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Species Richness and Abundance of Freshwater Mussels in Tributaries of the Lower Pearl River Basin

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SPECIES RICHNESS AND ABUNDANCE OF FRESHWATER MUSSELS IN
TRIBUTARIES OF THE LOWER PEARL RIVER BASIN

A Thesis

Submitted to the Graduate Faculty of
the Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Renewable Natural Resources

by
Corinne Nicole Bird
B.S., Louisiana State University, 2014
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Abstract

Freshwater mussels play a vital role in their ecosystems, influencing processes such as nutrient cycling and water filtration. In addition, they provide and improve habitat for other organisms. North America is home to the most species-rich freshwater bivalve fauna in the world, but most stream systems in the U.S. have been severely degraded, and future freshwater mussel extinction rates are estimated at 6.4 percent per decade. The Pearl River Basin is a significant area of aquatic species diversity and has a complex watershed land use mosaic, providing an excellent opportunity to investigate the relative importance of local and landscape level factors on freshwater mussel assemblages. The objectives of this study were to identify freshwater mussel species richness and relative abundance in tributary streams of the Pearl River Basin, and to identify relationships between microhabitat and landscape-level environmental variables and freshwater mussel diversity in these streams. Freshwater mussel and local habitat surveys were conducted on 36 tributary streams over two summers. In addition, percent area coverage of seven land use categories and seven geology categories were estimated for each sample site. Mussel surveys revealed nine species, with total abundance ranging from 0-66 mussels per sites and species richness ranging from 0-5 species per sites. Although there were relatively few mussels at survey sites, where mussels were present, substrate composition and water chemistry appeared to be important factors influencing richness and abundance. In general, associations between local habitat variables and landscape variables were low, suggesting that local habitat features were more important in explaining mussel assemblages encountered during this study. However, other studies have documented associations between mussel assemblage characteristics and landscape scale variables. Therefore, knowledge of land use and geology should be integrated with local habitat data to accurately assess population and assemblage

characteristics of these organisms in order to assist the informed development of effective management and conservation strategies for the Pearl River Basin.

Introduction

North America supports the most species rich freshwater bivalve fauna in the world, which includes 297 recorded species in the family Unionidae (Bogan 1993). Mussels are ecologically valuable to freshwater ecosystems, influencing processes such as nutrient cycling and water filtration (Vaughn et al. 2004; Atkinson et al. 2013), and stimulating production across trophic levels by transferring nutrients and energy from the water column (Vaughn et al. 2008). Moreover, the mere presence of bivalve shells provides and improves habitat for other organisms (Vaughn and Hakenkamp 2001; Gutierrez et al. 2003; Vaughn et al. 2008). Unfortunately, mussels are highly imperiled due to the innumerable threats that face freshwater ecosystems.

Over the last two centuries, most stream systems in the U.S. have been degraded from sedimentation and pollution, channel modification for navigation, flood control, altered drainage within the watershed, fragmentation and alteration from dam construction, and introduction of alien species (Bogan 1993). Because of these threats, 72% of native North American mussels are considered endangered, threatened, or of special concern, and only 70 species are considered stable (Williams et al. 1993). With recent extinction rates estimated at 1.2 percent per decade and future estimates of 6.4 percent per decade, freshwater mussels are heading toward an extinction crisis if the environmental quality of North American stream systems does not improve (Williams et al. 1993; Ricciardi and Rasmussen 1999). Improved conservation and management is necessary to conserve this rich component of North American freshwater biodiversity, and this in turn requires a better understanding of the environmental mechanisms that control freshwater mussel presence and abundance.

Numerous studies have assessed the effects of habitat characteristics on freshwater mussel assemblages at various spatial scales. At the landscape level, drainage basin area and

stream size have been used to assess species richness and abundance. Mussel richness in large river systems was found to be influenced more by the number of fish species present, while in smaller streams unionid richness was related more to drainage basin area (Watters 1992). Stream size, likely strongly correlated with basin area, has also been directly linked to mussel assemblage composition. Species richness and abundance have been found to increase as a function of environmental and hydrologic forces associated with stream size, with the largest segments containing the highest diversity and abundance of unionids (Gangloff and Feminella 2007; Ford et al. 2016).

Other landscape scale studies have used GIS and regression analyses to determine relationships among mussel density, species richness, and various land-use or geological watershed characteristics. Agriculturally dominated watersheds have been found to negatively impact mussel populations, causing declines in mussel density and richness as well as completely extirpating mussels from areas that supported productive mussel populations prior to agricultural activity (Arbuckle and Downing 2002; Poole and Downing 2004). In these studies, freshwater mussel diversity was impacted from siltation, destabilization of stream substrate, and a lack of streamside woodlands, all caused by intensive agricultural land use. An increase in urban/anthropogenically disturbed land area has also been associated with many changes to natural lotic systems, including increased runoff and more erratic hydrology, which can negatively impact mussel assemblage abundance and diversity (Allen 2004). In addition to land use, geology has been used to explain freshwater mussel distributions. Surface geology was found to regulate hydrology, slope and turbidity of streams within a study area in Michigan, thereby indirectly influencing mussel distribution and abundance (Strayer 1983).

At local spatial scales, substrate and its relationship with hydraulic variables have been examined in studies of mussel diversity, species composition and distribution. Shear stress, in particular, and its relationship to substrate stability has been found to play an important role in freshwater mussel abundance. Mussels are more common in silt than sand or gravel, favoring the more hydrologically stable environment these sediments (and lower velocities) produce (Brown and Banks 2001; Vaughn 2010). Mussels have been reported in low abundance at sites subject to high shear stress, suggesting that mussels favor areas that protect them from high flows (Strayer 1999; Howard and Cuffey 2003; Gangloff and Feminella 2007). Although substrate characteristics were suggested as the best physical parameters for describing habitat for *Margaritifera margaritifera* (Hastie et al. 2000), the relative importance of bed sediment composition and stream hydraulics in determining mussel assemblage composition and abundance have not been adequately clarified (Layzer and Madison 1995; Brim Box and Mossa 1999; Brim Box et al. 2002).

An alternative approach to identifying the structuring factors involved in freshwater mussel distribution has involved assessment of the effect of various environmental factors on mussel viability. These studies can help identify how processes, such as an increase in suspended solids from sedimentation or rising temperatures from global climate change, might limit mussel distribution. According to the U.S. Environmental Protection Agency, excessive sediment impairs over 40% of the nation's river miles (Brim Box and Mossa 1999) and erosion/sedimentation has been cited as the greatest threat to aquatic biota in North America (Waters 1995). Negative metabolic and physiologic effects on mussels have been linked to increased exposure to suspended solids, including an increase in metabolic demand and a switch to non-protein body stores for metabolism, reductions in filter feeding, gill clogging, and a

decrease in available light for photosynthetic production of unionid food items (Aldridge et al. 1987; Brim Box and Moss 1999). In addition to fine sediment, stream temperature, often elevated by riparian vegetation removal, also has significant impacts on mussel physiology. Metabolic expenditures and feeding rates were negatively affected by an increase in water temperature, which also forced mussels to rely on stored fuels to supply metabolic needs (Aldridge et al. 1995; Ganser et al. 2015). Dissolved oxygen levels have also been found to have effects on mussels, with declining DO causing an increase in physiological stress and eventual mortality if mussels are exposed to hypoxic conditions for long periods of time (Sparks and Strayer 1998; Haag and Warren Jr. 2008; Gagnon et al. 2011). Such studies are particularly important for understanding the physiological basis of observed mussel distributions, as well as understanding, predicting and mitigating changes in stream environments that affect mussel viability, growth, and distribution.

