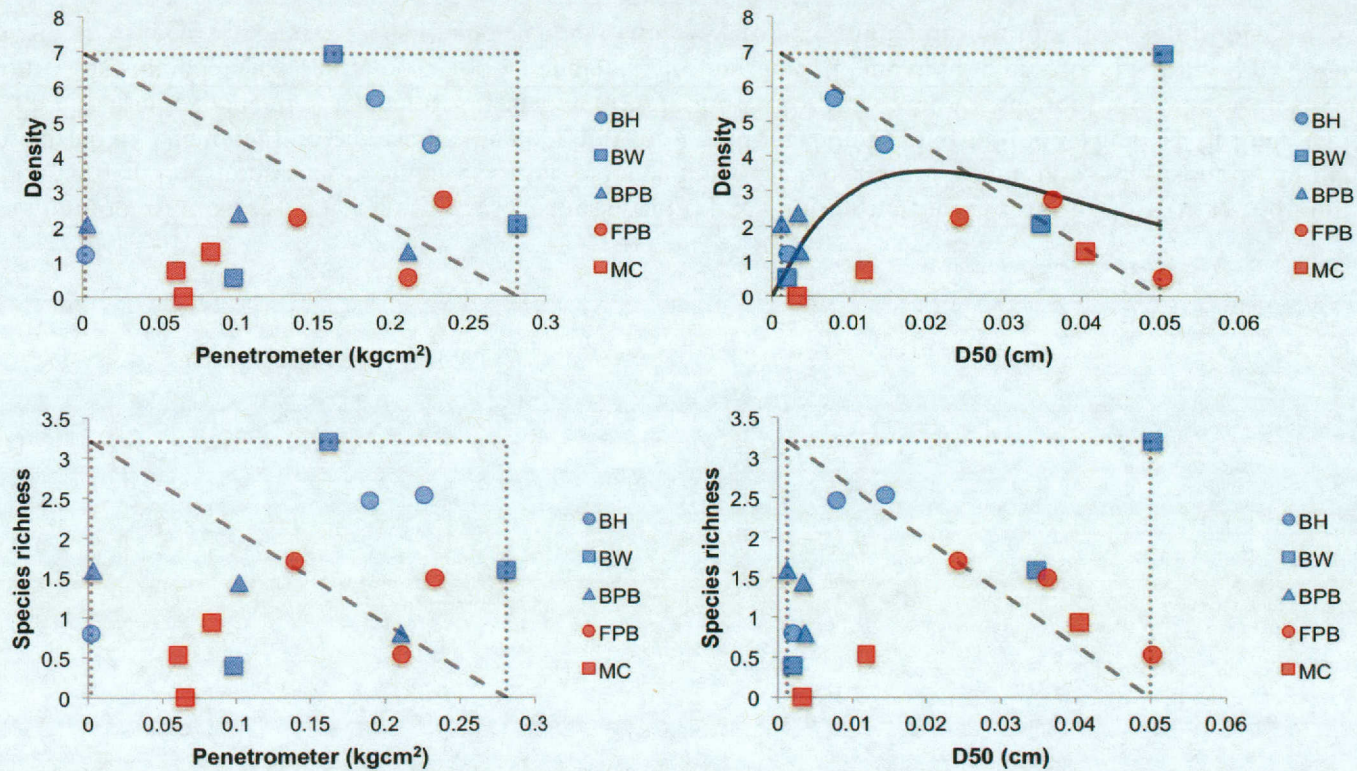


Figure 14. Mean number of mussels (top) and species richness (bottom) per sample site for substrate compactness (i.e., penetrometer) and particle size (i.e., D50). Dashed vertical lines represent boundaries of the independent variable and horizontal lines represent the upper boundary of the independent variable. Diagonal line represents the cut point for a random (50:50) distribution. The best-fit line, using nonlinear regression fit with either an exponential, Richer or linear function, is shown for reference for scatter plots where sites were determined to be non-randomly distributed based on the post-hoc  $\chi^2$  analysis. Points shaded blue denote protected habitat types, whereas points shaded red indicate unprotected habitats. Density and richness values were determined during low flow sampling.

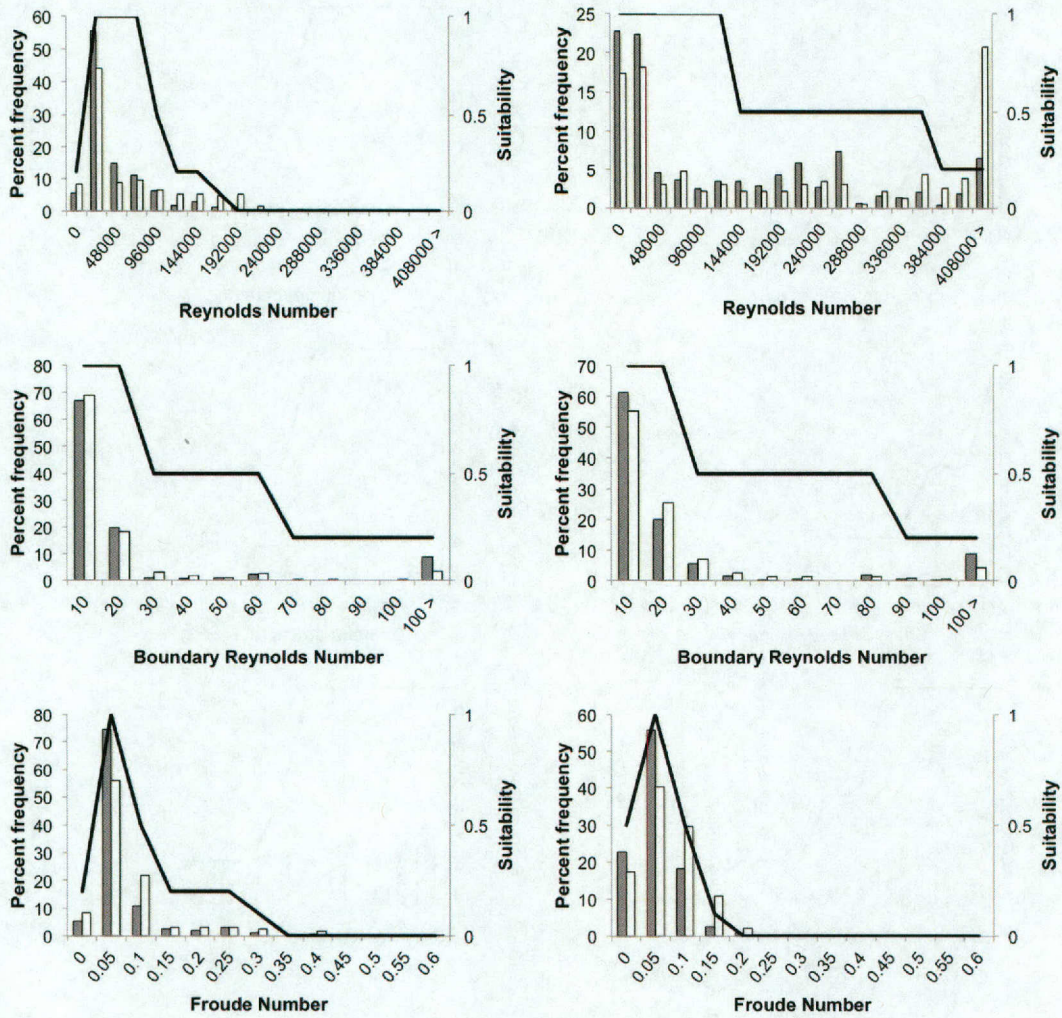


Figure 15. Percent frequency of occurrence (grey bars), habitat availability (white bars), and NPTL values (solid line; 95% confidence level) for Reynolds number, Boundary Reynolds number, and Froude number during low (left) and high (right) flows. Suitability criteria are shown only for all mussels and are based on abundance data from quantitative sampling. The number of observations used were: N = 524.

