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HABITAT ASSOCIATIONS AND DETECTABILITY OF THREE UNIONID
SPECIES ALONG THE UPPER SABINE RIVER IN EAST TEXAS

By

JARED DICKSON

A thesis submitted in partial fulfillment
Of the requirements for the degree of
Masters of Biology
Department of Biology

Srini Kambhampati, Ph.D., Committee Chair

College of Arts and Sciences

The University of Texas at Tyler
May 2018

The University of Texas at Tyler
Tyler, Texas
This is to certify that the Master's Thesis of

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January 19, 2018
for the Masters of Biology degree

Approvals:



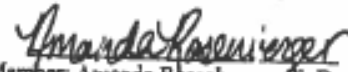
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Table of Contents

List of Tables	ii
List of Figures	iii
Abstract	iv
Chapter One	1
Chapter Two.....	7
Introduction.....	7
Methods.....	10
Study Area	10
Study Species	11
Site Selection	11
Sampling Technique	12
Covariate Measurement	13
Data Analysis	14
Results.....	16
Discussion.....	21
Conclusions.....	24
References.....	25
Appendix.....	30

List of Tables

Table 1. Sites and abundances of target species from the upper Sabine River of summers 2015 and 2016, locations indicate point of river access 18

Table 2. Best fit models of *Fusconaia askewi*, *Lampsilis satura*, and *Potamilus amphicaenus*. Models include occupancy rate (ψ), the habitat covariate, and detection (p). Also given are the parameter estimates of each covariate and its associated p-value 20

Table 3. Dates and locations of sites sampled in the upper Sabine river in East Texas. Julian dates, one of the detection covariates, were coded for in program R and represented the month/day/year dates which are notated in the table 30

Table 4. Occupancy covariates measured at each site in the upper Sabine in East Texas. Locations are included to distinguish each sites respective covariate measurements 31

Table 5. Occupancy covariates measured at each site in the upper Sabine in East Texas. Years' experience represent the second detection covariate collected. Locations are included to distinguish each sites respective covariate measurements 32

Table 6. Occupancy covariates measured at each site in the upper Sabine in East Texas. Locations are included to distinguish each sites respective covariate measurements. Mesohabitats were determined from field notes that included observations of water surface roughness within the delineated sample area 33

List of Figures

Figure 1. Map of the study area within the upper Sabine River. Points indicate the 5km segments where field surveys were conducted and lie between highways 69 (south of Mineola) and 79 (north of Carthage).....	15
Figure 2. Cumulative detection probabilities of the three focal species collected in the Sabine River. Values indicate the probability of detecting a species when a given number of 0.25m ² quadrats are searched at a site. These probabilities are conditional on the species presence. The 0.8 cutoff represents the desired probability of detection.....	17
Figure 3. Estimated detection probabilities for target species sampled in the upper Sabine River. Error bars represent standard error	19
Figure 4. Occupancy for target species collected in the upper Sabine River. Naive occupancy is indicated by non-shaded bars. Error bars represent standard error.....	19
Figure 5. Figure 5. Nonmetric multidimensional scaling (Stress = 0, Global R = 0.024, P = 0.47) for the sampled 5km river segments of the upper Sabine River. Numbers represents the corresponding segment as they were numerated from upstream to downstream. Each segment is represented in the non-dimensional space based on their habitat characteristics, for example, those close together are more similar than those sites further apart	21

Abstract

HABITAT ASSOCIATIONS AND DETECTABILITY OF THREE UNIONID SPECIES ALONG THE UPPER SABINE RIVER IN EAST TEXAS

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Thesis Chair: Srini Kambhampati

The University of Texas at Tyler
May 2018

East Texas contains the highest diversity of mussels in the state. Of the 37 species in East Texas, six are listed by the state as threatened and three have been proposed for listing as threatened or endangered under the U.S. Endangered Species Act. Although diverse, mussel populations are declining and few studies exist that establish habitat relationships identifying determinants of mussel distributions in the upper Sabine River. I explored potential habitat preferences of three state listed species using an occupancy modeling approach, including the Texas Pigtoe, *Fusconaia askewi*, Sandbank Pocketbook, *Lampsilis satura*, and Texas Heelsplitter, *Potamilus amphicaenus*. Thirty sites, along a 225km section of the upper Sabine River between US Highways 69 (Smith County) and 79 (Panola County) were surveyed with 0.25m² quadrats to estimate the occupancy of target species. *F. askewi* was the most abundant species, accounting for 92.3% of the collected mussels. Detection estimates based on sampling a 0.25m² quadrat ranged among species from 0.11 to 0.71. I found no significant relationship between occupancy estimates and reach-level occupancy covariates, suggesting that mussels associate with larger scale habitat variables or other river processes. To further investigate the potential for habitat selection, non-metric multidimensional scaling was used to plot habitat data in a multidimensional space. An ANOSIM was performed to test for significant relationships between the habitat data and species presence. Although this study was not successful for elucidating habitat preferences, it provided insight into the level of effort required to detect target species.

Chapter One

A LITERATURE REVIEW OF FRESHWATER MUSSELS AND OCCUPANCY MODELING

Freshwater mussels (Order: Unionida) represent a diverse taxa in North America with approximately 300 species in the families Unionidae and Margaritiferadae (Williams et al. 1993, Howells 2010). Although diverse, freshwater mussels are considered one of the most imperiled group of organisms. Williams et al. (1993) reported 55% of North American mussels as extinct or imperiled, and Negishi et al. (2012) considered 70% of North American mussel species endangered, threatened, or of special concern. Texas has 52 described mussel species and, of these, 15 are listed as state threatened by the Texas Parks and Wildlife Department and a small number are presumed extinct or extirpated from their environments (Howells 1996, Howells 2010, Randklev et al. 2013). East Texas lies within the Piney Woods ecoregion and contains 37 species that represent most of the mussel biodiversity in the state. Six species in East Texas are listed as state threatened: Texas Pigtoe (*Fusconaia askewi*), Triangle Pigtoe (*F. lananensis*), Southern Hickorynut (*Obovaria arkansasensis*), Louisiana Pigtoe (*Pleurobema riddellii*), Sandbank Pocketbook (*Lampsilis satura*), and Texas Heelsplitter (*Potamilus amphichaenus*).

The overarching goal of my thesis is to determine habitat associations of three state listed mussels in East Texas using occupancy modeling. Because of the imperiled status of Texas mussels it is important to identify and understand these relationships. Habitat association data can provide valuable information to state and federal agencies. For example, this information could aid in establishing new areas to search for mussel populations based on habitat surveys. In

this chapter, I and providing a review of relevant information that forms a basis for this work. This review begins by describing freshwater mussel ecology, including reproductive strategies, followed by a discussion on the occupancy modeling framework used in this thesis. Topics in this discussion include model assumptions, detection/non-detection, covariates, and data collection under this modeling framework.

Mussel Ecology

Mussels can form beds of dense local populations sometimes exceeding 100 animals/m² and reaching a total biomass greater than other benthic communities (Howells 2014, Strayer 2008). They also provide important ecosystem services that contribute to the structure and function of freshwater systems. As suspension-feeders (filter-feeders), mussels influence water chemistry and clarity (Haag 2012, Howells 1996, Strayer 2008), altering the amount and composition of suspended particles, including phytoplankton (Howells 1996). Waste products produced by mussels can also enhance algae and macroinvertebrate communities (Strayer 2008). In addition, mussels serve as prey items for fish and terrestrial species, acting as a vector for converting fine particles into biomass edible for higher trophic levels in aquatic food webs.