Studies that have assessed multiple habitat characteristics have also been successful in identifying mechanisms underlying mussel abundance and distribution. In Louisiana, mussels have been found to be more common in second order streams with elevated specific conductance and water hardness, density related to differences in water depth, substrate size, substrate compaction, water velocity, and substrate stability (Johnson and Brown 2000). In Michigan, densities of all mussel species in a study area were negatively correlated with larger sediment particle size and higher percent aquatic vegetation, however, a positive relationship was found with percent coverage of woody debris (Harriger and Moerke 2009). Spatial distribution of an endangered mussel species in Scotland has shown broadly similar habitat preferences between adults and juveniles. Adults were found over a wide range of physical conditions, however, highlighting the importance of addressing multiple microhabitat parameters (Hastie et al. 2000).

These examples stress the importance of measuring numerous small-scale habitat characteristics when evaluating freshwater mussel distributions, as different species and life stages may require different habitat attributes.

Spatial scale, i.e., microhabitat, reach, stream, and watershed, is an important aspect of studies that are designed to determine factors that most strongly influence mussel abundance and distribution. Analyses based on a combination of multi-scale habitat variables were shown to provide a better explanation of mussel distribution and abundance in Michigan streams, rather than analyses based solely on local habitat features (McRae et al. 2004). Conversely, other studies have found mussel assemblage composition and abundance are better explained by larger scale habitat variables, such as patterns of variability in the fish community or agricultural land use, rather than reach or microhabitat scale features (Haag and Warren 1998; Pandolfo et al. 2016). In southeastern Louisiana, Bambarger (2006) found patterns of mussel species richness and abundance were related to a combination of habitat variables across multiple spatial scales. Hydrologic variability, defined by geology and land-use in addition to fine sediment, was found to influence freshwater mussel assemblage composition within the surveyed area (Bambarger 2006). This type of approach, which combines information from multiple scales into one model for predicting mussel presence and diversity, is likely to be the most effective in explaining broad-scale mussel distribution patterns.

The Pearl River Basin encompasses a 22,690-km² watershed in central and southwest Mississippi and southeast Louisiana (Lang 1972). Historically, the eastern Florida Parishes occurring in this basin were dominated by rolling hills of extensive longleaf pine, which have been severely depleted from land conversion, development, and timber production (Holcomb 2015). Streams in this area are characterized by fine substrates, low gradients, and low

concentrations of dissolved substances (Felley 1992). Land use within the basin is predominately agriculture and forestry, with increasing urbanization from metropolitan development in the New Orleans area (Holcomb 2015). Erosion and sedimentation are the prime contributors to aquatic pollution in this system, and together with historic gravel mining have greatly altered environments in the Pearl and Bogue Chitto rivers (Holcomb 2015). The Louisiana Department of Wildlife and Fisheries describes the Pearl Basin as a significant area of aquatic species diversity. This diversity, together with the complex land use mosaic in the basin, provide an excellent opportunity to investigate the relative importance of local (e.g., turbidity) and landscape (e.g., agricultural land use) level factors in the distribution and abundance of freshwater mussels.

The goal of this study was to assist the informed development of effective habitat management and conservation strategies for the Pearl River Basin mussel biota. Specifically, my project identified: (1) freshwater mussel species richness and relative abundance in tributary streams in the Pearl River Basin; and (2) relationships between microhabitat and landscape-level environmental variables and freshwater mussel diversity in these stream systems.

Methods

Study Site

My study focused on 36 streams in the lower Pearl River Basin of southeast Louisiana and southwest Mississippi. The Pearl River flows 584 km from Ross Barnett Reservoir through the East (77 km) and West (71 km) Pearl River outlets to Lake Borgne, which is connected to the Gulf of Mexico by the Mississippi Sound (Lang 1972). The Bogue Chitto River is the largest western tributary to the lower Pearl River, draining portions of southwestern Mississippi and southeastern Louisiana. The Pearl River Basin supports about 40 species of freshwater mussels,

including the federally threatened inflated heelsplitter, *Potamilus inflatus*, with the mainstem supporting about 29 mussel species (USFWS 2014). My study focused on potentially additional species found in tributary streams located throughout the remainder of the southern portion of the basin. Because these streams occur on private land, perennial streams were sampled based on their accessibility and their location to the main stem of the river. During summer 2015 and 2016, 36 total sites were sampled (Figure 1).

Mussel Survey

Multiple studies have assessed differences in qualitative versus quantitative mussel sampling methods and found that there is no statistical difference between the two in determining species richness and relative abundance (Miller and Payne 1993; Obermeyer 1998). Because the goal of this survey was to assess overall species diversity rather than locate cryptic species, a timed visual and tactile mussel survey (rather than an exhaustive area-based survey) was carried out at each site. Two surveyors snorkeled along each stream bank for 45 minutes collecting all mussels in the wade-able portion of each study stream, with reaches ranging from 40 to 90 meters in length depending on stream width. All collected mussels were placed in mesh bags until the completion of the survey, at which time identifiable mussels were returned to the stream, and unidentified taxa were placed on ice and returned to the laboratory for identification based on Stern (1976) and Turgeon et al. (1998). These data allowed for calculation of catch per unit effort and relative abundance for each species, as well as richness and evenness of the assemblage at each study site. Eighteen sites were surveyed during summer 2015, with nine sites yielding mussels. In 2016, these nine sites were re-sampled, along with nine new sites, for a total of 36 sites over the two years.

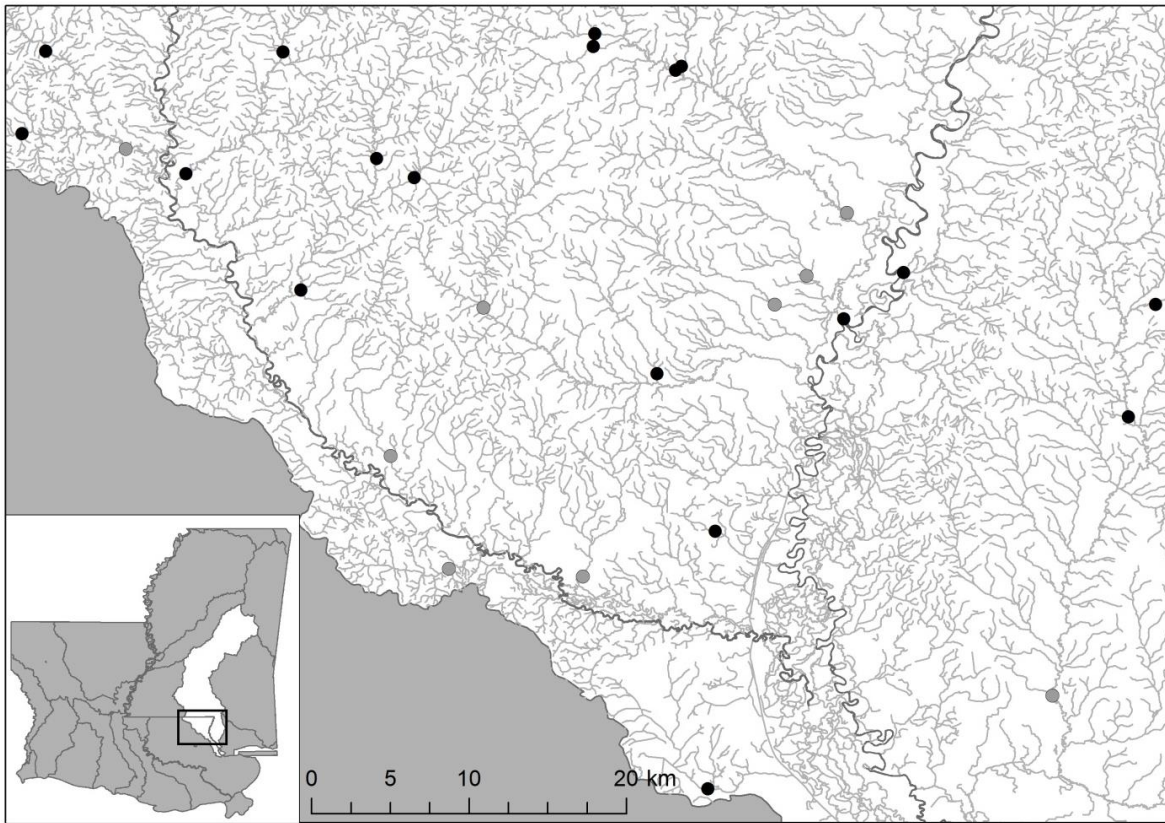


Figure 1. Tributary stream sites sampled during 2015 and 2016 in the Pearl River Basin, shown in white. Grey circles indicate sites that were re-sampled during the second sampling period.