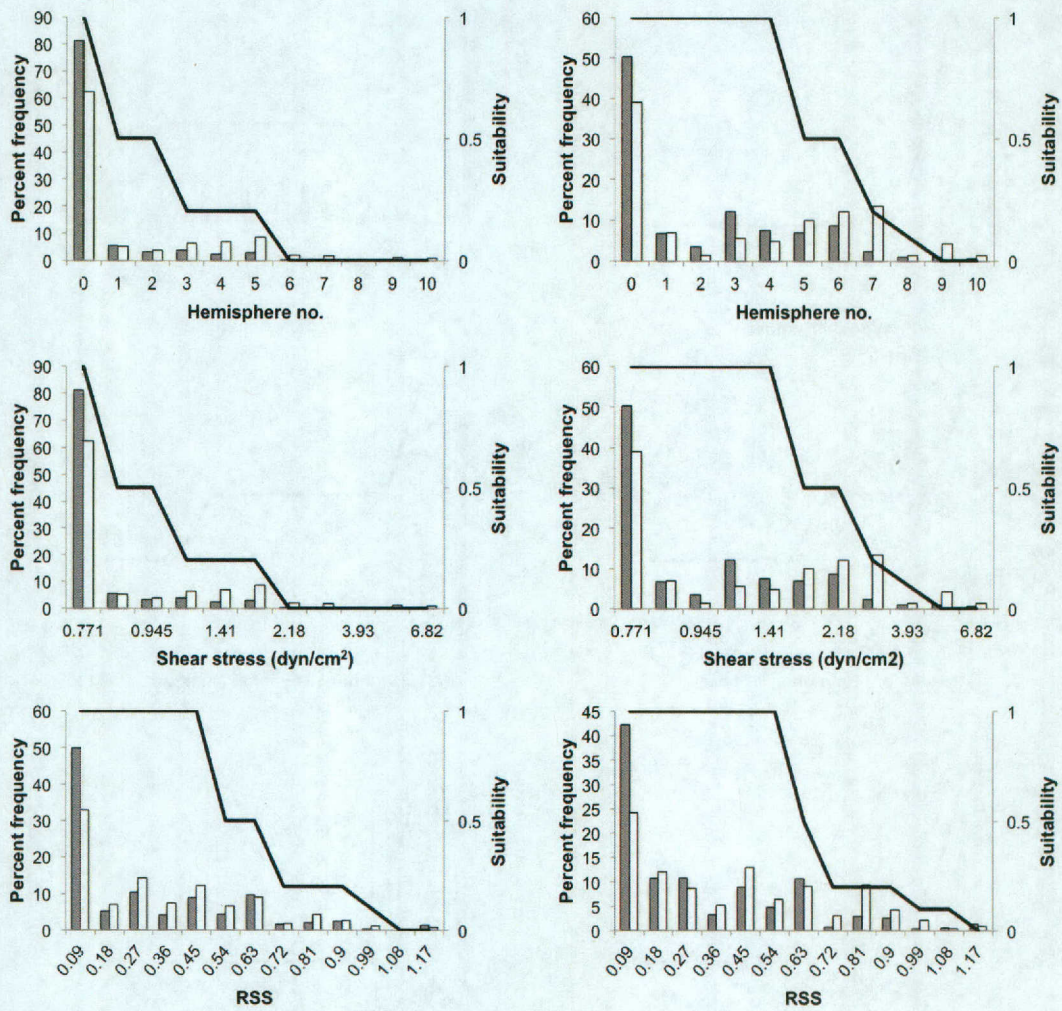


Figure 16. Percent frequency of occurrence (grey bars), habitat availability (white bars), and NPTL values (solid line; 95% confidence level) for Hemisphere no., shear stress, and RSS during low (left) and high (right) flows. Suitability criteria are shown only for all mussels and are based on abundance data from quantitative sampling. The number of observations used were: N = 524.

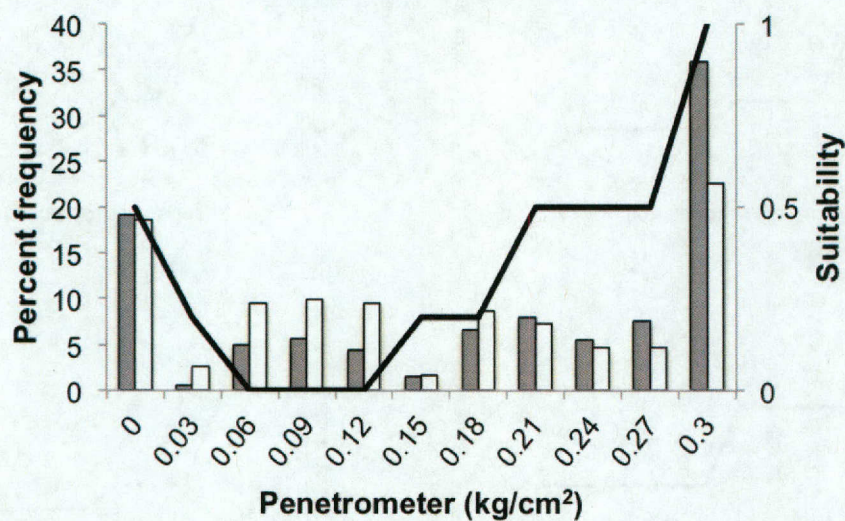
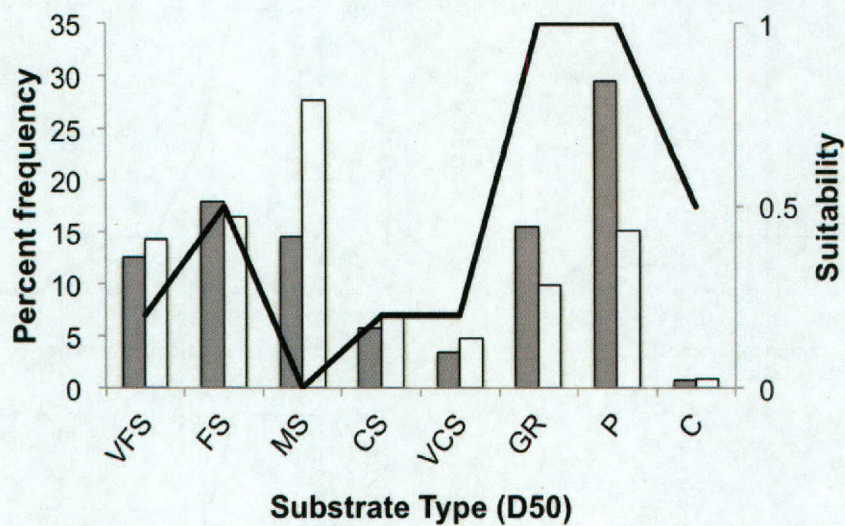


Figure 17. Percent frequency of occurrence (grey bars), habitat availability (white bars), and Strauss linear index values (solid line) for substrate type and compactness (Penetrometer). Suitability criteria are shown only for all mussels and are based on abundance data from quantitative sampling. The number of observations used were: N = 524. Acronyms for each substrate type correspond to the following: (VFS) very fine sand; (MS) medium sand; (CS) coarse sand; (VCS) very coarse sand; (GR) granule; (P) pebble; and (C) cobble.