Mussel species exhibit a complex life history and reproductive strategy in which their mode of distribution is dependent on the presence of an obligate host fish species (Haag 2012, Howells 1996). Males release sperm directly into the water column that females inhale through their incurrent siphons (Howells 1996). Eggs are fertilized within modified gill structures called marsupial pouches. Some species of mussels are gravid for only a short period of time, over a season, while others maintain their brood for several months. These brooding strategies are, respectively, tachytictic (short-term) and bradytictic (long term) (Gascho & Stoeckel 2016, Haag

2012, Howells 1996). To offset reduction in water velocity and respiratory efficiency, many bradytictic species will develop secondary water tubes while brooding takes place within the primary water tubes (Gascho & Stoeckel 2016). Short term brooders lack secondary water tubes, maintaining their brood throughout the gills (Gascho & Stoeckel 2016). After maturation from egg to mussel larvae, called glochidia, they are released to encyst onto the gills of a fish host. Several methods of glochidial distribution exist: mantle lures, conglutinates, and direct release of glochidia into the water column (Davis & Layzer 2012, Howells 1996, McIvor & Aldridge 2007, Strayer 2008). Mantle lures are elaborate representations that mimic prey items or that attract prey items of host fish species (Strayer 2008). Conglutinates are packets of glochidia that resemble worm-like food items. When a fish attempts to consume a conglutinate, it bursts releasing the glochidia to attach to their host. (Davis & Layzer 2012). Glochidia will parasitize on their host fish for several days to several months (Haag 2014, Strayer 2008). After metamorphosis into juveniles, they excyst and drop to the substrate presumably far from the parent mussel (McIvor & Aldrige 2007). This movement by fish is the primary mode of dispersal for North American mussels. Despite conservation efforts to preserve these important animals, mussel numbers are still in decline (Haag 2014). This is exacerbated by their inconspicuous nature; cryptic mussels are a challenge for ecological surveys that contrast areas where the animals are present versus areas where the species is absent.

Occupancy Modeling

Occupancy estimation is a model-based approach that employs repeat sampling techniques to account for imperfect detection of this kind (MacKenzie & Royle 2005, Wisniewski et al. 2014), where the probability of detection is conditional on the species presence and effort expended (MacKenzie et al. 2011, Wisniewski et al. 2014). Occupancy is defined as

the probability of a species presence, considering its overall naive occupancy (presence, non-detection), habitat associations, and detectability given the level of effort expended. This approach was designed to estimate the proportion of sites occupied by a species of interest using multiple visits or sampling events while simultaneously estimating probability of detection over a range of habitat conditions and sampling effort (MacKenzie et al. 2002, 2003, 2004).

As with most statistical methods assumptions exist for occupancy models, including site closure, no change of occupancy over the sampling event, and site independence (Bailey & Adams 2005, Crossland et al. 2005). Site independence is the most difficult assumption to meet in surveys of single rivers or within single drainages; however study design can separate sites spatially and stratify sampling to minimize site dependence or, alternatively, include a spatial covariate to account for any spatial dependence among sites (Bailey & Adams 2005, Crossland et al. 2005).

MacKenzie et al. (2002) used computer simulations to demonstrate that this approach can provide unbiased estimates of occupancy. Nonetheless, effort required to estimate occupancy is a function of detection probability. Because of this relationship, more effort is required at lower detection probabilities to provide a degree of certainty in absence data (MacKenzie et al. 2005). It is important to note that, if each site is sampled only once, an unbiased estimate of occupancy is not possible to obtain (Crossland et al. 2005). To meet model assumptions, an investigator may schedule surveys during periods when the system of interest is most likely to be closed (i.e., no immigration or emigration) (Bailey & Adams 2005, Watson et al. 2009). Also, understanding the movement ecology of the species of interest will aid in selecting sites within the sample area to maintain independence (Bailey & Adams 2005, MacKenzie & Royle 2005). To coincide with meeting model assumptions, sites should be chosen with a probability-based sampling scheme in

mind (Bailey & Adams 2005). These sampling schemes can include a simple-random sample, stratified-random sample, or systematic-random sample, among others. (MacKenzie & Royle 2005).

When estimating occupancy and detectability, an investigator aims to identify factors that are responsible for observed variation. These factors, called covariates, can include anything that would cause heterogeneity in either occupancy or detectability. Site or occupancy covariates are those that are related to or are indicative of the study species habitat use preferences (Watson et al. 2009). Occupancy covariates are measured once per site and can include factors such as canopy cover, substrate type in a river, flow regime, etc. Detection covariates are factors that affect sampling effectiveness, and therefore, detection probability of the study species when they are present in a site. They can include factors such as date, surveyor experience water clarity, time of day, etc. (MacKenzie et al. 2003, MacKenzie & Royle 2005, Watson et al. 2009). Occupancy and detection covariates are selected based on individual study objectives and considered separately.

During surveys, species detection/non-detection is recorded as a binary detection history of either a 1 or 0 (MacKenzie et al. 2002, Wisniewski et al. 2014, Royle & Nichols 2003). 1 indicates presence or detection and 0 non-detection. An example of a detection history collected during a sampling event with three repeat samples is 101. This indicates that the species was detected at the first and third survey, but not during the second. Without incorporating covariates, raw detectability can be calculated as a binomial probability per survey with the equation $\hat{p} = x/n$ where n is the total number surveys conducted at a location where the species was present and x is the number of those surveys where the species was detected (Mackenzie et

al. 2005). Based on the method of repeat sampling, it may seem intuitive to survey as many sites as possible to estimate detection and occupancy. However, this may not be the most efficient use of allocated resources and ultimately result in a less precise estimate of occupancy (MacKenzie & Royle 2005). Results from a simulated study by MacKenzie & Royle (2005) lend to a general strategy of when occupancy is low, survey more sites, and when occupancy is high, effort should be concentrated on repeated surveys. If the occupancy status for a low-density species is under investigation, surveying a greater amount of sites yields a more reliable detection estimate. With high occupancy, reliable information can be gathered from fewer sites using repeat samples. Occupancy modeling serves to provide an unbiased estimate of occupancy and detection based on the measured covariates and effort allocated to the study of the species under investigation.

Chapter Two

HABITAT ASSOCIATIONS AND DETECTABILITY OF THREE UNIONID SPECIES ALONG THE UPPER SABINE RIVER IN EAST TEXAS

Introduction

Freshwater mussels (Order: Unionida) represent a diverse taxa in North America with approximately 300 species in the families Unionidae and Margaritiferadae (Williams et al. 1993, Howells 2010). Although diverse, freshwater mussels are highly imperiled. Williams et al. (1993) reported 55% of North American mussels as extinct or imperiled, and Negishi et al. (2012) considered 70% of North American mussel species endangered, threatened, or of special concern. Causes for imperilment include degradation of freshwater habitats from anthropogenic disturbances and/or pollution (Ford et al. 2009), which promote deterioration of streams and negatively impact stream inhabitants (Burlakova et al. 2011, Falfushynska et al. 2014, Strayer 2008). Human alterations to stream hydrology, sedimentation, increased nutrient loads, changes to thermal and light regimes, and channelization alter mussel habitats (Strayer 2008) and impact other important components of the stream invertebrate community (Jardine et al. 2013). Mussels exhibit a complex life history in which their mode of distribution is dependent on the presence of the appropriate host fish species. With this complex system of distribution; aquatic infrastructure, such as impoundments, can have negative in-stream effects, including impediment

of the movement of host fish and loss of habitat (Borthagaray & Carranza 2007, Randklev et al. 2013).

Texas has 52 described mussel species and of these, 15 are listed as state threatened by the Texas Parks and Wildlife Department, and a small number are presumed extinct or extirpated (Howells 1996, Howells 2010, Randklev et al. 2013). With its large diversity of unionids, Texas ranks fourth in mussel species extinctions, a product of damming, pollution, land use, water extraction, and the introduction of invasive species (Burlakova et al. 2011, Watters 1995, Howells 1996). Because of their sensitivity to water quality, it has been suggested that the abundances and distributions of all native Texas mussels have declined, and over half have a conservation status of major concern (Howells 2014).