Microhabitat Survey

Cross-stream transects were placed every 10 meters along the entire stream reach that was surveyed for mussels, and flow velocity (cm/s) and water depth (cm) were collected at 25%, 50%, and 75% of stream width along each transect. I also recorded stream width (m), bank height (m), bank angle, dominant vegetation, and canopy density (%), as well as DO (mg/L), specific conductance (mmhos/cm), turbidity (NTU), pH, and temperature ($^{\circ}$ C) measured with a handheld YSI multiprobe. Three sediment samples were collected across the width of the stream and brought back to the laboratory for dry-sieving, which has been shown to obtain more accurate results than wet-sieving (McMahon et al. 1996). Samples were dried and hand-shaken

in a stack of sieves for seven minutes, with the contents remaining on each sieve weighed to determine percentage composition by weight according to a modified Wentworth classification for substrate particle sizes (Cummins 1962). Percent composition of pebble (>16.0 mm), gravel (2.0-4.0 mm)(4.0-8.0 mm)(8.0-16.0 mm), very course sand (1.0-2.0 mm), course sand (0.5-1.0 mm), medium sand (0.25-0.5 mm), fine sand (0.125-0.25 mm), very fine sand (0.0625-0.125 mm), and silt (<0.0625 mm) was estimated for each site. In addition, distance (km) downstream from each site to the mainstem was estimated with ArcMap 3.2 (Environmental Systems Research Institute, www.esri.com).

Land-Use and Geology

All landscape variables were estimated with ArcMap 3.2 and Spatial Analyst (Environmental Systems Research Institute, www.esri.com). Percent area coverage (km²) of 7 land use categories were estimated for the drainage area upstream of each sample site with NOAA 2010 C-CAP Regional Land Cover (NOAA, 2010). These habitat categories are condensed from NOAA's original 22 category types that occur in the study watershed (Table 1). Similarly, percent area coverage (km²) of: (1) Prairie Terrace, (2) High Terrace, (3) Alluvium, (4) Deweyville Terrace, (5) Citronelle Formation, (6) Pascagoula and Hattiesburg Formation, and (7) Coastal Deposits were also estimated within the same drainage area, based on study site locations within the geology shapefile (United States Geological Survey, Louisiana and Mississippi geology shapefile).

Table 1. Condensed land use categories estimated at each sample site and NOAA regional land use categories, 2010.

Condensed Land Use Categories	NOAA Land Use Categories
Developed	Developed – High Intensity
	Developed – Medium Intensity
	Developed – Low Intensity
	Developed – Open Space
Agriculture	Cultivated Crops
	Pasture/Hay
	Grasslands//Herbaceous
	Scrub/Shrub
Deciduous Forest	Deciduous Forest
Evergreen Forest	Evergreen Forest
Mixed Forest	Mixed Forest
Wetland	Palustrine Forested Wetlands
	Palustrine Scrub/Shrub Wetlands
	Palustrine Emergent Wetlands
	Estuarine Forested Wetlands
	Estuarine Scrub/Shrub Wetlands
	Estuarine Emergent Wetlands
Barren/Open	Unconsolidated Shore
	Barren Land
	Open Water
	Palustrine Aquatic Bed
	Estuarine Aquatic Bed

Statistical Analysis

Mussel catch-per-unit-effort (number per search; CPUE) and species richness were analyzed separately first with generalized linear models and subsequently, because of apparent interactivity among explanatory variables, by generalized additive models. First, for mussel CPUE, generalized linear models (PROC GENMOD, SAS Vers. 4.3, Cary, NC) were constructed with substrate size (percent less than each Wentworth scale size class, e.g., percent less than 0.5 mm), DO concentration, specific conductance, pH, and turbidity as explanatory fixed effects. Candidate generalized linear models included the untransformed data with the normal probability distribution, log link transformed data with the normal probability distribution, log link transformed data with the Poisson probability distribution, and log link transformed data with the negative binomial probability distribution, with the best fitting model selected by the χ^2/df (\hat{c}) fit statistic (Kéry and Royle 2016). Next, backward stepwise model selection compared generalized linear models with linear and quadratic substrate size classes, DO, specific conductance, pH, and turbidity as explanatory fixed effects with small sample Akaike's Information Criterion (AICc) as the model selection criterion. Finally, because the model selection process suggested potential interactivity among explanatory fixed effects, a generalized additive model (GAM) (PROC GAM, SAS Vers. 4.3, Cary, NC) was constructed to fit a nonlinear relationship between percent substrate less than 16 mm (<16) with percent substrate less than 0.5 mm (<5) by a thin-plate spline with back-calculated smoothing based on the deviation of predicted from observed values. For mussel species richness, the same process was followed by determining the best fitting generalized linear model, backward selection with the best fitting generalized linear model, and construction of a GAM, which included percent substrate less than 0.5 mm and DO concentration.

Stream physical and chemical variables associated with either CPUE or species richness were compared to watershed land cover and geology, expressed in terms of percent composition (e.g., % forested wetland and % high terrace) by canonical ordination. Several ordinations were compared for this analysis (e.g., canonical correlation analysis, canonical correspondence analysis, and nonmetric multidimensional scaling), and the canonical ordination best meeting axis length and STRESS2 criteria was selected.

Results

Mussel Survey

Over the course of the study, I collected a total of 174 mussels belonging to 9 different species at 19 of the 36 sites, with 17 sites yielding no mussels. Total abundance averaged 4.83 (\pm 1.96SE) mussels (range 0-66) per site, with the greatest abundance occurring at Silver Creek. On average, species richness was 1.22 (\pm 0.26SE) species (range 0-5) per site, with the greatest species richness occurring at Deer Lick Creek, Silver Creek, and Miller Creek, all of which contained five species. The most abundant species were *Villosa lienosa* (38.9% frequency of occurrence), *Elliptio crassidens* (2.8% frequency of occurrence), and *Pleurobema beadleianum* (25% frequency of occurrence), the former of which was also the most widely distributed species (14 sites; Table 2). None of the mussels collected were federally listed species, however, of the nine species encountered, *Anodontoides radiatus*, *Elliptio crassidens*, *Pleurobema beadleianum*, and *Villosa vibex* are considered species of conservation concern in Louisiana (Holcomb 2015). Mussels were not present at all sites that were re-sampled during the second sampling season, which yielded mussels during the first season. Neither multivariate analyses nor single species models could be performed on these data due to the low frequency of occurrence of the species at each site.