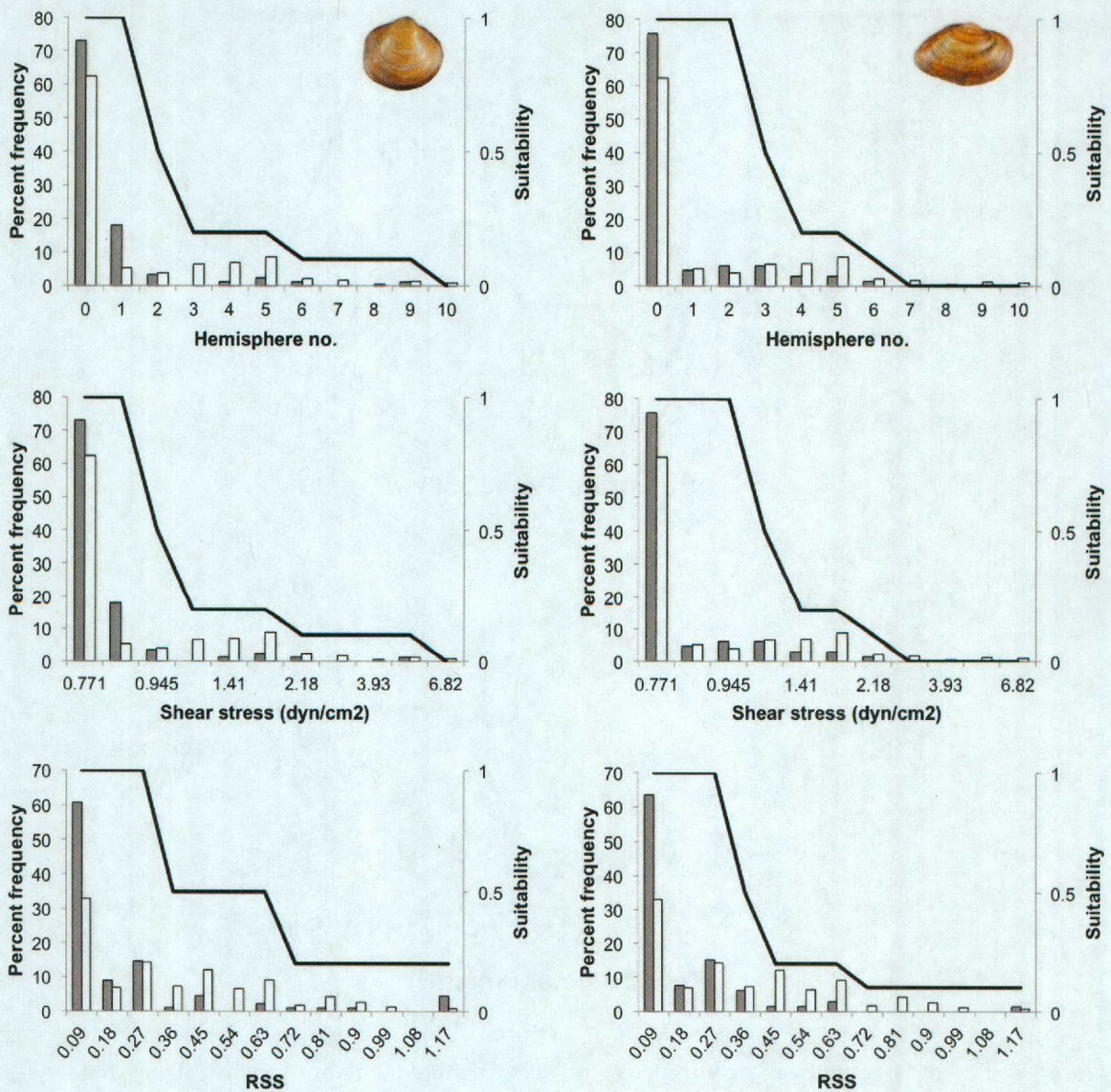


Figure 18. Percent frequency of occurrence (grey bars), habitat availability (white bars), and NPTL values (solid line; 95% confidence level) for Hemisphere no., shear stress, and RSS during low flows. Suitability criteria are shown for *Q. houstonensis* (left) and *T. macrodon* (right). Suitability criteria are based on abundance data from quantitative sampling. The number of observations used were: N = 89 (*Q. houstonensis*) and N = 66 (*T. macrodon*).



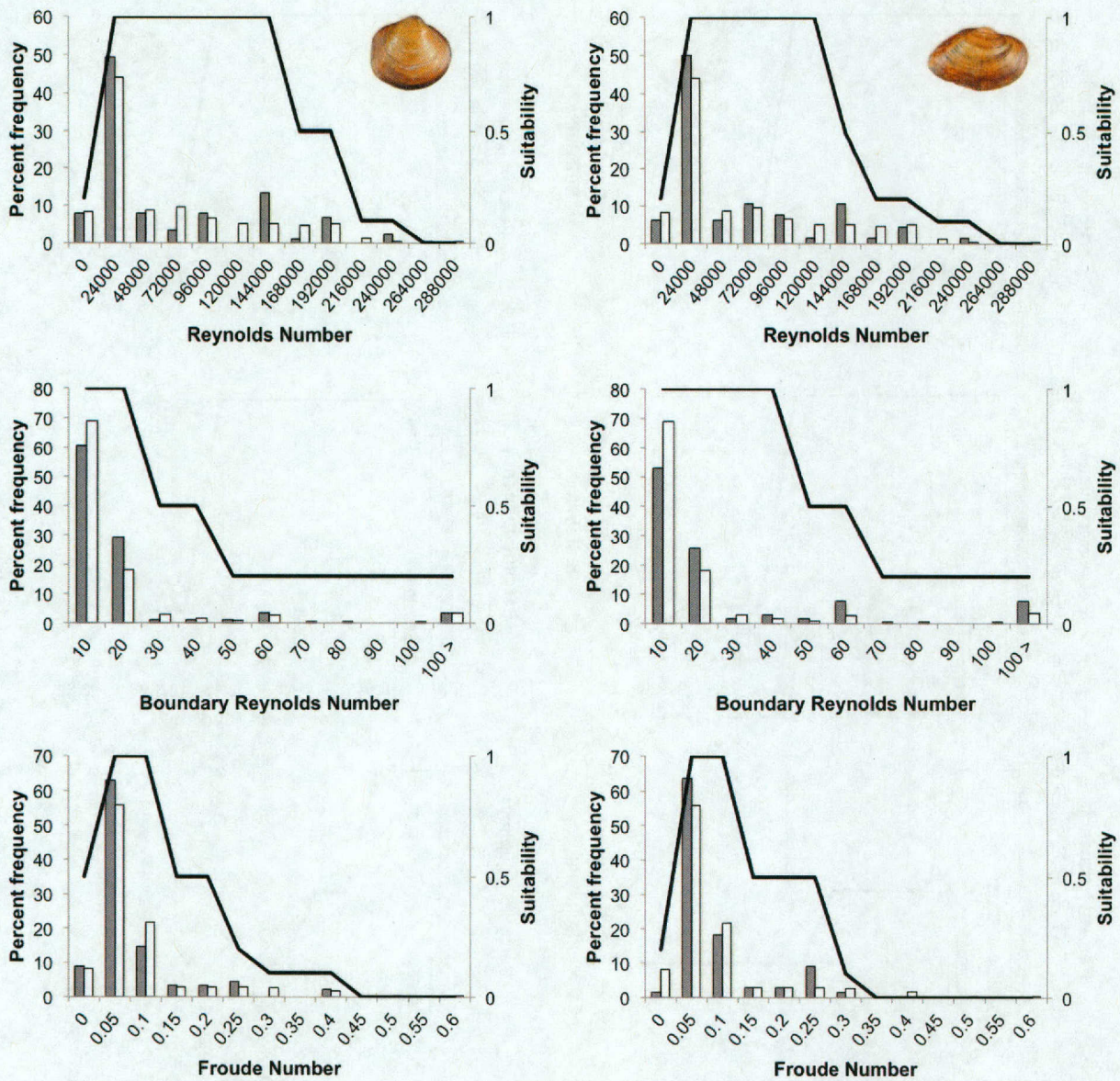


Figure 19. Percent frequency of occurrence (grey bars), habitat availability (white bars), and NPTL values (solid line; 95% confidence level) for Reynolds number, Boundary Reynolds number, and Froude number during low flows. Suitability criteria are shown for *Q. houstonensis* (left) and *T. macrodon* (right). Suitability criteria are based on abundance data from quantitative sampling. The number of observations used were: N = 89 (*Q. houstonensis*) and N = 66 (*T. macrodon*).