East Texas lies within the Piney Woods ecoregion, and its abundant rivers, creeks, and streams contain 37 species that represents most of the mussel biodiversity in the state. Of these, six are listed as state threatened: *Fusconaia askewi*, *Fusconaia lananensis*, *Obovaria arkansasensis*, *Pleurobema riddellii*, *Lampsilis satura*, and *Potamilus amphichaenus*. Although widespread, these systems are at risk and are impacted by the aforementioned types of disturbances (Howells 1996, Randklev et al. 2013, Burlakova et al. 2011). A limited amount of information related to the current distribution and health of mussel populations, and an inaccessibility of historical data according to the National Native Mussel Conservation Committee (NNMCC) (Arnold et al. 2013) pose a challenge to managers tasked with preserving this native fauna. Particularly in the Sabine River, a limited amount of surveys have taken place and fundamental information on species in this system is lacking (Arnold et al. 2013, Ford et al. 2009, Randklev et al. 2016).

Occupancy estimation is a model-based approach useful for ecological and wildlife studies posing questions about species distribution, potential range, habitat associations, and population dynamics (Mackenzie et al. 2002, 2003, 2004, and 2005). Although occupancy models require a sufficient amount of data to conduct, which is a challenge for relative rare and cryptic species, they also account for imperfect detection, a reality in any mussel study (Chestnut et al. 2014, Mackenzie et al. 2003, 2005). Factors that influence the spatial heterogeneity of species abundances and presence/absence data are used jointly to estimate the probability of species presence while accounting for imperfect detection (Wisniewski et al. 2014 and Bailey & Adams 2005). Detection probability is the probability of observing the species if present during a given survey (Mackenzie et al. 2002, 2011, Wisniewski et al. 2014). Using repeated sampling or surveys, detection probabilities can be estimated as a proportion, $\hat{p} = x/n$ where n is the total number of surveys conducted at a location and x is the number of those surveys where the species was detected (Mackenzie et al. 2005). Replicate samples can be acquired by visiting a site multiple times across seasons to estimate temporal variations in detection or by conducting repeated samples at one visit to measure spatial variations in detection. Through repeated sampling, a binary detection history is constructed for the target species where presence (1) and absence (0) are recorded. Non-detection does not necessarily imply non-occupancy because the species could be present and not detected; detection probability may also change based on site-specific characteristics, and habitat covariates can be included for estimates of detection probability. (Mackenzie et al. 2002, 2003, 2004). For mussels, occupancy models can provide insight to their status, habitat use, and distributions (Wisniewski et al. 2014).

Mussels are influenced by abiotic environmental conditions that mediate spatial distributions; therefore, they may respond to stream habitat variables such as water depth, water

velocity, and the substrate in which they bury (Howells 1996 and Troia et al. 2015). In this study, my goal was to provide information on the distribution and habitat associations of three state listed mussel species: *F. askewi*, *L. satura*, and *P. amphichaenus*. Our project objectives were to: 1) Identify areas that are considered suitable for target species, 2) Determine covariates that could influence occupancy and detection, and 3) use habitat models to determine habitat use patterns of the three species.

Materials and Methods

Study Area

The Sabine River originates in north-central Texas in the counties of Hunt, Collin, and Van Zandt. From there it flows southeastwardly dividing Texas and Louisiana, moving southward to Sabine Lake, encompassing a total drainage area of about 25,100 km² (Neck 1986). Sabine Lake is an estuary of the Gulf of Mexico in which both the Sabine and Neches rivers flows. Two large reservoirs are located along the main stem of the Sabine River, Lake Tawakoni and Toledo Bend. Lake Fork Creek, a tributary of the Sabine River, located in Wood, Rains, and Hopkins counties east of Lake Tawakoni flows into the river, supplying much of the initial flow (Ford et al. 2009). Sites were selected (see below) along a section of the upper Sabine River between US highways 69 near Lindale, TX (Smith County) and 79 near Carthage TX (Panola County) (Figure 1). The first portion of the study area flows alongside the Old Sabine Bottom Wildlife Management Area, which creates the northern border. Two TPWD mussel sanctuaries

are encompassed by this section of the river, the first is located below the bridge at Highway 14 to Highway 155, and the second is located between Highways 43 and 59 (Ford et al. 2009).

Study Species

Target species include the Sandbank pocketbook (*L. satura*), Texas pigtoe (*F. askewi*), and the Texas heelsplitter (*P. amphichaenus*), all of which occur in the Sabine River. *F. askewi* is the most numerous, while *L. satura* and *P. amphichaenus* are much more rare (Howells 2014). All three species are listed by TPWD as state threatened; however, *P. amphichaenus* is proposed for federal listing by the United States Fish and Wildlife Service (USFWS) (Howells 2014). Past studies indicate that all three species generally occur in mud, sand, gravel or a mix and areas of low to moderate water velocity (Howells 1996, 2014).

Site Selection

Thirty sites were selected in high suitability areas for the three target species according to MaxEnt analysis by Symonds (2015). Data layers used by the author of the previous study included: soil type, vegetation type, groundwater recharge, geology type, landform, and land cover diversity (Symonds 2015). Individual maps were created for each species to assess their distributional overlap within the sampling area. Suitability scores, produced by MaxEnt, were divided into quartiles and scores within the top 25% were used to delineate sample sites. All

target species shared distributions within the selected portion of the river according to the MaxEnt maps. This section of the river was then divided into five kilometer segments resulting in a total of 45 possible sampling sites. Fifteen of those sites were selected randomly. Two sites within the 5km segment were sampled. Each of the two locations were separated by at least 1km to account for pseudoreplication (Wisniewski et al. 2014, Hurlbert 1984).

Sampling Technique

Eighteen sites were sampled from July 2015 to September 2015 and 12 sites from July 2016 to August 2016 during base flow conditions. In the field, sampling sites were established based on signs of mussel presence (shells on the bank and river morphology), and an informal survey was conducted to define the perimeter of the mussel bed (Ford et al. 2009). All sites were delineated to 50 meters, and each survey consisted of three independent surveys. Ten 0.25m² quadrats were sampled during each survey for a total of 30 samples at each site. Independence of samples was ensured by using separate observers for each sampling event, at least three observers per site. Quadrat placement was determined by a systematic random sampling approach, which allows for adequate spatial coverage with three random starts as outlined in Strayer et al. (2003). Beginning points were determined from a random number generator, and the distance between each quadrat was calculated with the equation: $d = \sqrt{\frac{L \cdot W}{n/k}}$ at each study site along the river. L and W are the length and width of the study site, respectively, n is the number of quadrats, and k is the number of random starts (Strayer et al. 2003). Detection/non-detection surveys were conducted at each sampled site.

Covariate Measurements

Fifteen site-level habitat covariates were assessed within each sample reach to model occupancy of the target species, accounting for detectability. Occupancy covariates were measured along a transect perpendicular to flow (D'Ambrosio et al. 2014). Mean velocity, mean depth, width, bank angle, and substrate composition were measured or recorded at each site. The coefficient of variation of depth was calculated to measure heterogeneity in depth at each site. Channel width was measured from the water's edge at both banks with a meter tape. Substrate composition was visually estimated, and categories were recorded as percent coverage (Clapcott et al. 2011). Substrate categories included: clay, gravel, cobble, boulder, bedrock, and woody debris. A sites mesohabitat was classified as slackwater or swiftwater which applied to pools/edgewater and riffles/runs, respectively and are determined based on the surface roughness of the water. (Wisniewski et al. 2014). Bank angles were measured with a clinometer from the thalweg of the river, at the water surface, to the top of the bank at the point of bank full. Detection covariates included measurements of observer experience, expressed as years of mussel survey experience and the Julian date of each sampling period.