Microhabitat Survey

Trees were the most common dominant vegetation among sites, with an average percent canopy cover of 72.6% ($\pm 2.42SE$) (Table 3). The most abundant sediment size classes were 0.25-0.5 mm and 0.5-1.0 mm, comprising 66% of the total amount of sediment processed. The least abundant size class was <0.125 mm, making up only 0.85% of the total amount of sediment processed (Table 4). There was a considerable range in several habitat characteristics among the study sites, particularly turbidity, stream width, distance to the mainstem, and upstream watershed area.

Table 2. Mussel species collected at each site and their frequency of occurrence in Pearl River tributary streams during 2015 and 2016.

Site	<i>A. radiatus</i>	<i>E. crassidens</i>	<i>L. claibornensis</i>	<i>P. dombeyanus</i>	<i>P. beadleianum</i>
House Creek	–	–	–	–	–
Talley’s Creek	–	–	3	–	–
Bogue Lusa Creek	–	–	–	–	8
Mill Creek	–	–	–	–	–
Peters Creek	–	–	–	–	6
Adams Creek	–	–	1	–	–
Pushepatapa Creek	–	–	–	–	5
Silver Springs Creek	–	–	1	–	–
West Hobolochitto Creek	–	–	–	1	–
Talley’s Creek	–	–	1	–	–
Mill Creek	–	–	–	–	–
West Hobolochitto Creek	–	–	–	–	–
Pushepatapa Creek	–	–	–	–	1
Bogue Lusa Creek	–	–	–	–	2
Adams Creek	–	–	–	–	–
Deer Lick Creek	1	–	1	–	2
Silver Creek	3	52	–	–	3
Crains Creek	–	–	–	–	1
Miller Creek	1	–	1	–	6
Total	5	52	8	1	34
Frequency of occurrence	8.3%	2.8%	16.7%	2.8%	25%

Table 2 continued.

Site	<i>Q. refulgens</i>	<i>U. declivis</i>	<i>V. lienosa</i>	<i>V. vibex</i>
House Creek	–	–	2	2
Talley’s Creek	–	3	7	–
Bogue Lusa Creek	–	–	7	–
Mill Creek	–	–	4	–
Peters Creek	–	–	2	–
Adams Creek	–	–	1	1
Pushepatapa Creek	–	–	–	–
Silver Springs Creek	–	–	–	–
West Hobolochitto Creek	2	–	1	–
Talley’s Creek	–	3	21	–
Mill Creek	–	–	1	–
West Hobolochitto Creek	1	–	–	–
Pushepatapa Creek	–	–	–	–
Bogue Lusa Creek	–	–	1	–
Adams Creek	–	–	1	1
Deer Lick Creek	–	–	2	1
Silver Creek	–	–	7	1
Crains Creek	–	–	–	–
Miller Creek	–	–	1	1
Total	3	6	58	7
Frequency of occurrence	5.6%	5.6%	38.9%	16.7%

Table 3. Maximum, minimum, and mean values for measured physical and water quality variables in Pearl River tributary streams during 2015 and 2016.

Variable	Maximum	Minimum	Mean (\pm SE)
Temp (°C)	29.33	22.09	24.40 (\pm 0.35)
D.O. (mg/L)	9.59	3.93	7.20 (\pm 0.32)
SpCond (mmhos/cm)	0.06	0.02	0.04 (\pm 0.00)
pH	8.32	5.50	7.43 (\pm 0.12)
Turbidity (NTU)	59.60	0.30	10.09 (\pm 2.71)
Depth (cm)	121	0	46.31 (\pm 1.27)
Flow Velocity (cm/s)	83.8	0	14.05 (\pm 0.66)
Bank Height (m)	5	0.17	1.45 (\pm 0.04)
Bank Angle (°)	90	2.7	42.35 (\pm 1.36)
Stream Width (m)	21.5	0.4	7.85 (\pm 0.34)
Watershed Area (km ²)	515.25	3.13	72.14 (\pm 20.94)
Distance to mainstem (km)	44.01	0.05	12.36 (\pm 2.22)
Canopy Density (%)	100	6.25	72.63 (\pm 2.42)

Table 4. Percent substrate less than each size class in Pearl River tributary streams during 2015 and 2016.

Site	% < 16mm	% < 8mm	% < 4mm	% < 2mm	% < 1mm	% < 0.5mm	% < 0.25mm	% < .125mm
11	99.93	99.88	99.31	98.07	90.42	72.26	14.21	0.55
Adam's Creek	99.19	98.65	97.62	95.61	92.95	62.24	10.72	0.36
Ben's Creek	99.53	99.09	98.12	96.13	92.27	44.85	8.10	0.20
Bogue Lusa Creek	99.75	99.66	99.48	98.72	95.40	35.34	4.87	0.16
Crains Creek	99.16	97.61	96.45	95.10	92.68	32.60	2.86	0.02
Deer Lick Creek	88.55	74.28	65.93	61.04	56.24	27.35	2.27	0.05
Hays Creek	81.74	57.87	41.73	30.70	25.50	14.71	4.09	0.21
Hays Creek 2	91.52	86.27	83.57	81.80	78.11	18.44	1.67	0.02
House Creek	99.50	99.40	99.19	98.52	97.63	57.39	10.17	0.15
Lawrence Creek	76.99	65.03	55.82	49.98	46.10	14.35	2.35	0.03
Lawrence Creek 2	99.98	99.98	99.97	99.93	99.66	20.43	1.96	0.02
Mill Creek	82.82	58.30	45.11	38.49	35.01	21.48	2.70	0.02
Miller Creek	99.20	99.09	98.78	98.33	97.19	29.88	3.05	0.03
Peter's Creek	99.62	99.10	97.47	96.38	94.73	29.60	7.85	0.41
Peter's Cutoff	98.80	98.12	95.58	91.40	87.40	51.96	18.36	0.66
Pushapatapa 2	90.08	73.65	61.50	54.27	43.90	9.33	1.74	0.02
Pushapatapa Creek	99.75	99.25	98.31	95.91	90.84	41.47	9.16	0.18
Sal's Branch	88.87	59.09	36.58	23.50	17.52	6.19	1.01	0.04
Silver Creek	72.68	61.77	57.54	52.44	35.59	4.71	1.00	0.04
Silver Springs Creek	94.56	87.21	83.83	82.08	72.98	41.84	12.75	0.25
Stubbs Creek	95.15	85.85	74.03	60.24	48.14	22.53	2.53	0.06
Talisheek Creek	99.58	99.47	99.39	99.04	96.41	48.89	5.02	0.09
Talley's Creek	91.63	67.06	54.59	47.56	44.67	38.46	5.45	0.07
Thomas Creek	96.32	93.85	90.11	87.12	78.41	49.07	6.48	0.11
West Hobolochitto 2	99.72	98.48	95.69	92.51	88.53	39.64	3.91	0.07
West Hobolochitto Creek	100.09	100.01	99.80	99.30	97.60	53.84	5.64	0.07
White Sands Creek	97.76	94.31	91.30	88.64	84.38	46.81	5.71	0.12

Mussel CPUE and Richness

The final backward-selected generalized linear model(log link, negative binomial distribution) for mussel CPUE included a quadratic term for percent substrate less than 16 mm ($2.9 \pm 1.97SE \text{ } \mu\text{t}16 - 1.006 \pm 1.003SE \text{ } \mu\text{t}16$), a quadratic term for percent substrate less than 0.5 mm ($1.30 \pm 1.07SE \text{ } \mu\text{t}5 - 1.003 \pm 1.009SE \text{ } \mu\text{t}5$), and specific conductance ($-0.95 \pm 1.01SE$ mmhos/cm). The GAM confirmed the interactivity of percent substrate less than 16 mm with percent substrate less than 0.5 mm (Analysis of Deviance $P = 0.02$; Figure 4). The final

generalized linear model for mussel richness (log link, Poisson distribution) included a quadratic term for percent substrate less than 0.5 mm ($1.13 \pm 1.06SE$ $\%lt5 - 1.002 \pm 1.008SE$ $\%lt5$), and specific conductance ($-0.81 \pm 1.09SE$ mmhos/cm). The generalized additive model confirmed the interaction between percent substrate less than 0.5 mm with DO (Analysis of Deviance $P = 0.02$; Figure 5).