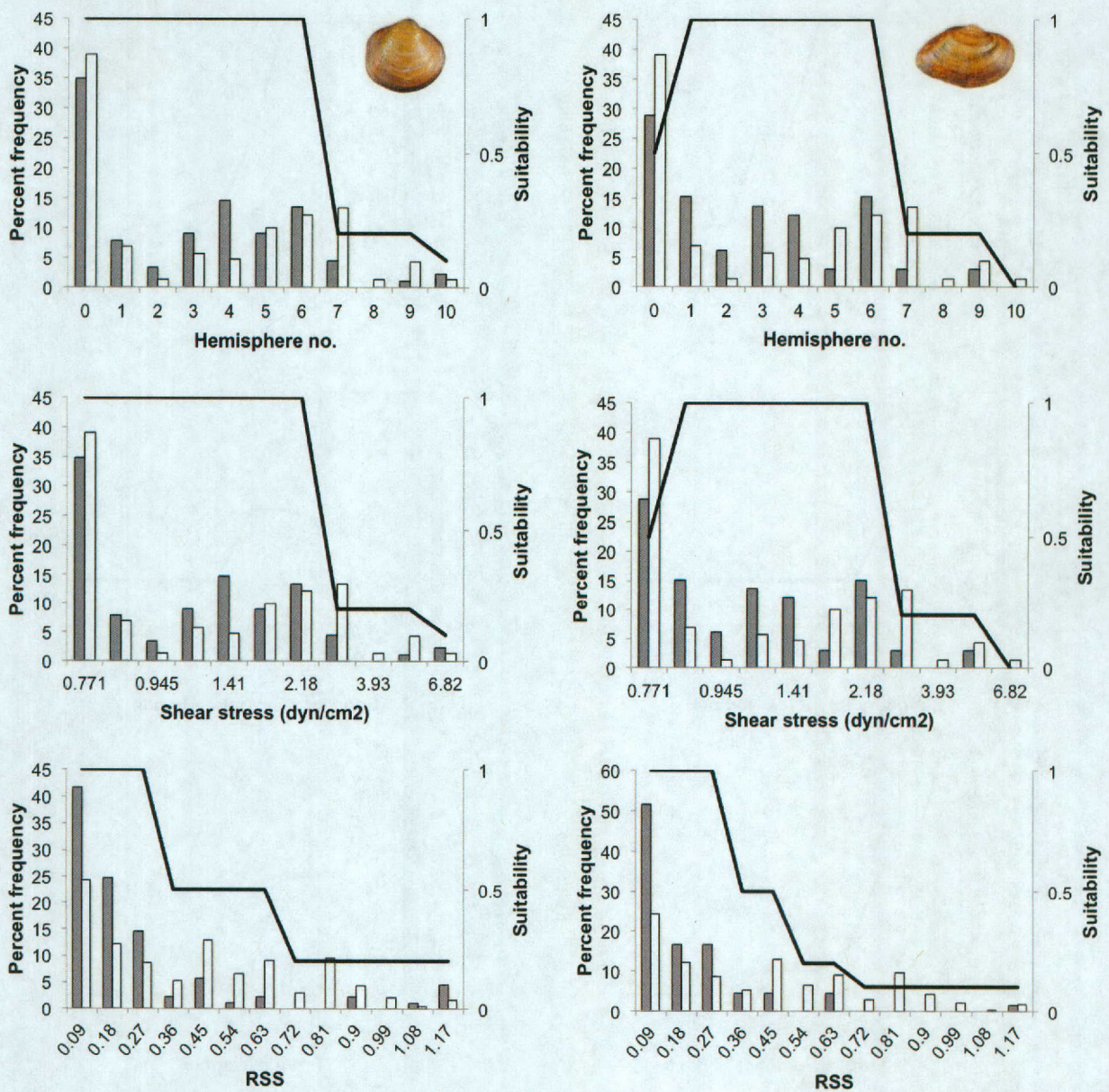


Figure 20. Percent frequency of occurrence (grey bars), habitat availability (white bars), and NPTL values (solid line; 95% confidence level) for Hemisphere no., shear stress, and RSS during high flows. Suitability criteria are shown for *Q. houstonensis* (left) and *T. macrodon* (right). Suitability criteria are based on abundance data from quantitative sampling. The number of observations used were: N = 89 (*Q. houstonensis*) and N = 66 (*T. macrodon*).

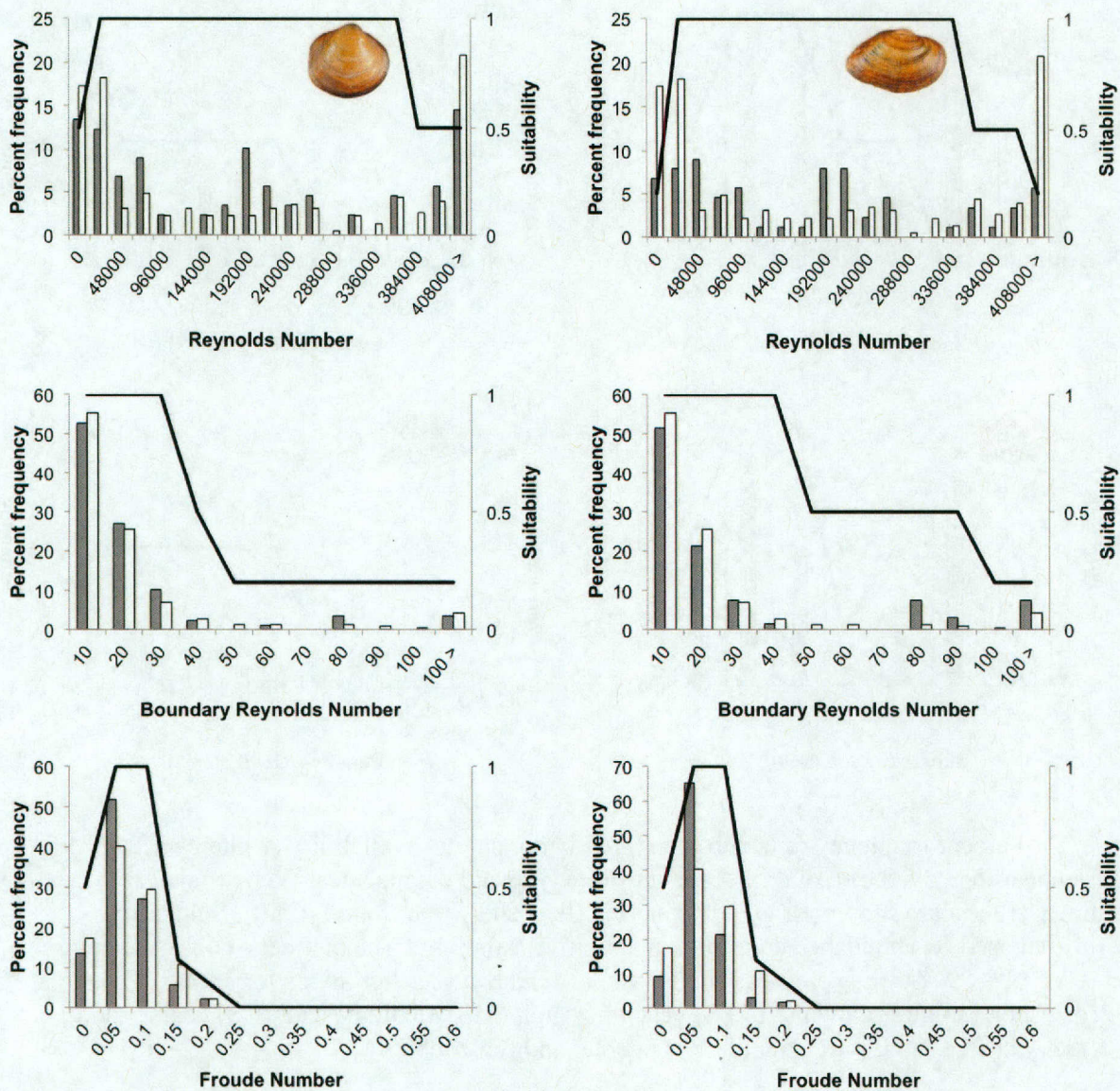


Figure 21. Percent frequency of occurrence (grey bars), habitat availability (white bars), and NPTL values (solid line; 95% confidence level) for Reynolds number, Boundary Reynolds number, and Froude number during high flows. Suitability criteria are shown for *Q. houstonensis* (left) and *T. macrodon* (right). Suitability criteria are based on abundance data from quantitative sampling. The number of observations used were: N = 89 (*Q. houstonensis*) and N = 66 (*T. macrodon*).