Data Analysis

Occupancy and detection estimates were generated using single-season occupancy models for each species (MacKenzie et al. 2006). Probabilities of occupancy and detection were estimated in relation to site-level characteristics collected during sampling. The R package “DiversityOccupancy (R Core Team 2016, Corcoran & Kesler 2016)” was used to develop the models from a series of data sets. The associated data sets were categorized as occupancy and detection covariates and as the binomial presence/absence data. This package uses information theory using a multimodel inference approach with model averaging to produce the most likely model or model set (Grueber et al. 2011). Models are selected from a set based on Akaike Information Criterion (AIC_c), corrected for small sample size. To avoid collinearity in predictor variables, a Pearson’s correlation analysis was conducted. Variables with a correlation coefficient of $|r| < 0.7$ were selected for analysis (Dormann et al. 2013).

To supplement the results of the occupancy model, data were compiled by surveyed river segments to conduct a non-metric multidimensional scaling (NMDS) analysis using a Bray-Curtis similarity measure. This allowed for a comparison of the measured habitat covariates at each river segment and displays segment characteristics in a multidimensional space. Because NMDS uses a rank correlation approach, an analysis of similarity (ANOSIM) was performed to test for significant relationships of species presence, using count data, and habitat covariates.

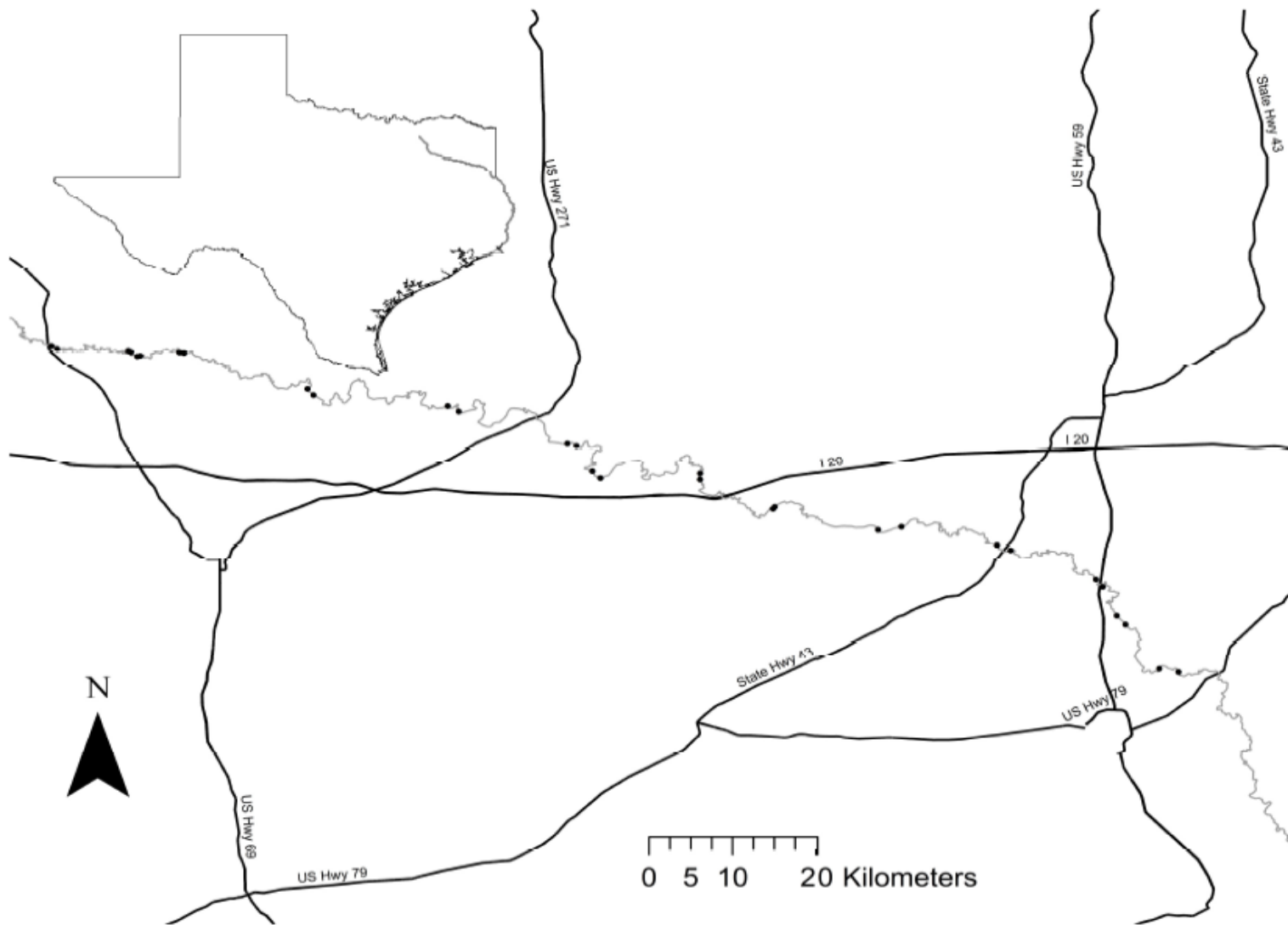


Figure 1. Map of the study area within the upper Sabine River. Points indicate where field surveys were conducted and lie between highways 69 (upper left) and 79 (lower right).

Results

Two-hundred and thirty four live individuals of the three target species were collected across the 30 sample sites in the upper Sabine River, which encompassed 900 quadrat samples and 328.3 person hours (Table 1). The Texas Pigtoe (*F. askewi*) was collected at 13 sites and was the most abundant of our target species throughout the sampled region of the river. The Sandbank Pocketbook (*L. satura*) was collected at six sites, where detection represented a single individual collection, with the exception of one site, where seven individuals were collected. The Texas Heelplitter (*P. amphicaenus*) was collected at four sites; three of these individuals were detected in a single quadrat at a site near highway 59 (Panola county).

To determine the effectiveness of the sampling scheme, cumulative detection probabilities were calculated based on 30 quadrat samples, which exceeded 0.8 for all species, suggesting this method was adequate to detect these species when present (Figure 2). The highest detection probability per unit effort, described as a quadrat sample, was observed for *F. askewi* $0.71 \pm (0.34)$, while *P. amphicaenus* was the lowest $0.11 (\pm 0.06)$ (Figure 3), and *L. satura* had a detection probability of $0.15 (\pm 0.03)$ for a single quadrat sample when present at the site (Figure 3). Models did not show a relationship between habitat variables and detection probabilities. Naïve occupancies were most similar between *P. amphicaenus* and *L. satura*, at 0.13 and 0.2 of the sites, respectively (Figure 4). *Fusconaia askewi* had the highest naïve occupancy of 0.43 (Figure 4). A Pearson's correlation test with a cutoff score of 0.7 identified mean water velocity and mean bank angle as highly correlated. Mean bank angle was not included in the models and mean water velocity was retained. Once a juvenile mussel excysts from its fish host, it must establish itself within the sediment. Extreme water velocities can impede or completely disrupt

this process, thus creating an area where mussels cannot establish (Strayer 2008), therefore it was determined that mean water velocity was the most relevant habitat variable to include in the models. Models indicated no effects on occupancy in response to the habitat variables ($P > 0.05$ for all occupancy covariates; Table 2). Models failed to produce realistic results for *P. amphicaenus*, presumably because of a lack of data, and are therefore not presented. Null models were selected for both detection and occupancy for all species, resulting in no weighted models produced. The NMDS (Stress = 0, Global R = 0.024, $P = 0.47$) illustrates no clustering, which would indicate no dissimilarity of community structure among the sampled river segments (Figure 5). An analysis of similarity (ANOSIM) indicated no relationship of habitat variables and species presence ($P = 0.21$).