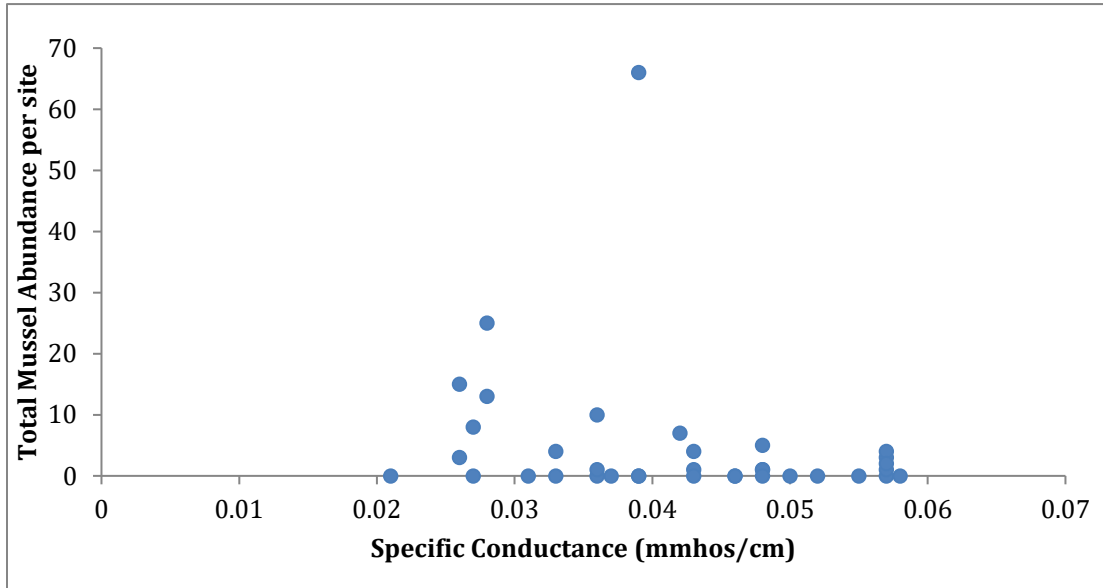


Figure 2. Relationship between mussel catch per unit effort and specific conductance in Pearl River tributary streams sampled in 2015 and 2016.

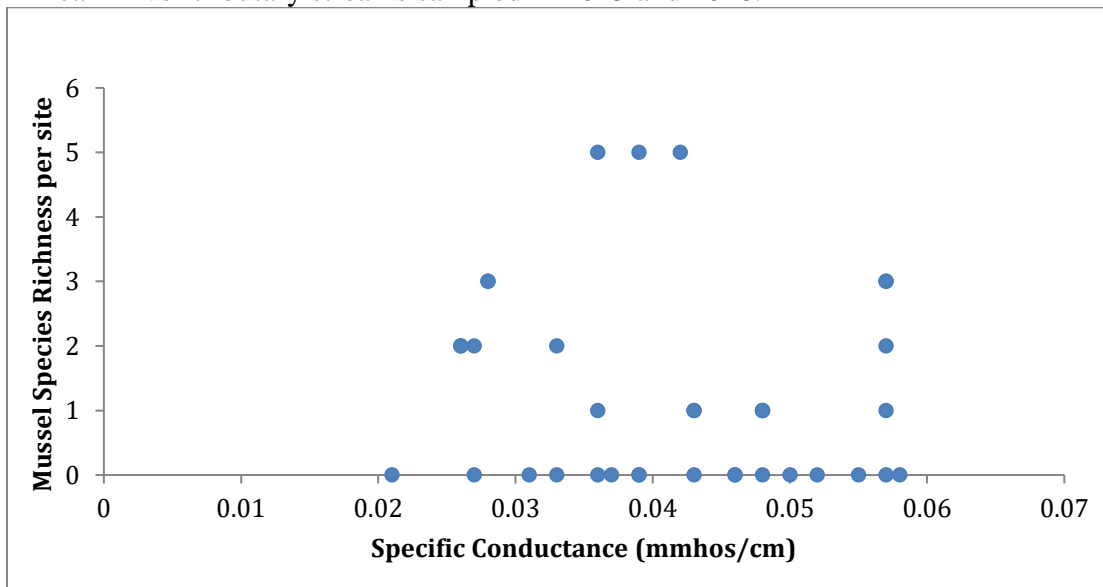


Figure 3. Relationship between mussel species richness and specific conductance in Pearl River tributary streams sampled in 2015 and 2016.

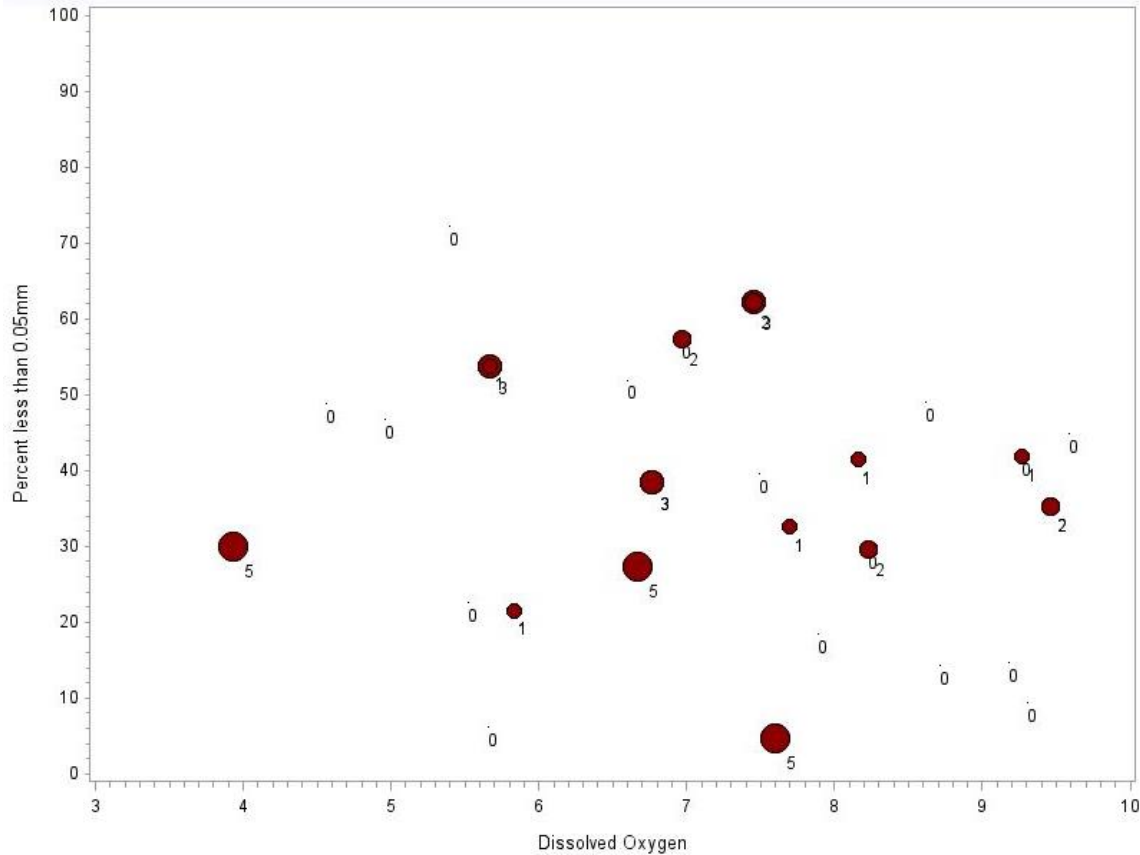


Figure 5. Predicted mussel species richness based on the interaction of percent substrate less than 0.5 mm (pct_lt5) with DO based on mussel collections in Pearl River tributary streams sampled in 2015 and 2016.