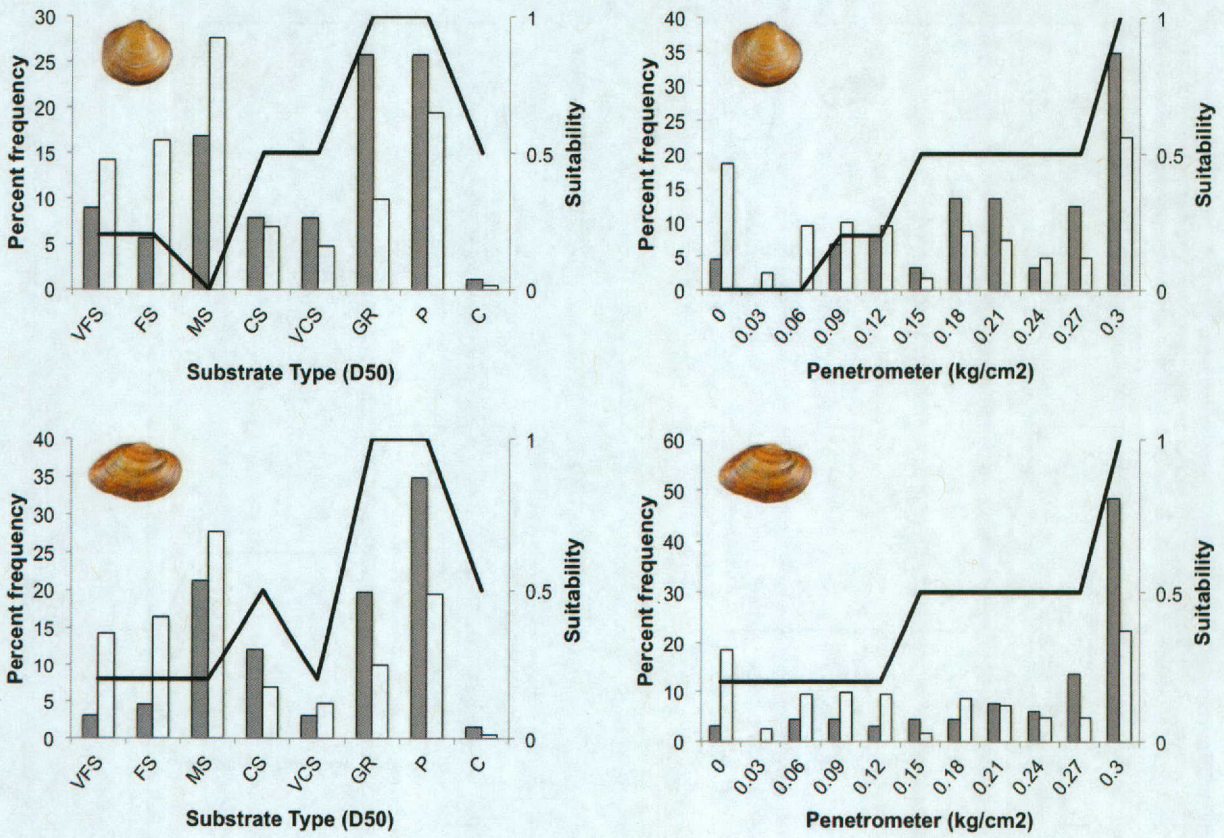


Figure 22. Percent frequency of occurrence (grey bars), habitat availability (white bars), and Strauss linear index values (solid line) for substrate type and compactness (Penetrometer). Suitability criteria are shown for *Q. houstonensis* (left) and *T. macrodon* (right). Suitability criteria are based on abundance data from quantitative sampling. The number of observations used were: N = 89 (*Q. houstonensis*) and N = 66 (*T. macrodon*). Acronyms for each substrate type correspond to the following: (VFS) very fine sand; (MS) medium sand; (CS) coarse sand; (VCS) very coarse sand; (GR) granule; (P) pebble; and (C) cobble.

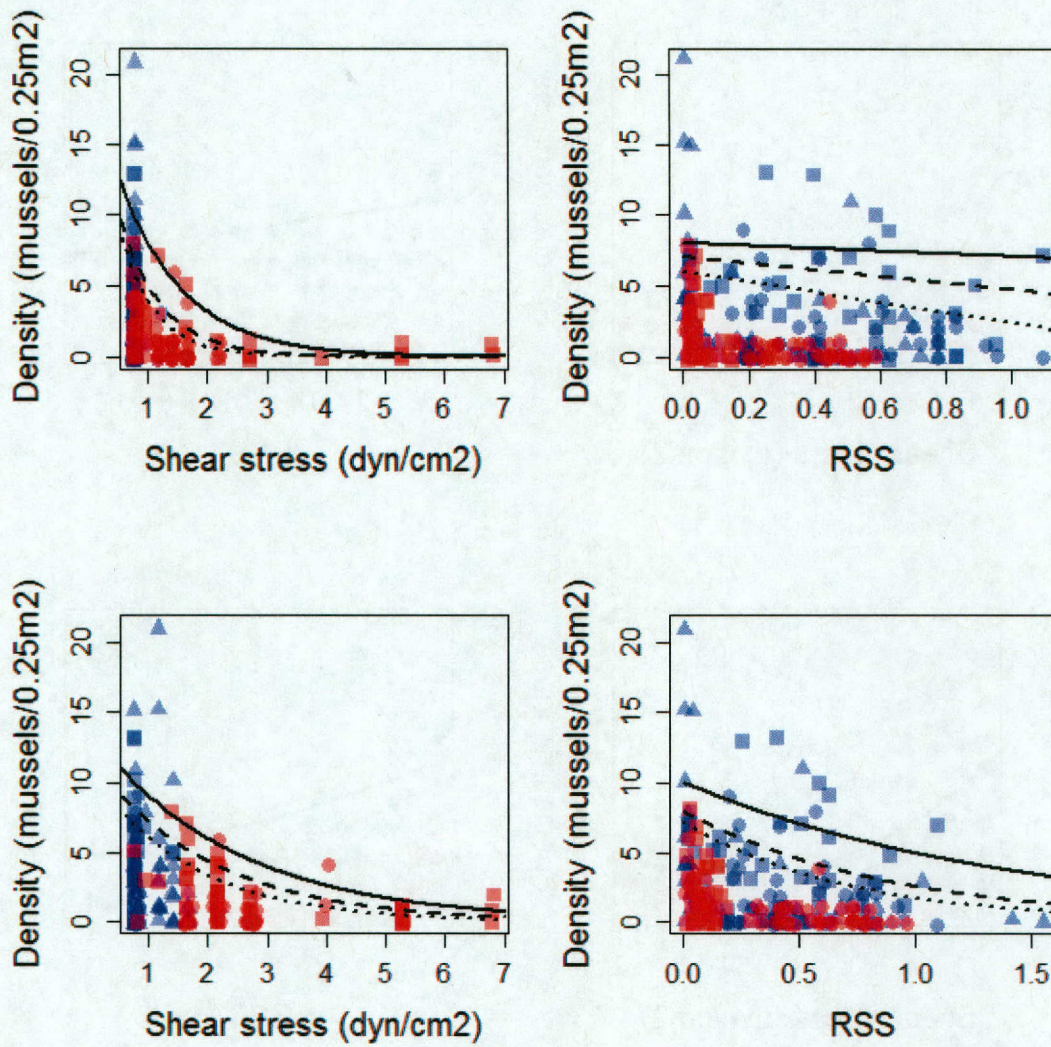


Figure 23. Quantile regression models for mussel density for Shear stress ( $\tau$ , dynes/cm<sup>2</sup>) and Relative shear stress (RSS) at low (top) and high (bottom) flows. Solid, dashed, and dotted lines represent 95<sup>th</sup>, 90<sup>th</sup>, and 85<sup>th</sup> quantile regression lines, respectively. Points shaded blue denote 0.25 m<sup>2</sup> quadrats from protected habitat types (i.e., BH = bank habitat; BPB = immediately downstream of point bar; and BW = backwater), whereas points shaded red indicate quadrats from exposed habitat types (i.e., MC = mid-channel and FPB = front of point bars).