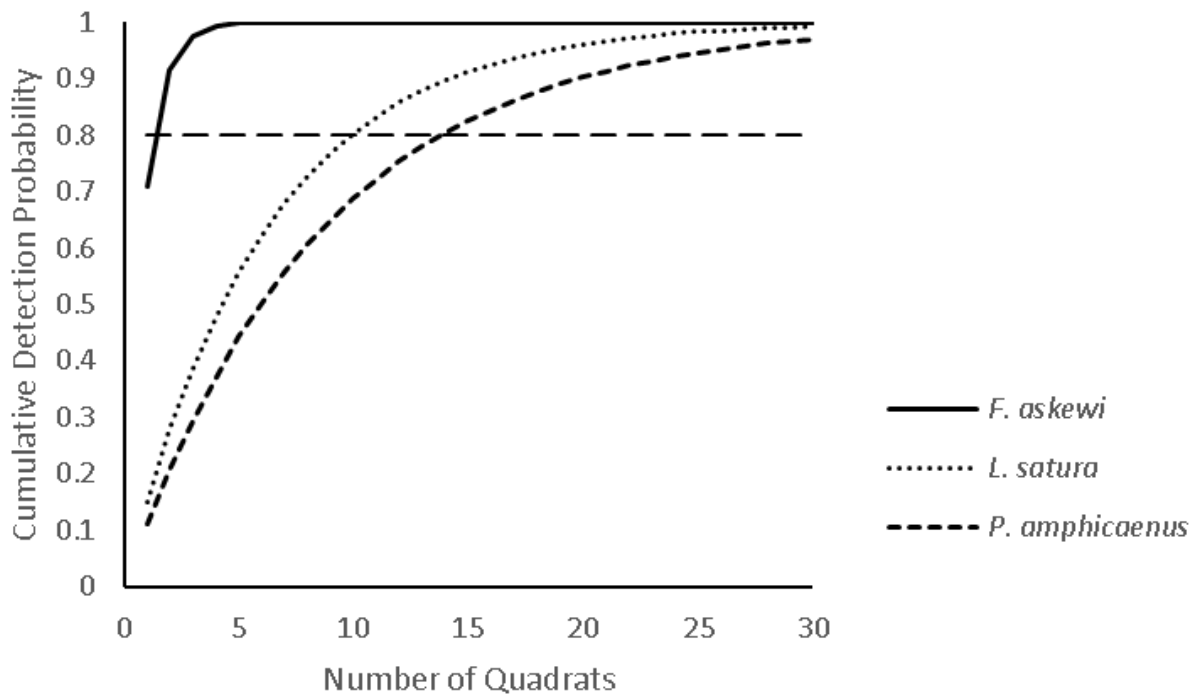


Figure 2. Cumulative detection probabilities of the three focal species collected in the Sabine River. Values indicate the probability of detecting a species when a given number of 0.25m² quadrats are searched at a site. These probabilities are conditional on the species presence. The 0.8 cutoff represents the desired probability of detection.

Table 1. Sites and raw capture data of target species from the upper Sabine River in summers 2015 and 2016, locations indicate point of river access

Sites	Location	Latitude	Longitude	# Surveyors	Total Time (min)	<i>F. askewi</i>	<i>L. saturata</i>	<i>P. amphicaenus</i>
1	HWY 69	32.61	-95.48	3	60	0	0	0
2	HWY 69	32.61	-95.48	3	60	0	0	0
3	HWY 14	32.55	-95.20	5	125	0	0	0
4	HWY 14	32.56	-95.21	5	135	0	0	0
5	HWY 271	32.50	-94.93	3	180	27	1	0
6	HWY 271	32.49	-94.92	3	48	0	0	0
7	HWY 149	32.42	-94.70	3	126	3	1	0
8	HWY 149	32.42	-94.70	3	135	6	0	0
9	Hoard RD	32.60	-95.39	4	160	0	0	0
10	Hoard RD	32.60	-95.39	4	188	0	0	0
11	Hoard RD	32.61	-95.40	4	188	1	0	0
12	Hoard RD	32.60	-95.40	4	96	0	0	0
13	HWY 43	32.38	-94.47	3	72	0	0	0
14	HWY 43	32.37	-94.45	3	246	6	0	0
15	HWY 59	32.34	-94.36	3	123	5	0	0
16	HWY 59	32.33	-94.35	3	78	0	0	3
17	HWY 1794	32.28	-94.33	3	45	0	0	1
18	HWY 1794	32.29	-94.34	3	120	0	0	0
19	Old Sabine WMA	32.60	-95.34	3	120	2	0	0
20	Old Sabine WMA	32.60	-95.34	3	105	0	0	0
21	up from 271	32.54	-95.06	3	450	62	7	0
22	up from 272	32.53	-95.04	3	105	6	1	0
23	HWY 31	32.45	-94.78	3	90	0	0	1
24	HWY 31	32.46	-94.78	3	105	1	0	0
25	HWY 79	32.23	-94.27	3	90	0	0	0
26	HWY 79	32.23	-94.29	3	90	0	0	0
27	HWY 42	32.45	-94.89	3	225	46	1	0
28	HWY 42	32.46	-94.90	3	165	50	1	0
29	down from 149	32.40	-94.59	3	150	0	0	0
30	down from 149	32.40	-94.57	3	60	1	0	1

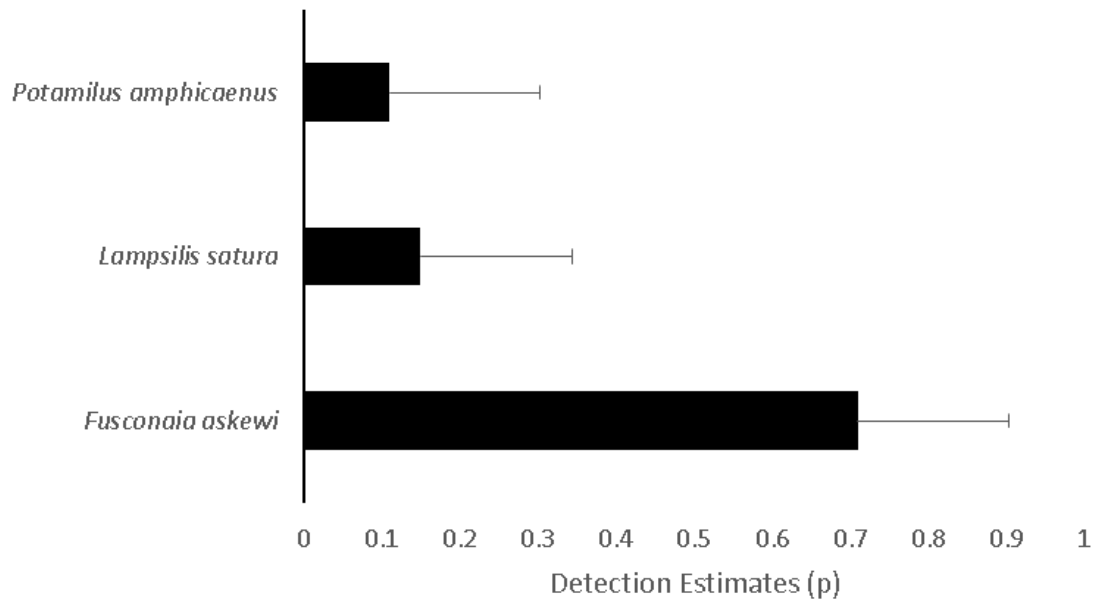


Figure 3. Estimated detection probabilities collected at 30 sites, calculated from the occupancy model for target species sampled in the upper Sabine River. These surveys are based on a survey of 30 quadrats. Error bars represent standard error.

