Final Report
Endangered Species Research Projects for Freshwater Mussels, Region 2, East Texas
Texas Comptroller of Public Accounts

Project Leader
Dr. Neil B. Ford
Department of Biology
University of Texas at Tyler
Tyler, TX 75799
(903) 566-7249
nford@uttyler.edu

Co-Principal Investigators:
Dr. Lance Williams
Ms. Marsha G. Williams
Dr. Josh Banta
Dr. John Placyk
Dr. Harmonie Hawley

Reporting Period

September 1, 2015 – Dec. 31, 2016

Executive Summary

The goal of this project was to obtain a database of information on the 6 state-threatened species of mussels found in the northeast Texas region (Region 2). We used predicted distribution maps (generated from ecological niche models, and our 6+ years of new mussel data generated from East Texas rivers) to identify sites of highly suitable habitat for the target species. These maps were used to determine our field survey locations, which took 2 forms: (1) Determine population level information for each species, including information about population size, age distribution, and recruitment at sites of greater abundance, and (2) Conduct additional distributional surveys to refine the maps. Surveys were conducted in the fall of 2015 and summer of 2016, at 21 sites in the Angelina River, 11 sites in the Attoyac Bayou, 125 sites in the Neches River, 76 sites in the Sabine River, 13 sites in the Cypress Creek basin, 17 sites in the Sulphur River, 3 sites in the San Jacinto River and 7 sites in the Trinity River. In total we have collected 31,130 individual mussels of 35 species of which 26,371 were live. We recorded 1853 live and 243 dead mussels of 22 species in the Angelina River and Attoyac Bayou, 10,122 live and 972 dead mussels of 28 species from the Neches River, 460 live and 294 recently dead mussels of 19 species from the Cypress Creek basin, 2215 live and 1139 dead mussels of 19 species from the Sabine, 940 live and 95 dead mussels of 21 species from the Sulphur River, and 1124 live and 679 dead mussels of 16 species in the Trinity River.

We found all 6 species of mussels listed as state-threatened. Three of those have been petitioned for federal listing. The highest mussel diversity was 28 species and occurred within the upper Neches, which corresponds to the location of a new USFW refuge. We collected population information (density, age structure, growth and mortality) on the 6 species in locations where our maps indicated that abundances were high. In 7 locations mark-recapture was conducted on Texas, Triangle and Louisiana pigtoes. The Texas Pigtoe appears stable but is difficult to separate visually from the rare Triangle Pigtoe, which appears to only occur in the Angelina River, the Attoyac Bayou and lower Neches River. The distribution of the Southern Hickorynut is restricted to only a very limited reach on the Neches River. The Louisiana Pigtoe is only doing well in the upper Neches River. The Sandbank Pocketbook and the Texas Heelsplitter are rare everywhere and primarily found only in the Neches and Sabine Rivers.

We collected detailed habitat data in the upper Sabine River to examine hydrologic factors (velocity, shear stress) known to be involved in mussel habitat selection. We also used landscape environmental layers to create habitat suitability maps (MaxEnt) for the 6 species. This information will be useful in determining critical habitat and areas of high conservation value that could be used by the USFWS for planning and potential listing of species. We used the MaxEnt maps of each species habitat suitability to identify potential anthropogenic impacts (dams, oil rigs) on mussel communities in rivers of East Texas. It did appear that dams did impact the

presence of the threatened species but the occurrence of oil rigs did not. Although zebra mussels have been confirmed in the Trinity and Red River and their lakes, we found no evidence in our surveys that they have spread to the other east Texas rivers.

We addressed genetic questions regarding the taxonomic status of the three Pigtoe species and also the relationships of the Texas Heelsplitter and Pink Papershells. Louisiana Pigtoes are distinct in all genes examined from Texas and Triangle Pigtoes. However, the latter two are not different in the genes examined (ND1, COX1, 16S, ITS1). Texas Heelsplitters are genetically distinct from Pink Papershells but a morphologically intermediate individual from Lake Lewisville exhibited genes of both species. Such information will be critical to determine which species should be protected by the state of Texas and the USFWS.