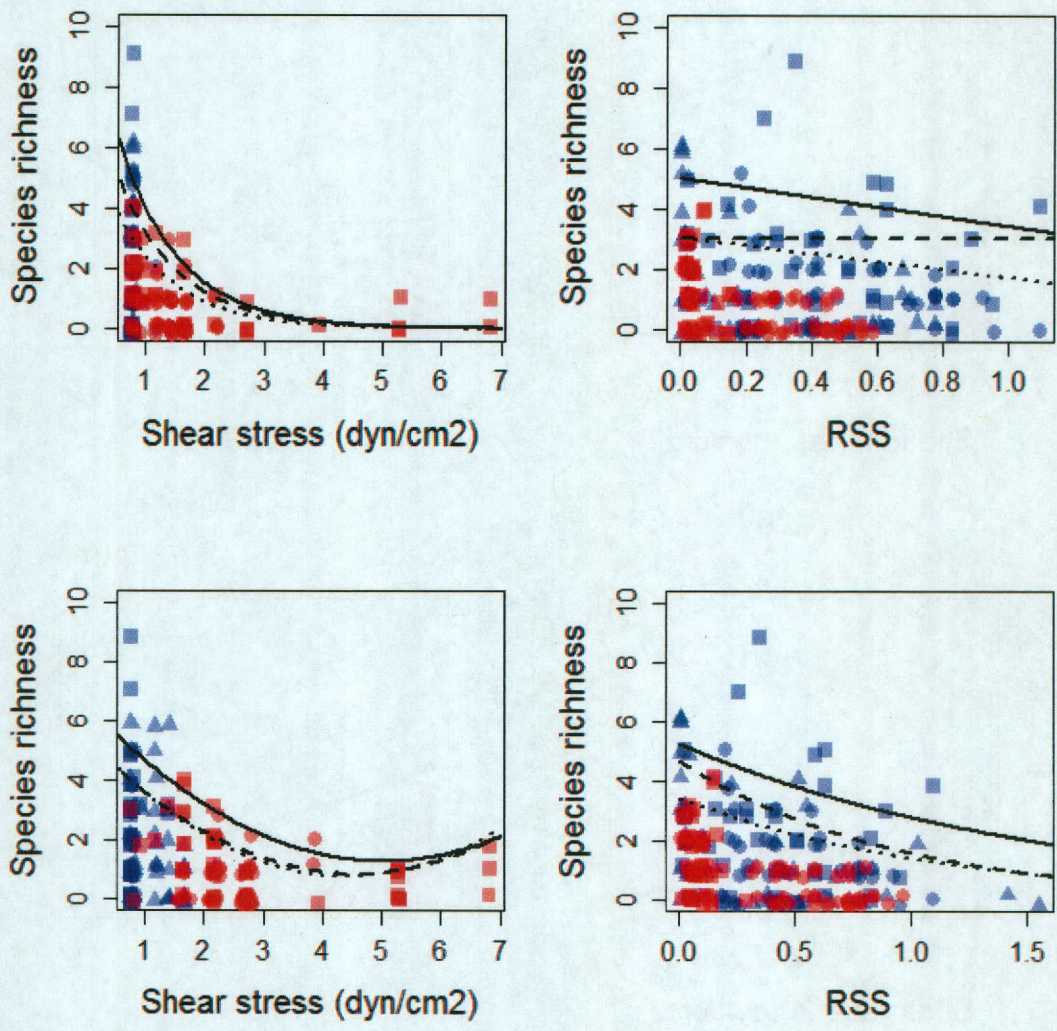


Figure 24. Quantile regression models for mussel species richness for Shear stress ( $\tau$ , dynes/cm<sup>2</sup>) and Relative shear stress (RSS) at low (top) and high (bottom) flows. Solid, dashed, and dotted lines represent 95<sup>th</sup>, 90<sup>th</sup>, and 85<sup>th</sup> quantile regression lines, respectively. Points shaded blue denote 0.25 m<sup>2</sup> quadrats from protected habitat types (i.e., BH = bank habitat; BPB = immediately downstream of point bar; and BW = backwater), whereas points shaded red indicate quadrats from exposed habitat types (i.e., MC = mid-channel and FPB = front of point bars).

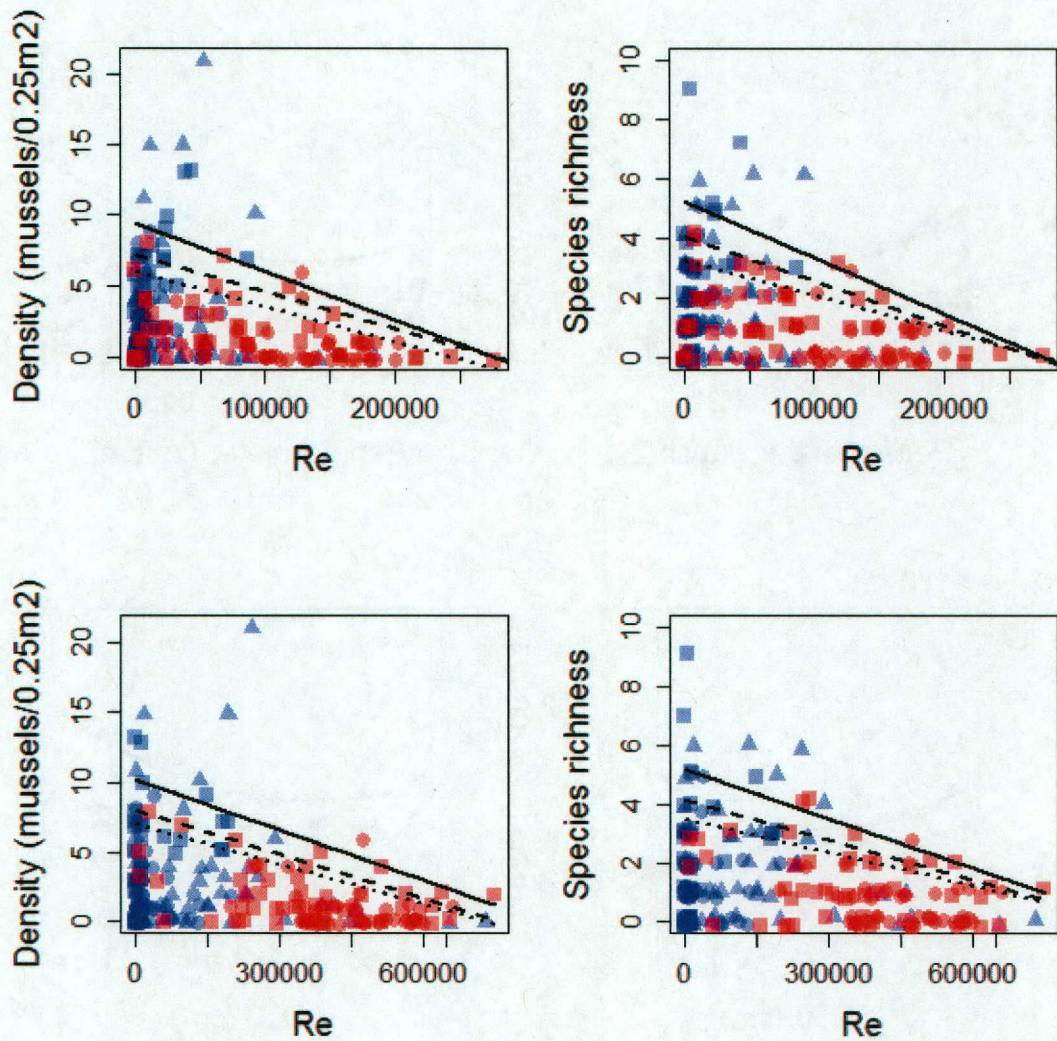


Figure 25. Quantile regression models for Reynolds number ( $Re$ ) for mussel density (left) and species richness (right) at low (top) and high (bottom) flows. Solid, dashed, and dotted lines represent 95<sup>th</sup>, 90<sup>th</sup>, and 85<sup>th</sup> quantile regression lines, respectively. Points shaded blue denote 0.25 m<sup>2</sup> quadrats from protected habitat types (i.e., BH = bank habitat; BPB = immediately downstream of point bar; and BW = backwater), whereas points shaded red indicate quadrats from exposed habitat types (i.e., MC = mid-channel and FPB = front of point bars).