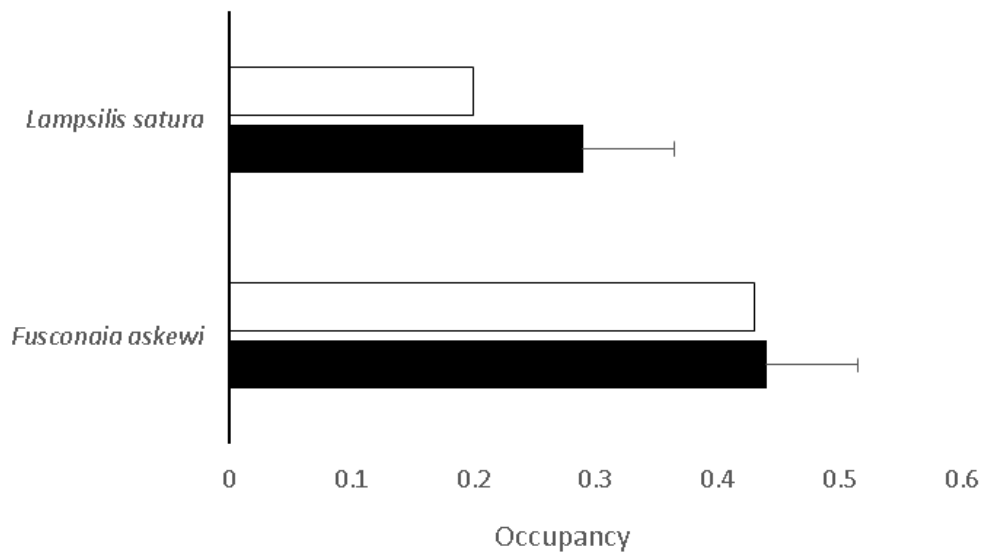


Figure 4. Occupancy for target species collected in the upper Sabine River. Naive occupancy is indicated by non-shaded bars, and shaded bars represent model predictions of occupancy. Error bars represent standard error.

Table 2. Best fit models of *Fusconaia askewi*, *Lampsilis satura*, and *Potamilus amphicaenus*. Models include occupancy rate (ψ), the habitat covariate, and detection (p). Also given are the parameter estimates of each covariate and its associated p-value.

Parameter	Estimate	SE	P-value
<i>Fusconaia askewi</i>			
Detection (p)			
Intercept	0.92	0.34	< 0.01
Occupancy (Ψ)			
Intercept	274.63	238.12	0.25
COV Depth	-78.68	99.32	0.43
Max Bank Angle	-1.74	2.11	0.41
Sand	-2.27	2.13	0.23
Woody Debris	-4.25	4.69	0.37
<i>Lampsilis satura</i>			
Detection (p)			
Intercept	-4.90	1.79	< 0.01
Occupancy (Ψ)			
Intercept	-43.66	129.74	0.74
Width	4.16	9.98	0.68
Width2	-0.09	0.15	0.57
<i>Potamilus amphicaenus</i>			
Detection (p)			
Intercept	1.894	1.659	0.25

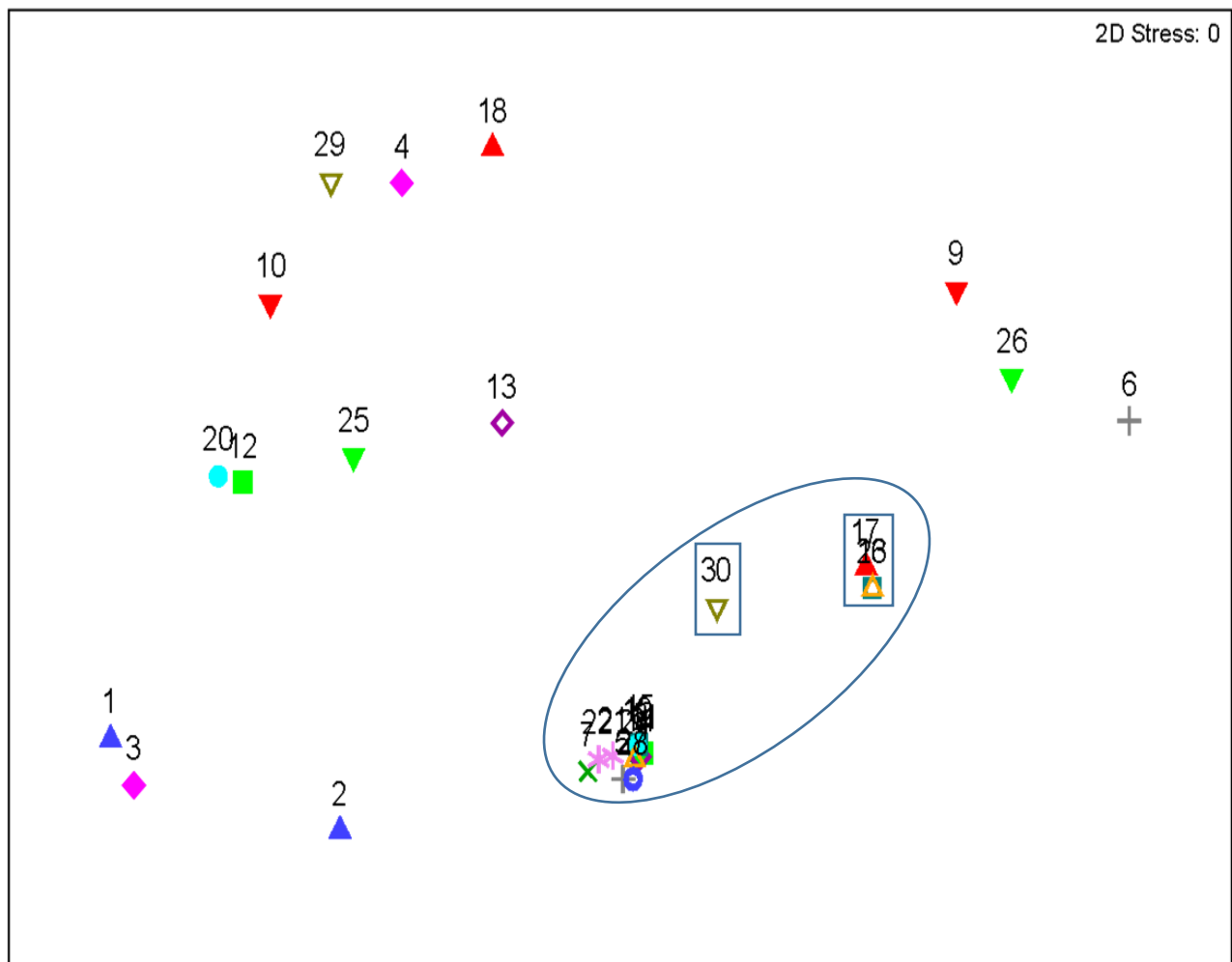


Figure 5. Nonmetric multidimensional scaling (Stress = 0, Global R = 0.024, P = 0.47) for the sampled 5km river segments of the upper Sabine River. Numbers represent the corresponding segment as they were numerated from upstream to downstream. Each segment is represented in the non-dimensional space based on their habitat characteristics, for example, those close together are more similar than those sites further apart. Sites contained within the oval are those that were occupied by at least one of the focal species. Those outlined by a rectangle represent all sites occupied by *P. amphicaenus*.