We also present a summary of the current status of each species as concluded from our surveys.

Literature Review

Freshwater mussels (Family: Unionidae) are extremely imperiled in North America with over 70% endangered, threatened, or presumed extinct (Williams et al., 1993; Ehlo and Layzer, 2014). Population declines in mussels have been linked to historic overharvest, habitat degradation from impoundments and pollution, lost of host fish species and the introduction of exotic species (Neves et al., 1997; Dudgeon et al., 2006). In Texas the same habitat destruction from impoundments, contaminants and invasive species have occurred with the result that of the 52 described species of unionids, 15 species were designated as State Threatened (TPWD, 2009) and of those 9 are being considered for protection under the Endangered species Act (ESA). Of those 6 species are found in the larger rivers of eastern Texas; the Southern Hickorynut, *Obovaria arkansasensis*, the Louisiana Pigtoe, *Pleurobema riddellii*, the Texas Pigtoe, *Fusconaia askewi*, the Triangle Pigtoe, *Fusconaia lananensis*, the Sandbank Pocketbook, *Lampsilis satura*, and the Texas Heelsplitter, *Potamilus amphichaenus* (TPWD, 2010). Two of those, the Louisiana Pigtoe and Texas Heelsplitter, have been petitioned to be listed by the United States Fish and Wildlife Service (USFWS) for protection under the Endangered Species Act with status reviews planned in 2017 (USFWS, 2011).

The Triangle Pigtoe, *Fusconaia lananensis,* is endemic to the Neches drainage basin of east Texas and has been recorded in the Angelina River, Attoyac Bayou and southern tributaries of the Neches such as Village Creek (Howells, 2006; Karatayev and Burlakova, 2007a, b). It is a problematic species as it is difficult to distinguish in the field from the Texas pigtoe, *F. askewi* with which it co-occurs (Howells, 2010b). This makes the evaluation of the current records difficult, as misidentifications of this species are very likely. Most of the historical records are from Village Creek but we found a number of presumptive Triangle Pigtoes in the Angelina River. It is not

abundant in any sites. It was found in rocky riffles of fast waters.

The Texas Pigtoe, *Fusconaia askewi*, is endemic to east Texas rivers but is not common in any except the upper Sabine River where it sometimes is abundant.   In the Sabine basin below Toledo Bend Dam, the Neches, Angelina, Cypress Creek and Trinity River drainages it is much less abundant and is often the least common species (Troia, 2014; Randklev et al., 2011; Ford et al., 2015). In our recent surveys the upper Neches and the upper Sabine rivers are by far where most occur. Specimens from Cypress Creek, Sulphur and Trinity River basins differ in morphology from the *F. askewi* collected from the Sabine and Neches Rivers and we have tentatively identified some from the Sulphur River as *F. flava* (Ford, et al., 2014) Most have been found in shallow rocky riffles. Erosion of riverbanks is problematic in these rivers and sand and silt often covers existing mussel beds.

The Sandbank Pocketbook, *Lampsilis satura*, is endemic to eastern Texas and western Louisiana (Vidrine, 1993). It is found in rivers in gravel or sand substrates. In Texas it occurs in the Neches and Sabine basins including the Angelina River and Village Creek. Although widely distributed it is uncommon at each site. There appear to be no locations that exhibit significant populations. They are often the species with the lowest rank abundance. The limited records from the Trinity and San Jacinto Rivers need confirmation as misidentification with other species occurs. Potentially Sandbank Pocketbooks could also be found in the Sulphur River or other Red River drainages. Where it is found it occurs in a variety of habitats including sand. It is relatively mobile and is sometimes stranded when floodwaters recede.