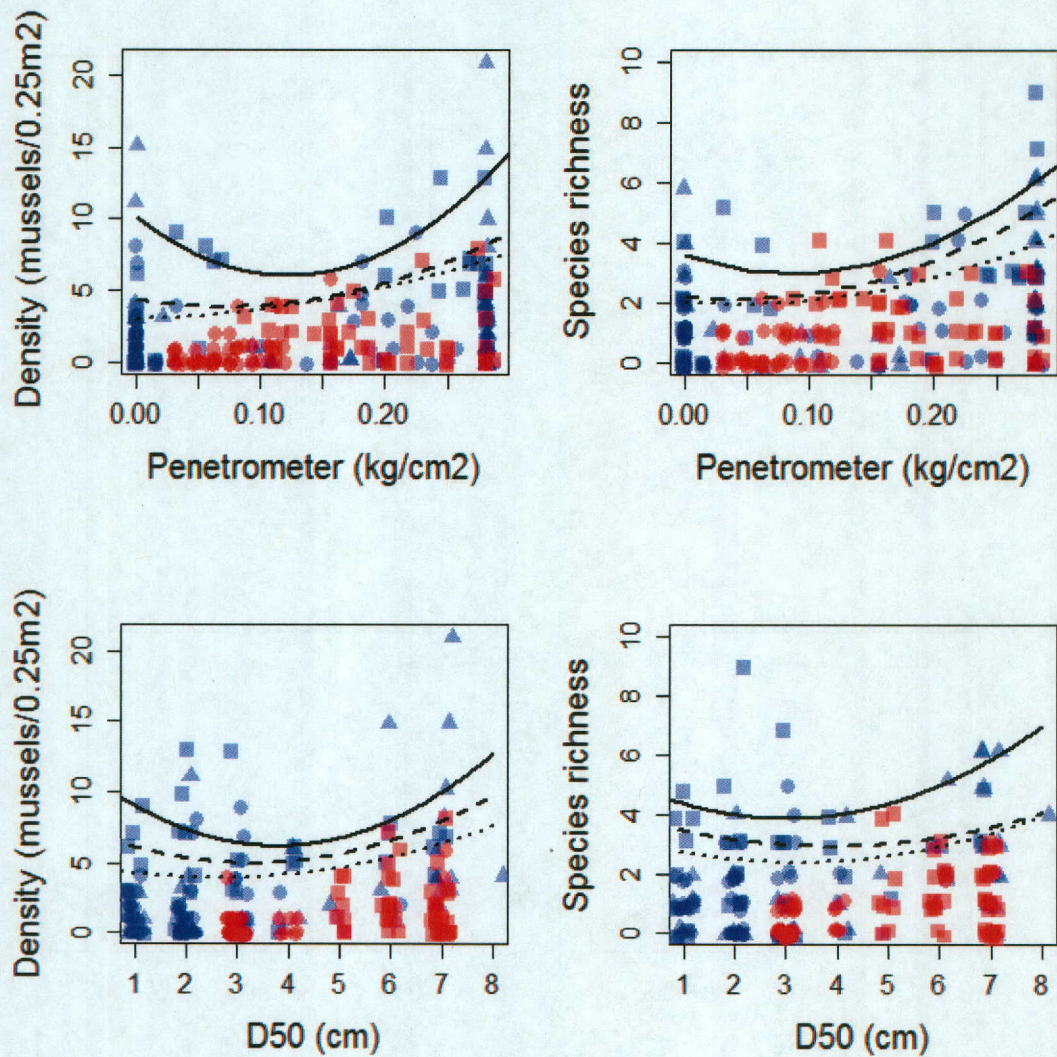


Figure 26. Quantile regression models for substrate compactness (Penetrometer, kg/cm<sup>2</sup>) and substrate type based on D50 (mm) for mussel density (left) and species richness (right). Solid, dashed, and dotted lines represent 95<sup>th</sup>, 90<sup>th</sup>, and 85<sup>th</sup> quantile regression lines, respectively. Points shaded blue denote 0.25 m<sup>2</sup> quadrats from protected habitat types (i.e., BH = bank habitat; BPB = immediately downstream of point bar; and BW = backwater), whereas points shaded red indicate quadrats from exposed habitat types (i.e., MC = mid-channel and FPB = front of point bars). Substrate codes denote the following: (1) very fine sand; (2) medium sand; (3) coarse sand; (4) very coarse sand; (5) very coarse sand; (6) granule (7) pebble; and (8) cobble