Land-Use and Geology

Agriculture was the most abundant land use category, comprising 49% of the total area upstream of sample sites, followed by Evergreen Forest (22%) and Wetlands (22%; Table 5). Deciduous Forest was the least abundant land use category, making up only 0.6 percent of the total area upstream of sample sites. Of the Louisiana geology types, High Terraces was the most extensive type, comprising 67% of the total sample area in Louisiana. Deweyville Terraces was the least abundant type, making up 0.18% of the total sample area. Of the Mississippi geology types, Pascagoula Hattiesburg was most extensive, making up 73% of the total area sampled, with Coastal Deposits being the least extensive at 1.61%.

Three statistically significant canonical variates (CVs) correlating land cover and geology with stream characteristics were identified by canonical correlation analysis (overall Wilk's Lambda = 0.007, $F_{48, 71.4} = 3.83$, $P < 0.001$, CV 1 approximate $F_{48, 71.4} = 3.83$, $P < 0.001$, CV 2 approximate $F_{33, 56.7} = 2.88$, $P < 0.001$, CV 3 approximate $F_{20, 40} = 2.50$, $P = 0.007$). The first canonical variate contrasted low percent < 0.5 mm substrate with high percent Developed land cover, low percent Evergreen Forest, and high percent High Terrace geology (Table 6). The second canonical variate identified high DO and high percent < 16 mm substrate as important organizing variables, but did not correlate high DO with any land cover or geology. The third canonical variate correlated high specific conductance with high percent Pascagoula and Citronelle geology.

Table 5. Maximum, minimum, and average percent land cover and geology above each mussel sampling site in Pearl River tributary streams sampled in 2015 and 2016.

Variable	Maximum	Minimum	Average	Standard Error
Land Cover Types				
% Developed	3.44%	0.58%	1.69%	0.00
% Agriculture	63.18%	23.12%	50.21%	0.02
% Deciduous Forest	1.71%	0.14%	0.64%	0.00
% Evergreen Forest	36.79%	9.09%	20.98%	0.01
% Mixed Forest	8.07%	0.79%	3.90%	0.00
% Wetland	53.40%	10.02%	21.56%	0.02
% Barren	8.09%	0.16%	1.02%	0.00
Geology				
% Alluvium	57.06%	1.39%	20.55%	0.02
% Prairie Terraces	98.61%	0.00%	22.84%	0.07
% High Terraces	95.74%	0.00%	56.48%	0.07
% Deweyville Terraces	2.02%	0.00%	0.13%	0.00
% Pascagoula Hattiesburg	85.32%	58.61%	69.82%	0.06
% Citronelle Formation	32.43%	0.00%	19.83%	0.08
% Coastal Deposits	41.39%	0.00%	10.35%	0.10

Table 6. Correlations between the stream physical and chemical characteristics land cover and geology with canonical variates 1-3. Shaded correlations were interpretable.

Variable	Canonical Variate 1	Canonical Variate 2	Canonical Variate 3
Stream characteristics			
% less than 16 mm substrate	-0.48	0.52	0.08
% less than 0.5 mm substrate	-0.96	0.20	0.01
Dissolved oxygen	0.24	0.72	-0.52
Specific conductance	-0.15	0.12	0.94
Land Cover Types			
% Developed	0.54	0.15	0.17
% Agriculture	0.36	0.29	-0.11
% Deciduous Forest	0.34	0.25	0.09
% Evergreen Forest	-0.56	0.04	-0.03
% Mixed Forest	0.14	0.23	0.21
% Wetland	0.06	-0.29	-0.66
% Barren	-0.22	-0.49	0.04
Geology			
% Prairie Terrace	-0.30	-0.35	0.06
% Alluvium	0.32	0.13	-0.63
% High Terrace	0.61	0.30	0.03
% Deweyville	-0.38	0.18	-0.19
% Pascagoula	-0.38	0.03	0.13
% Citronelle	-0.26	0.01	0.15
% Coastal	-0.45	-0.18	0.03

Discussion

Freshwater mussel diversity, abundance, richness, and distribution have been shown to be positively related to river and stream size (Strayer 1983; Strayer 1993; Haag and Warren 1998; Gangloff and Feminella 2007; Daniel and Brown 2013; Ford et al. 2016). In this study, stream width averaged less than 8 meters, which likely contributed to low catch-per-unit-effort and species richness. Low richness precluded calculation of species diversity, although I was confident that the field collections provided representative estimates of density and species

composition at the study sites. Absence of mussels at a site could have been due to anthropogenic stressors that were not measured, such as chemical toxins (Watters 2000; Nobles and Zhang 2015). Low abundance and species richness were also found in a study analyzing mussel assemblages in the Lake Pontchartrain watershed. Abundance averaged only 6.3 individuals per site (4.83 in this study) in the Bogue Chitto sub-segment of the study, and compared to other segments had significantly lower species richness, averaging only 1.5 species per site (1.22 in this study)(Bambarger 2006). However, where mussels were present, substrate composition and water chemistry appeared to be important factors determining species abundance and distribution.

Freshwater mussel catch-per-unit-effort was influenced by two substrate size classes, percent substrate < 16 mm and percent substrate < 0.5 mm. Interactivity between these substrate size classes suggests that an optimal mix of substrate characteristics existed, which in turn supported a greater abundance of mussels. Freshwater mussels have been shown to favor finer sediments, based on the idea that reduced habitat disturbance (e.g., velocity and turbulence) has a positive effect on mussel presence, species richness, and abundance. This reduction in disturbance favors substrates composed mostly of fine sediments, rather than sand or gravel (Brown and Banks 2001; Brim Box et al. 2002; Daniel and Brown 2013). In contrast, juvenile mussels have been associated positively with larger sediments, such as sand, gravel, and boulder (Brown and Johnson 2000; Hastie et al. 2000; McRae et al. 2004; Geist and Auerswald 2007). These studies suggest that overall, adult mussel abundance and richness are associated with finer sediment, although substrate associations can vary among species and life stages. Some studies suggest an optimal combination of substrate characteristics can support freshwater mussel species of varying substrate preferences (e.g., lacustrine mussels; Harman 1972). Substrate

heterogeneity in streams has also been positively linked with macroinvertebrate assemblage structure (Beisel et al. 2000; Boyero 2003; Milesi 2016). In this study, substantial substrate heterogeneity (gravel) was only apparent in 8 streams (Table 4), with most sites exhibiting varying mixtures of fine substrates.