The Southern Hickorynut, *Obovaria arkansasensis*, is a species of the southeastern United States that is rare throughout its range (Williams et al., 2008). In Texas this species has a very limited distribution and currently is known from only a short reach in the Neches River (Troia, 2014) and Village Creek near Beaumont including areas of the Big Thicket National Park (Bordelon and Harrel, 2004; Symonds, 2015). It appears to be found in habitats that are connected with the floodplain (i.e., rivers with low banks). Rivers of east Texas are being undercut by waters from dams and so are developing steeper banks which are likely impacting this species.

The Louisiana Pigtoe, *Pleurobema riddelli*, occurs in eastern Texas (Howells et al., 1996) into Louisiana (Vidrine, 2008) and in the Red River tributaries in Arkansas (Howells, 2010). It is very uncommon in all drainages with only the upper Neches River recording more than just a few specimens. A few have been recorded in the Neches River below Town Bluff Dam, in Village Creek, a tributary of the Neches, in the Angelina River near Nacogdoches and two recent records in the Sabine River. We also have found individuals in the upper Trinity River and both the Little Cypress and Big Cypress Creeks. Because Louisiana Pigtoes are rare in all localities they may have been missed in passing surveys. They are found in a variety of habitats but most are associated with cobble and rocks. This species is being evaluated by USFWS for potential listing in 2017.

The Texas Heelsplitter, *Potamilus amphichaenus*, is endemic to Texas and Louisiana in bigger rivers such as the Sabine and Neches. It is also known from the Trinity River but may hybridize with *P. ohienis*, the Pink Papershell, in impoundments of that drainage. It is the rarest of the state-listed East Texas mussels. Presently, it has small populations upstream of Sam Rayburn Reservoir (Karatayev and Burlakova, 2007) and the upper Neches River and below Town Bluff Dam. Its largest numbers are in the upper Sabine River but it rarely has been seen in numbers at any single location. Very few living specimens have been found anywhere but have been recorded primarily in sand in slow moving waters of larger rivers. It is a species that can adapt to reservoirs.

Introduction

In 2008 we began surveys of the five major rivers in northeastern Texas under grants from TPWD, USFWS, TxDOT and the National Park Service (Troia et al., 2014, Ford et al., 2012; 2014, Symonds, 2015). From 2010 to 2014 we surveyed sites on the Sabine, Neches, Sulphur, Cypress Creek, and Trinity Rivers and produced more current distributional and relative abundance data for all six state-threatened species. These data were used to create ecological niche models to produce predictive distribution maps for each species (Dunithan, 2012, Ford, 2013, Heffentrager, 2013, Williams et al., 2013, Symonds, 2015). We also conducted genetic analysis of the *Fusconaia* from the Sulphur River and Cypress Creek basins. Two specimens were identified as *F. flava*, the Wabash pigtoe (Ford et al., 2014).

Our studies indicate several issues relative to the understanding of the conservation status of East Texas mussels. First the Pigtoes (*Fusconaia spp.* and *Pleurobema riddellii*) are difficult to identify in the field. Conservation decisions rely on the ability of biologists and their stakeholders to be able to distinguish one species from another. Even using internal characteristics, identification of these species can be challenging. Distributions, abundance, and other ecological information for the species are confounded by misidentifications. In southeast Texas, the Triangle Pigtoe may actually be the Texas Pigtoe (Burlakova et al., 2012) and in the Trinity, Sulphur and Cypress Creek drainages some Texas Pigtoes may actually be Wabash Pigtoe (*F. flava*) (Ford et al., 2014). Resolving the taxonomy of these species is critical so that accurate information concerning the need for listing is available. A similar identification problem is present in the Trinity River basin for the Texas Heelsplitter. Potentially it overlaps with the Pink Papershell (*P. ohiensis*), which is extremely difficult to distinguish from the Texas Heelsplitter. Because this area of overlap includes the Dallas/Fort Worth metroplex, where accurate information is critical for construction activities, a genetic method to distinguish this species in the field is warranted.