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Supplemental material

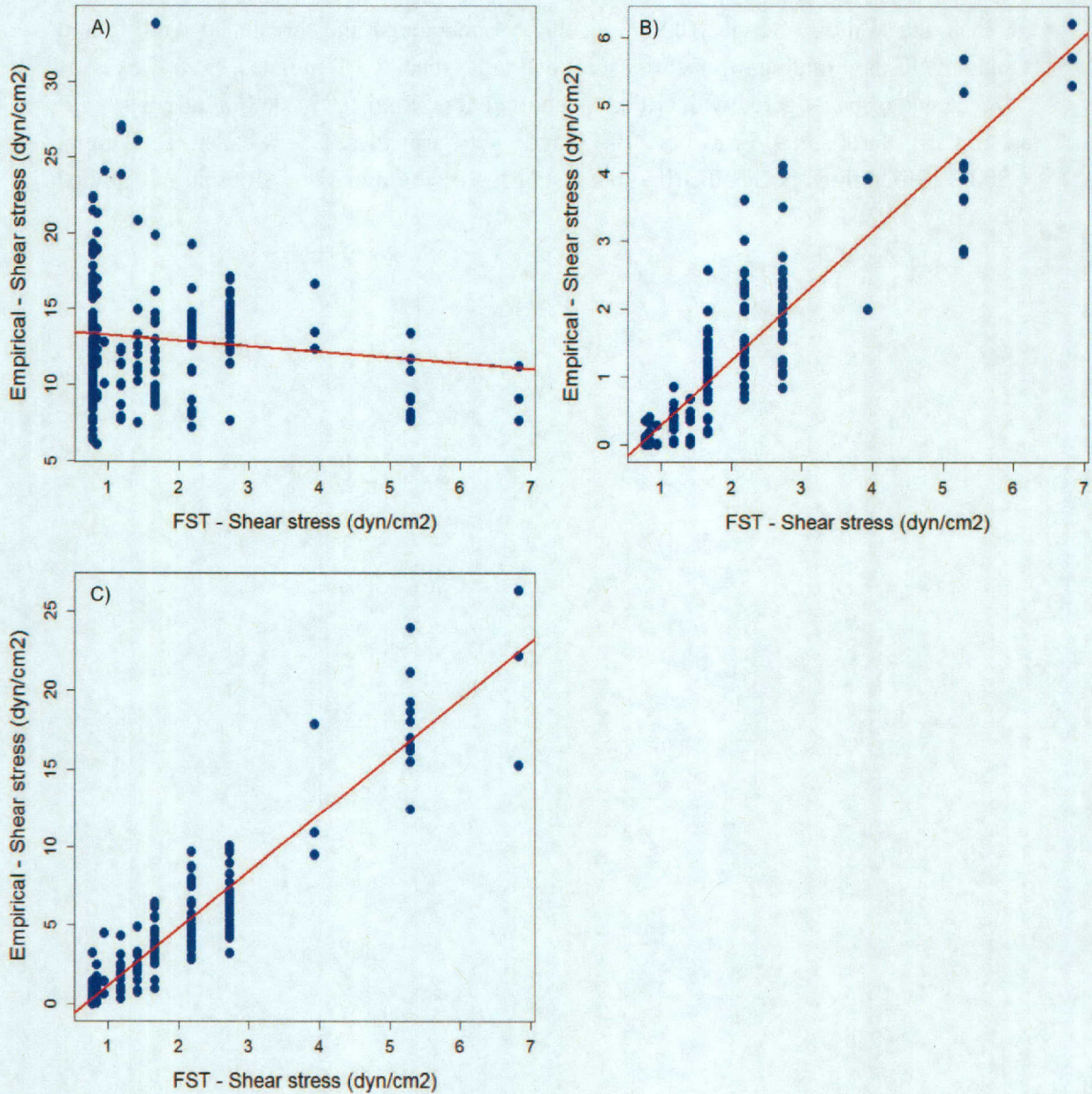


Figure A. Relationship between empirically derived shear stress (Empirical) and point-specific shear stress (FST). Empirically derived values of shear stress in panel (A) were calculated using the following formula:  $\tau = gSd$ ; where  $g$  is the acceleration due to gravity ( $980 \text{ cm/s}^2$ ),  $S$  is slope of the water surface, and  $d$  is water depth (cm). For panels (B) and (C), empirical shear stress was obtained using the following:  $U/5.75 \log_{10}(12d/k_s)$ ; where  $U$  is currently velocity (cm/s) measured at the bed surface (for panel B) or at  $0.6 \times$  depth (for panel C),  $d$  is water depth (cm), and  $k_s$  is bed roughness (see Table 3). The best-fit line, using OLS regression is shown for reference: (A)  $R^2 = 0.01$ ,  $p = 0.08$ , (B)  $R^2 = 0.84$ ,  $p < 0.001$ , and (C)  $R^2 = 0.89$ ,  $p < 0.001$ .

## Appendix A – Response to TWDB Comments

### REQUIRED CHANGES TO TASK 2 REPORT

1. Please reference “TWDB Contract No. 1104831145” on the cover of the report.  
**Response: addressed**
2. Please check the report for typos such as the following and correct as necessary:
  - a. Page 5, 2<sup>nd</sup> paragraph, 1<sup>st</sup> sentence, “water depth velocity and shear stress” should be “water depth, velocity, and shear stress.”
  - b. Page 6, 1<sup>st</sup> paragraph, 7<sup>th</sup> sentence, “additional line evidence” should be “additional line of evidence.”
  - c. Page 6, 3<sup>rd</sup> paragraph, 1<sup>st</sup> sentence, “(Dufrêne Legendre 1997)” should be ““(Dufrêne and Legendre 1997).”
  - d. Page 7, 1<sup>st</sup> paragraph, 2<sup>nd</sup> sentence, “(Dufrêne Legendre 1997)” should be ““(Dufrêne and Legendre 1997).”
  - e. Page 27, Figure 6, four occurrences of “BPS” should be “BPB.”
  - f. Page 28, Figure 7, four occurrences of “BPS” should be “BPB.”
  - g. Page 28, Figure 7, title, “(DH)” should be “(BH).”

**Response: all addressed**

3. In the last sentence on page 3 and first sentence on page 4, the authors note that “the nearest on-channel reservoir is Lake Whitney, which is located several hundred kilometers upstream.” As shown in Figure 1 on page 22, several tributaries to the Brazos River are impounded within 100 kilometers of two of the sites. Please note the proximity to the study area of reservoirs on tributaries to the Brazos River.

**Response: the last impoundment on the mainstem Brazos River has been edited to show that Lake Brazos in Waco is the last mainstem impoundment on the Brazos River. Some clarifying language was added to also indicate that there are several tributaries of the Brazos River within the study area as requested, but the specific distances of these tributary impoundments is not relevant to this study since the work was being conducted on the mainstem of the Brazos River so were not included.**

4. Please provide a definition for “CPUE” before this acronym is used in the text on page 4.

**Response: addressed**

5. The last sentence on page 4 and the first sentence of page 5 mention that water velocity “was measured at the level of the bed surface.” From a theoretical standpoint, when the bed of the channel is not moving, the velocity of water at the bottom of the water column (right next to the bottom) should be zero for all flow rates. In practice, electromagnetic current meters (like the OTT MF Pro used in this study) sample a cone shaped volume of water in front of the electromagnetic head. Measuring the water velocity with the head at its lowest setting on the wading rod therefore provides an average velocity for a volume of water of *unknown* height above the channel bottom. Because the height where the velocity measurement is taken is unknown, this velocity value is useless for estimating bed shear. Please clarify if water velocity was only measured at an unknown height above the bottom of the water column or if average column velocity was also measured. If average column velocities were measured, please provide them with the data associated with this report.

**Response: Clarification has been provided that given the equipment limitations for sampling current velocity at the bed, these measurements are the best available estimates of current velocity near the bed and are more reflective of the current**

velocities acting on the bed than an average column velocity would provide. This is discussed in the "Habitat Sampling" section of the report.

6. The 1<sup>st</sup> sentence of the 3<sup>rd</sup> paragraph on page 8 states that "The suitability curves constructed from the timed-search data for all species of each mesohabitat type indicate that mussels are primarily using bank and behind point bar habitats." Later in this paragraph, the authors note that *Q. houstonensis* prefer bank, backwater, and front of point bar habitats while *T. macrodon* prefer bank habitats. Data from Tables 4 and 5 (on pages 16 and 17) also seem to support the premise that different species of mussels prefer different mesohabitats. For example, *L. fragilis* appear to prefer mid-channel habitat and *C. tampicoensis* seem to prefer downstream of point bar habitat. Please clarify if the authors believe it is appropriate to characterize mussel habitat in general (all species combined) or if individual species have different habitat preferences that should be considered.

**Response: depends on the management/conservation question. If the goal is to manage assemblages or communities then aggregating the data is probably a better option. However, if the goal is to develop species-specific management plans then obviously individual species habitat preferences would be most appropriate. Finally, some of the analyses require fairly large sample sizes (e.g., quantile regression), which is a problem for some mussel species, especially those such as *T. macrodon*, and so one must aggregate the data in order evaluate mussel-flow relationships using some of the analyses presented in this report.**

7. The 2<sup>nd</sup> sentence of the 4<sup>th</sup> paragraph on page 10 states that "Mussel abundance and species richness was generally the same across all three reaches, and the dominant species within each were also similar." This is an important observation from the data. In looking at the data in Table 4, there appears to be a significant difference in mussel mesohabitat utilization across the three sites. Particularly, when looking at CPUE for mussels in general (all species combined), it appears that mussels are primarily utilizing bank and downstream of point bar mesohabitats at the Mussel Shoals site, bank and backwater mesohabitats at the Washington on the Brazos site, and upstream and downstream of point bar mesohabitats at the Wildcat Bend site. Please comment as to whether this difference in mesohabitat utilization across the sites is significant.

**Response: Yes, many of those differences are significant (see Table 6; Indicator Species Analysis).**

8. In Table 3 on page 15, the definition of velocity used in the formulae for Froude and Reynolds numbers is incorrect. The caption of the table indicates that velocity (U) used in these formulae is the "mean boundary velocity." This is incorrect. The velocity used in these formulae is the "mean **column** velocity." Please correct this error. If mean column velocity was not measured as part of this study, Froude and Reynolds numbers should not be calculated.

**Response: The reviewers are correct that mean column velocity was used to calculate Froude and Reynolds, this is now explicitly stated in table caption.**

9. Please provide documentation related to study methods and results related to macroinvertebrate distributions and abundances.

**Response: A paragraph describing the invertebrate collections are now provided.**

### **SUGGESTED CHANGES TO TASK 2 REPORT**

10. In the title for Tables 4 and 5, on pages 16 and 17, the “front-of-point bar” and “back-of-point bar” habitats are referred to as “immediately upstream of point bar” and “immediately downstream of point bar,” respectively. The description of habitat as being upstream or downstream of a point bar seems to be more readily understandable (as compared to “front-of” or “back-of” a point bar). Please consider using the designations “upstream of point bar” and “downstream of point bar” to refer to these mesohabitats throughout the document.

**Response: We see this may be confusing at first, but given our explanation of the habitat types and the examples of each habitat type in Figure 5 should make it clear.**