Mussel catch-per-unit-effort also was associated with low specific conductance. There are conflicting literature reports on these relationships, as mussel assemblages have been associated with both high (Brown and Johnson 2000) and low (McRae et al. 2004) specific conductance. Overall, freshwater mussels were uncommon throughout the Pearl River tributaries, occurring at only 53% of sample sites, with only 26% yielding 10 or more mussels. This low abundance and generally uncommon pattern could be attributed to limited habitat suitability, as results suggested a threshold of 0.05 mmhos/cm, at which point mussel abundance declined (Figure 2). There were 21 sites (58% of the total) that exhibited water quality and sediment ranges positively associated with mussel CPUE. However, mussels were not present at all sites within this habitat range. This suggests that there were additional factors (e.g., lack of colonization, lack of fish hosts, anthropogenic alterations in water quality) beyond those measured in my study that could significantly influence mussel distribution and abundance in these tributary streams.

Trends in mussel species richness reflected those seen in the analysis of CPUE, with richness increasing with percent substrate < 0.5 mm and low specific conductance, with an additional positive relationship with high DO. The positive association of species richness with finer substrates and lower specific conductance have already been discussed (Brown and Banks 2001; Brim Box et al. 2002; Daniel and Brown 2013). My statistical analysis also revealed an interaction between percent < 0.5 mm and high levels of DO. Depleted DO concentrations can

have negative impacts on freshwater mussels, leading to stress and eventual mortality (Belanger 1991; Sparks and Strayer 1998; Haag and Warren Jr. 2008; Gagnon et al. 2011). The negative impact of low DO levels on mussel physiology could explain the observed richness/DO association. However, none of the measured DO concentrations were hypoxic (~4 mg/L), and DO did not appear to be a limiting factor regarding mussel distribution and abundance, although I did not measure DO over a 24-h cycle, which could have revealed much lower nocturnal DO levels.

Overall, only 1 or 2 species dominated the mussel assemblages in the study streams. It seems unlikely that the low species richness could be due to competition, as healthy mussel communities typically occur as multispecies assemblages (Bauer et al. 1991; Vaughn 1997; Vaughn et al. 2008). Rare mussel species have been shown to benefit energetically from living in species-rich communities (Vaughn et al. 2008), and although food limitation and competition have been documented, the relative importance and spatial and temporal dynamics of these interactions require further study (Vaughn et al. 2008). Freshwater mussels have a parasitic larval life stage in which they rely on specific fish species, and it has been well documented in the literature that host fish may play an important role in mussel distributions (Smith 1985; Bogan 1993; Haag and Warren Jr. 1998). It may be that a lack of suitable fish hosts contributed to the low species richness found in this study, and future research in these systems should incorporate assessment of fish assemblage composition and abundance in addition to invertebrate surveys.

Multivariate analysis revealed correlations between land use, geology, substrate, and specific conductance. However, most local habitat variables were not correlated with land use or geology. Low percentages of substrate < 0.5 mm were associated with a high percentage of

developed land cover, low percentages of Evergreen Forest, and a high percentage of High Terrace geology. High Terraces are the oldest geologic formations in the Florida Parishes and consist of relatively larger sediment types (Mossa and Autin 1986). High Terrace watersheds are often characterized by variable hydrologic regimes and associated soil erosion, bank failures, flashy stream flow and high rates of sediment displacement (Lenat and Crawford 1994; Arbuckle and Downing 2002). Increased urban/disturbed land area is also associated with similar stream effects related to increased runoff and more erratic hydrology (Allan 2004). Unstable hydrology increases the potential for dislodgement of mussels from the substrate, which reduces their chances of survival (Lenat and Crawford 1994; Arbuckle and Downing 2002). A similar negative relationship between benthic aquatic insects and substrate instability also was observed in some of my study watersheds (Markos et al. 2016), suggesting that hydrology, particularly flashy hydrographs resulting from watershed development, may be an important factor influencing mussel assemblages in Pearl River tributaries.

Both mussel richness and catch-per-unit-effort were positively associated with the percentage of < 0.5 mm substrate. Urban and agricultural development in the tributary watersheds has likely increased peak discharges in most of these streams, contributing to extirpation and reduced colonization of mussels. The negative relationship between high terrace geology and mussel assemblage composition and abundance has also been found in previous mussel studies in Lake Pontchartrain and Pearl River drainages (Bambarger 2006). Conservation of topsoil, maintenance of stream buffer zones, and re-establishment of wetlands in these watersheds would likely decrease sediment transport, bank failures and scouring events (Bambarger 2006). Intact riparian forests would also improve delivery of large woody debris into these streams, which could help stabilize stream channels and increase habitat heterogeneity,

promoting development of downstream low-velocity, fine-sediment areas of particular benefit to mussels (Watters 2000; Bambarger 2006).

In these watersheds, specific conductance was negatively correlated with high percentage of wetland area and a low percentage of alluvium soils and was positively related to the percentage of Pascagoula and Citronelle geologies and a high percentage of Mixed Forest land use. However, these associations with landscape scale variables were weak, and a clear relationship in the literature between mussel assemblages and specific conductance is not evident. McRae et al. (2004) found a gradient of decreasing species diversity that coincided with increasing specific conductance, but suggested natural or anthropogenic causes could explain the association. Conversely, *Margaritifera hembelli*, a threatened mussel native to central Louisiana, was more common in streams with elevated specific conductance and water hardness (Brown and Johnson 2000). It was suggested that soft water, which has lower specific conductance and calcium content, may have been a limiting factor for shell deposition in this species. The effect of specific conductance (and its relationship with other environmental characteristics) on mussel distribution, assemblage composition, and abundance requires additional investigation in these systems.

Although elevated DO was identified as an organizing variable for mussels, it was not correlated with any land cover or geology categories. Coupled with low associations between local habitat variables and landscape variables in general, results suggest that local habitat variables (as influenced by surrounding environmental factors) are important in explaining freshwater mussel assemblages in streams of the Pearl River Basin. Studies based on local habitat variables such as hydrology, substrate, or water quality have been successful in explaining various aspects of freshwater mussel assemblages (Strayer 1999; Hastie et al. 2000;

Johnson and Brown 2000; Brown and Banks 2001; Howard and Cuffey 2003; Golladay et al. 2004; Kaller and Kelso 2006; Gangloff and Feminella 2007; Harriger and Moerke 2009; Allen and Vaughn 2010). However, associations have also been made between mussel assemblage characteristics and landscape scale variables, such as land use or geology. For example, agricultural land use has been shown to indirectly cause declines in mussel populations because of associated local scale events such as siltation, destabilization of the substrate, or elimination of suitable riparian habitat such as streamside woodlands (Arbuckle and Downing 2002; Poole and Downing 2004). Although surface geology has been used to explain freshwater mussel distribution (Strayer 1983), the proximal factors that were directly influencing mussel assemblages were hydrology, slope and turbidity of streams. Consequently, mussel assemblage distribution, composition, and abundance are likely influenced by both proximate (local scale) and ultimate (landscape scale) factors, the latter including both natural (e.g., geology) and anthropogenic (e.g., land conversion) components. Thus, knowledge of land use or geology must be integrated with local habitat data to accurately assess the population and assemblage characteristics of these organisms.

Silver Creek, the site that exhibited the highest abundance and species richness, proved to be an outlier in CPUE analysis. Although specific conductance and two substrate size classes were found to influence total abundance at each site, Silver Creek did not follow the same apparent patterns. Low specific conductance was found to positively influence abundance, steadily declining to a threshold at 0.05 mmhos/cm, whereby abundance appeared to drop off. However, specific conductance was 0.04 mmhos/cm at Silver Creek, much higher than this relationship would suggest for the site with the highest total abundance. Sediment samples taken at Silver Creek also showed low amounts of the two positively associated substrate size classes.

Clearly, something about this site is unique to provide the highest abundance and species richness of mussels sampled during this study and this site should be revisited in the future for further habitat analysis.