Discussion

Determining mussel habitat use is important to better understand mussel distributions and to develop conservation strategies (Harriger et al. 2009). Instream habitat influences aquatic assemblages, including fish, crayfish, and macroinvertebrates (Niraula et al. 2015). McRae et al. (2004) identified reach-level variables with which mussels associated, for example, habitat quality, flow stability, substratum, and conductivity. However, several accounts also are published that are inconclusive regarding microhabitat associations. Haag (2012) proposed that these negative results were the product of sampling within small systems (e.g., tributaries) with homogeneous habitats, resulting in poor contrast between occupied and unoccupied areas. Smaller stream systems tend to have less habitat diversity when compared to that of larger systems. Our study indicated no significant relationships between the target species and the measured occupancy covariates within the upper Sabine River.

Occupancy estimates indicate that *F. askewi* was the most widely distributed of the three species within the upper Sabine River; the model suggests an approximate 75% probability of detection for each quadrat when the species was present. *Fusconaia askewi* also accounted for the highest abundance (92.3% of target species observations). Abundance-induced heterogeneity in detection is most prevalent in small populations (Wisniewski et al. 2014). Because *F. askewi* occurrence was not associated with measured detection covariates, this species had a higher detection probability because it was locally abundant and well distributed throughout our study sites.

Occupancy estimates of *L. satura* suggest that this species was moderately distributed throughout the sampling region. This species was found within 20% of the sampled sites in the

upper Sabine. Models indicated an approximate 15% probability of detection per sample quadrat at sites where present, suggesting poor detectability for *L. Satura* unless sufficient effort is expended. Occupancy of *P. amphicaenus* indicate that this species was the rarest and most difficult to detect. It was collected in a total of four of our thirty sites, too rare for reliable modeling of habitat associations. For such rare organisms, more sites are needed, a requirement that must be balanced against effort required for detection (Crossland et al. 2005). *P. amphicaenus* is not only regionally rare, but is locally sparse, indicating the need for extensive sampling for detection. *P. amphicaenus* was collected from sites only within the lower portion of the sample area, in areas with large channel widths, at least one low bank, and sandy substratum. Further, when collected, *P. amphicaenus* was excavated from depths of approximately 10 cm and deeper within the sandy substrate. It could be expected that because of their opportunistic life history and observed burrowing behavior, these individuals occur in areas with bed instability and is responding to habitat variables and processes occurring at a larger scale (Haag 2012, Randklev et al. 2016). Results from an Analysis of Similarity (ANOSIM), produced from the NMDS, corroborate the results from the occupancy model indicating no relationships for these species with the habitat variables measured.

Because of their limited ability to migrate, mussels must be able to tolerate their immediate chemical and physical environment (Golladay et al. 2004), therefore they may associate with unique microhabitats within the areas in which they occur (Cao et al. 2015). Functional (life history) traits play a large role in the distribution of freshwater mussels within the river continuum (Troia et al. 2015). Because of the importance of these traits, greater understanding of mussel life histories could be crucial in determining habitat relationships (Vaughn 2012). For example, *P. amphicaenus* is classified as having an opportunistic life history

for a freshwater mussel, potentially allowing this species to occur within areas of habitat instability (Haag 2012 based on Winemiller & Rose 1992). Observations from the current study indicate that it only occurred in the lower reaches of the sampling area, areas prone to bank falls because of unstable sandy substrate (Ford et al. 2006). Given this and other studies that have shown the importance of hydro-geomorphological factors in mussel-habitat relationships (Atkinson et al. 2012), it may be necessary to include habitat variables incorporating these factors, particularly at larger scales, i.e., landscape and catchment scales, to elucidate habitat relationships of this species (McRae et al. 2004). A recent study published by Troia et al. (2015) describes a framework that links geomorphic processes and temporal variation that influence population dynamics of stream organisms, this framework is the process domains concept (PDC). Using the PDC to outline geomorphic processes at sample sites would be beneficial in understanding how natural disturbances at large spatial scales influence local mussel communities. In addition to investigating habitat relationships at multiple scales, the effects of dams should also be considered because of their ability to alter hydro-morphologic characteristics of the river (Neck 1986, Randklev et al. 2016, Troia et al. 2015, Wisniewski et al. 2013, 2014). The sampling region lies between two major impoundments of the Sabine River, with both upstream (Toledo Bend Reservoir) and downstream (Lake Tawakoni) effects. Recent studies have indicated the plausibility that the overlap of these upstream and downstream effects have reduced diversity and altered the distributions of mussel assemblages (Randklev et al. 2016).

Conclusions

This study investigated the habitat associations of three state listed freshwater mussel species sampling at the reach level using occupancy modeling. Although occupancy modeling failed to elucidate habitat relationships in our study, it did provide important information on the effort required to detect species when present in sampling sites. The number of 0.25m² quadrats required to detect *F. askewi* with >80% confidence, as derived from figure 2, is two. Ten and 14 quadrats were determined to be required to detect *L. satura* and *P. amphicaenus* with 80% confidence, respectively. Cumulative detection estimates across all sites in the current study were lowest for *P. amphicaenus* and *L. satura*. Because of low local abundances, much more effort should be allocated to the study of these two species in the form of sampling a greater amount of quadrats, and determining their distribution will require significant effort in terms of the number of sample sites (Crossland et al. 2005, MacKenzie & Royle 2005). From this study, I was able to produce a cumulative detection history which models detection probability based on the number of quadrats used to sample a site. Although, no habitat associations were found, these habitat variables should not completely dismissed. Freshwater mussels may associate with these variables in conjunction with others at different spatial scales.

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Appendix A. Habitat and detection covariates collected to model occupancy and detection in the upper Sabine River in East Texas.

Table 3. Dates and locations of sites sampled in the upper Sabine river in East Texas. Julian dates, one of the detection covariates, were coded for in program R and represented the month/day/year dates which are notated in the table.

Date	Location	Latitude	Longitude
JUL 15 2015	HWY 69	32.61178	-95.48229
JUL 15 2015	HWY 69	32.60849	-95.47613
JUL 31 2015	HWY 14	32.55308	-95.19980
JUL 31 2015	HWY 14	32.56044	-95.20637
AUG 06 2015	HWY 271	32.49687	-94.92624
AUG 06 2015	HWY 271	32.49415	-94.91649
AUG 07 2015	HWY 149	32.42143	-94.70282
AUG 07 2015	HWY 149	32.41932	-94.70470
AUG 10 2015	Hoard RD	32.60051	-95.38650
AUG 10 2015	Hoard RD	32.59997	-95.38980
AUG 10 2015	Hoard RD	32.60638	-95.39936
AUG 10 2015	Hoard RD	32.60486	-95.39647
AUG 27 2015	HWY 43	32.37727	-94.46508
AUG 27 2015	HWY 43	32.37108	-94.45031
SEP 1 2015	HWY 59	32.33534	-94.35844
SEP 1 2015	HWY 59	32.32674	-94.35106
SEP 8 2015	HWY 1794	32.28297	-94.32669
SEP 8 2015	HWY 1794	32.29333	-94.33596
JUL 15 2016	Old Sabine WMA	32.604529	-95.34423
JUL 15 2016	Old Sabine WMA	32.603714	-95.33941
JUL 19 2016	up from 271	32.540393	-95.05517
JUL 19 2016	up from 272	32.534124	-95.04336
JUL 21 2016	HWY 31	32.453355	-94.78323
JUL 21 2016	HWY 31	32.460448	-94.78317
JUL 26 2016	HWY 79	32.226142	-94.26945
JUL 26 2016	HWY 79	32.22998	-94.29026
JUL 28 2016	HWY 42	32.454849	-94.89059
JUL 28 2016	HWY 42	32.46308	-94.89934
AUG 11 2016	down from 149	32.395012	-94.59315
AUG 11 2016	down from 149	32.398615	-94.56798

Table 4. Occupancy covariates measured at each site in the upper Sabine in East Texas. Locations are included to distinguish each sites respective covariate measurements.