Previous mussel surveys in the Pearl River mainstem from 2012-2014 and during summer 2016 (concurrent with this study) revealed a total of 31 mussel species (Kayla Kimmel, Baton Rouge Fish and Wildlife Conservation Office, personal communication; LDWF 2014). Of the nine species encountered during my study, *Anodontoides radiatus*, and *Uniomerus declivis* were unique to tributary sites and were not found during the mainstem surveys (Table 7). Bambarger (2006) also found *U. declivis* and *Strophitus (=Anodontoides) radiatus* in Bogue Chitto tributaries. Together these results indicate that tributary streams in the Pearl River basin contribute to the overall mussel diversity of the lower Pearl River drainage by supporting small-stream species not found in mainstem habitats. Conservation of mussel biodiversity in the lower Pearl River basin should emphasize protection of the physicochemical and biological integrity of these tributary streams, as well as minimization of stream alterations that would restrict movements of glochidial fish host species.

Table 7. List of species collected from the Pearl River mainstem and species collected during 2015 and 2016 from Pearl River tributary streams. * indicate species unique to tributary streams.

Mainstem Species 2016	Mainstem Species LDWF 2012-2014	Tributary Species
<i>Amblema plicata</i>	<i>Amblema plicata</i>	<i>Anodontoides radiatus*</i>
<i>Anodonta suborbiculata</i>	<i>Anodonta suborbiculata</i>	<i>Elliptio crassidens</i>
<i>Arcidens confragosa</i>	<i>Arcidens confragosa</i>	<i>Lampsilis claibornensis</i>
<i>Elliptio crassidens</i>	<i>Elliptio crassidens</i>	<i>Plectomerus dombeyanus</i>
<i>Fusconaia flava</i>	<i>Fusconaia flava</i>	<i>Pleurobema beadleianum</i>
<i>Glebula rotundata</i>	<i>Fusconaia ebena</i>	<i>Quadrula refulgens</i>
<i>Lampsilis straminea</i>	<i>Glebula rotundata</i>	<i>Unio merus declivis*</i>
<i>Lampsilis ornata</i>	<i>Lampsilis claibornensis</i>	<i>Villosa lienosa</i>
<i>Lampsilis teres</i>	<i>Lampsilis straminea</i>	<i>Villosa vibex</i>
<i>Leptoidea fragilis</i>	<i>Lampsilis ornata</i>	
<i>Ligumia subrostrata</i>	<i>Lampsilis teres</i>	
<i>Obliquaria reflexa</i>	<i>Leptoidea fragilis</i>	
<i>Obovaria unicolor</i>	<i>Ligumia subrostrata</i>	
<i>Plectomerus dombeyanus</i>	<i>Obliquaria reflexa</i>	
<i>Pleurobema beadleianum</i>	<i>Obovaria unicolor</i>	
<i>Potamilus purpuratus</i>	<i>Plectomerus dombeyanus</i>	
<i>Pyganodon grandis</i>	<i>Pleurobema beadleianum</i>	
<i>Quadrula apiculata</i>	<i>Potamilus purpuratus</i>	
<i>Quadrula quadrula</i>	<i>Pyganodon grandis</i>	
<i>Quadrula refulgens</i>	<i>Quadrula apiculata</i>	
<i>Quadrula verrucosa</i>	<i>Quadrula quadrula</i>	
<i>Regina ebena</i>	<i>Quadrula refulgens</i>	
<i>Toxolasma parvus</i>	<i>Quadrula verrucosa</i>	
<i>Toxolasma texasiensis</i>	<i>Regina ebena</i>	
<i>Utterbackia imbecillis</i>	<i>Toxolasma parvus</i>	
<i>Villosa lienosa</i>	<i>Toxolasma texasiensis</i>	
<i>Villosa vibex</i>	<i>Tritogonia verrucosa</i>	
	<i>Truncilla donaciformis</i>	
	<i>Utterbackia imbecillis</i>	
	<i>Villosa lienosa</i>	
	<i>Villosa vibex</i>	

In summary, my study of freshwater mussel populations within streams of the Pearl River Basin revealed few mussels at the survey sites, which could be attributed to the small size of sample streams or lack of suitable habitat. However, where mussels were present, local habitat conditions appeared to be important in determining mussel richness and abundance. Greater

species richness was associated with higher proportions of finer substrate, with catch-per-unit-effort related to two differing substrate size classes, suggesting that substrate heterogeneity also played a role in mussel assemblage structure. Mussel associations with water quality were less clear, and the literature on mussel relationships to specific conductance is equivocal at best. Both CPUE and species richness were associated with low specific conductance, but the mechanisms behind this relationship are unknown. The higher suitability of finer substrate size classes was also related to higher levels of DO, although again, none of the DO levels appeared to be physiologically stressful. Freshwater mussels are widely but sporadically distributed in Pearl River tributaries, and it could be that longitudinal surveys in these streams from their confluence with the Pearl River to their headwaters could provide additional insights regarding the environmental factors that ultimately influence mussel assemblages in these streams.

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APPENDIX: GPS LOCATIONS

GPS Locations in decimal degree format (NAD27).

Site	Longitude	Latitude	Watershed Area (km²)	River
House Creek	-90.040444	30.662458	20.82	Bogue Chitto
Talley's Creek	-89.963925	30.657869	15.97	Bogue Chitto
Lawrence Creek	-90.125008	30.821258	128.76	Bogue Chitto
Sal's Branch	-89.888414	30.683603	3.38	Pearl
Talisheek Creek	-89.892617	30.53695	33.59	Pearl
Bogue Lusa Creek	-90.020811	30.811283	82.55	Pearl
Ben's Creek	-89.921544	30.7734	24.26	Pearl
Mill Creek	-90.073767	30.726806	25.64	Bogue Chitto
Peters Creek	-89.854344	30.813083	30.03	Pearl
Adams Creek	-89.836181	30.829414	33.42	Pearl
Pushepatapa Creek	-89.813219	30.865222	183.89	Pearl
Silver Springs Creek	-90.224975	30.901597	90.55	Bogue Chitto
West Hobolochitto Creek	-89.695883	30.590111	515.25	Pearl
Thomas Creek	-89.907633	30.948775	12.47	Pearl
Pushepatapa 2	-89.911033	30.946567	85.97	Pearl
West Hobolochitto 2	-89.652444	30.748797	297.21	Pearl
White Sands Creek	-89.636828	30.812953	52.96	Pearl
Hays Creek	-90.135278	30.956914	3.13	Bogue Chitto
Peter's Cutoff	-89.814984	30.804729	75.60	Pearl
11	-89.780783	30.831226	35.01	Pearl
Deer Lick Creek	-90.284287	30.910233	28.97	Bogue Chitto
Silver Creek	-90.270746	30.957422	30.32	Bogue Chitto
Hays Creek 2	-90.190647	30.887575	32.42	Bogue Chitto
Lawrence Creek 2	-90.081691	30.896203	50.34	Bogue Chitto
Crains Creek	-89.956981	30.967294	14.16	Pearl
Miller Creek	-90.060306	30.88534	30.47	Bogue Chitto
Stubbs Creek	-89.957995	30.959945	10.71	Pearl

VITA

Corinne Nicole Bird was born in Greenville, Texas but has lived most of her life in Mandeville, Louisiana. She attended Louisiana State University in Baton Rouge from August 2010 to May 2014 and earned her Baccalaureate in Renewable Natural Resources. She worked as a research associate at LSU in a freshwater ecology lab before she began her Master's degree. She now works as an environmental educator for the state's 4-H Youth Wetlands Education and Outreach Program.