Location	Velocity (m/s) RB	Velocity CL	Velocity LB	Bank Angle RB	Bank Angle LB	River Width (m)
HWY 69	0.4	0.62	0.45	15	17	18.7
HWY 69	0.01	0.27	0.25	14	19	15
HWY 14	0.43	0.67	0.25	11	43	20
HWY 14	0.49	0.31	0.03	14	16	27.1
HWY 271	0.87	0.68	0.51	9	22	26.1
HWY 271	0	0	0	26	22	37
HWY 149	0.13	0.24	0.4	14	2	17.4
HWY 149	0.45	0.74	0.43	11	22	15.8
Hoard RD	0	0	0	29	28	8.9
Hoard RD	0	0.01	0.01	34	16	8.2
Hoard RD	0.46	0.41	0.29	6	24	7.6
Hoard RD	0.11	0.11	0.03	30	18	10.7
HWY 43	0.57	0.35	0.38	11	22	35.2
HWY 43	0.33	0.45	0.55	17	23	37.8
HWY 59	0.63	0.39	0.25	4	19	45.7
HWY 59	0.15	0.09	0.05	3	22	7
HWY 1794	0.31	0.61	0.58	33	11	35.7
HWY 1794	0.62	0.64	0.91	26	34	35.5
Old Sabine WMA	0.71	0.74	0.62	23	14	18.7
Old Sabine WMA	0.62	0.55	0.36	33	10	11.8
up from 271	0.56	0.48	0.46	32	18	22
up from 272	0.17	0.53	0.06	32	26	24.8
HWY 31	0.09	0.26	0.34	27	25	25.6
HWY 31	0.16	0.14	0.13	34	27	22.6
HWY 79	0.45	0.38	0.35	9	18	45.75
HWY 79	0.37	0.96	0.58	23	8	39.3
HWY 42	0.52	0.75	0.65	30	30	19.4
HWY 42	0.34	0.29	0.27	21	31	20.3
down from 149	0	0.01	0.09	20	25	22
down from 149	0.03	0.01	0	29	28	21.1

Table 5. Occupancy covariates measured at each site in the upper Sabine in East Texas. Years' experience represent the second detection covariate collected. Locations are included to distinguish each sites respective covariate measurements.

Location	Average Experience (Yrs/searcher)	Depth(m) RB	Depth CL	Depth LB	Average depth	SD Depth
HWY 69	4	0.65	0.74	1.06	0.82	0.18
HWY 69	4	1.06	1.1	1.16	1.11	0.04
HWY 14	0.1	0.18	0.16	0.98	0.44	0.38
HWY 14	0.1	0.56	0.24	0.82	0.54	0.24
HWY 271	1	0.52	0.4	0.22	0.38	0.12
HWY 271	1	0.96	1.18	1.27	1.14	0.13
HWY 149	0.47	0.5	0.86	0.46	0.61	0.18
HWY 149	0.47	0.16	0.4	0.46	0.34	0.13
Hoard RD	0.18	0.68	1.08	0.4	0.72	0.28
Hoard RD	0.18	0.68	1.14	0.4	0.74	0.31
Hoard RD	0.3	0.38	0.28	0.16	0.27	0.09
Hoard RD	0.3	1.12	0.92	0.54	0.86	0.24
HWY 43	4.1	0.6	0.42	0.62	0.55	0.09
HWY 43	4.1	0.64	0.7	0.58	0.64	0.05
HWY 59	0.47	0.24	0.64	0.84	0.57	0.25
HWY 59	0.47	0.26	0.64	1.16	0.69	0.37
HWY 1794	7.2	0.74	0.48	0.22	0.48	0.21
HWY 1794	7.2	0.38	0.52	0.36	0.42	0.07
Old Sabine WMA	0.11	0.84	0.46	0.46	0.59	0.18
Old Sabine WMA	0.11	0.98	0.84	0.44	0.75	0.23
up from 271	0.25	0.4	0.54	0.2	0.38	0.14
up from 272	0.25	0.5	0.84	0.88	0.74	0.17
HWY 31	0.19	0.9	0.78	0.36	0.68	0.23
HWY 31	0.19	1.04	1.18	1.04	1.09	0.07
HWY 79	0.31	0.4	0.64	0.84	0.63	0.18
HWY 79	0.28	0.42	0.58	0.3	0.43	0.11
HWY 42	0.14	0.52	0.88	0.34	0.58	0.22
HWY 42	0.14	0.48	0.72	0.38	0.53	0.14
down from 149	3.89	1.3	0.86	0.7	0.95	0.25
down from 149	3.89	1.02	1.4	1.12	1.18	0.16

Table 6. Occupancy covariates measured at each site in the upper Sabine in East Texas. Locations are included to distinguish each sites respective covariate measurements. Mesohabitats were determined from field notes that included observations of water surface roughness within the delineated sample area.

Location	Distance between quadrats (m)	Substrate (%)	Mesohabitat
HWY 69	9.5	75-100 sand 0-25 woody debris	swiftwater
HWY 69	8.5	75-100 sand 0-25 woody debris	swiftwater
HWY 14	10	75-100 sand 0-25 woody debris	swiftwater
HWY 14	11.5	75-100 sand	swiftwater
HWY 271	11.5	75-100 small rock/cobble 0-25 sand	swiftwater
HWY 271	13.5	75-100 sand	slackwater
HWY 149	9.5	50-75 sand 25-50 small rock/cobble 0-25 woody debris	swiftwater
HWY 149	9	75-100 sand 0-25 small rock/cobble	swiftwater
Hoard RD	6.5	75-100 sand/silt 50-75 woody debris	slackwater
Hoard RD	6.5	0-25 sand 50-75 clay 25-50 woody debris	slackwater
Hoard RD	6	50-75 small rock/cobble 0-25 sand	swiftwater
Hoard RD	7.5	75-100 sand 50-75 woody debris	swiftwater
HWY 43	13	75-100 sand	swiftwater
HWY 43	13.5	25-50 small rock, 25-50 sand, 0-25 cobble 0-25 woody debris 0-25 silt-clay	swiftwater
HWY 59	15.5	50-75 small rock/cobble 0-25 sand 0-25 woody debris	swiftwater
HWY 59	6	75-100 sand	slackwater
HWY 1794	13	75-100 sand 0-25 woody debris	swiftwater
HWY 1794	13	75-100 sand 25-50 small rock 0-25 boulder 0-25 woody debris	swiftwater
Old Sabine WMA	9.5	75-100 sand 0-25 woody debris 0-25 clay	swiftwater
Old Sabine WMA	7.5	75-100 sand 0-25 woody debris 0-25 clay/silt	swiftwater
up from 271	10	25-50 small rock/cobble 75-100 sand 0-25 woody debris	swiftwater
up from 272	11	50-75 sand 25-50 gravel/cobble 0-25 bedrock	swiftwater
HWY 31	11	75-100 sand 0-25 woody debris	swiftwater
HWY 31	10.5	75-100 sand 25-50 woody debris 0-25 clay	slackwater
HWY 79	15	75-100 sand 0-25 woody debris	swiftwater
HWY 79	14	75-100 coarse sand 0-25 woody debris	swiftwater
HWY 42	9.5	75-100 small rock 0-25 cobble 0-25 sand/clay	swiftwater
HWY 42	10	25-50 cobble 25-50 sand 0-25 woody debris	swiftwater
down from 149	10.5	25-50 clay/silt 50-75 sand 0-25 woody debris	slackwater
down from 149	10	0-25 clay/silt 75-100 sand 0-25 woody debris	slackwater