Geographic patterns of species distributions, combined with information on the factors that contributed to their endangerment, are necessary for developing effective conservation strategies (Burlakova et al., 2011). For freshwater mussels in Texas, distribution information is available at a very broad scale, based on shell

material collected in early studies (Howells, 2006). Recent studies by university researchers, over the last 10 years, have increased our knowledge of the distribution of mussels in Texas, but many sections of rivers and streams have yet to be surveyed and quantitative data necessary for population analysis is generally lacking for east Texas species. This includes information such as age classes, recruitment of juveniles, and mortality rates (Jones et al., 2012). For the east Texas threatened species we located larger populations of several of the threatened species in the upper Neches and Sabine Rivers where we conducted standard population analysis (Vaughn et al., 1997). We also gathered habitat characteristics and data on other mussel species presence to develop a more comprehensive understanding of the ecology of these rare mussels. From the niche models produced from our earlier surveys (Williams et al., 2013) we selected sites in two of the major rivers that showed high levels of suitable habitat for each species, Texas Pigtoe, Triangle Pigtoe, Louisiana Pigtoe, Sandbank Pocketbook, Southern Hickorynut and Texas Heelsplitter, for population and habitat association studies. Sizes of individuals, rates of recruitment, survival at different ages and movement are all characteristics influencing the viability of a population (Jones et al., 2012, Vilella et al., 2004). Quantitative studies of individuals in these sites were begun in 2014 and continued through 2016.

Some additional distribution information was needed throughout all the rivers of east Texas to fill gaps in our knowledge of each species. We used the same ecological niche models to identify areas in which to conduct additional qualitative (timed) sampling. This information was then used to improve the niche models we used in the other objectives.

Objectives/Questions/Deliverables

To better guide the listing process for east Texas mussels obtaining the most current, accurate information on the distribution, basic ecology and habitat requirements of these species is necessary. Therefore our objectives for this project on East Texas mussels included the following:

1. Use predicted distribution maps (generated from ecological niche models, and our 6+ years of new mussel data generated from east Texas rivers) to identify sites of high mussel diversity, for the target species of concern.
2. Determine population level information for these species, including information about population size, age distribution, and recruitment at sites of greater diversity.
3. Conduct additional distributional surveys to refine areas of critical habitat.
4. Identify potential anthropogenic impacts on mussel communities in rivers of east Texas, including a habitat and water quality assessment using a multi-scale approach (i.e., digital landscape level analysis to on the ground measurement of habitat features in the rivers).

5. Address genetic questions regarding the taxonomic status of Pigtoes and the Texas Heelsplitter in east Texas.
6. Identify potential locations of exotic species that might impact mussel communities.
7. Identify critical habitat and areas of high conservation value for each of the six species that could be used by the USFWS for planning and potential listing of species.

Methods

We identified sites of high abundance for each threatened species from previous ground surveys and niche modeling as locations for population studies, mark-recapture and, if not surveyed before, new sites to survey to improve distributional information. Survey and study sites were accessed from public launch areas and we traveled to sampling locations via kayaks or boats. The studies were performed in the fall of 2014 and summers of 2015 and 2016. The following specific studies and analyses were undertaken:

Additional Distributional Surveys

Surveys sites were specifically chosen to fill in records for areas where we have not recently surveyed, with emphasis given to sites that appeared appropriate for mussels based on our ecological niche models for the threatened species. In addition during each of the surveys for population information we conducted timed surveys in nearby locations. We primarily concentrated on the Sabine and Neches Rivers as these two larger rivers have the most robust populations of the threatened species. However, we did conduct some more limited surveys on other rivers. When sites were located we sampled using a timed hand search in shallow areas or with surface compressed air equipment in deeper sites (Strayer et al., 1997; Strayer and Smith, 2003; Vaughn et al., 1997). Depending on the size of the area, we conducted between 1 and 2 person hour surveys. All live and recent dead Unionids collected were identified, counted, and then returned to the river except for a limited few for genetic analysis. The results of these surveys were combined with data from our previous work to produce current distribution maps and to use in ecological niche modeling (see below).

Population Sizes, Age Structure, & Mortality

During all surveys, individuals of any threatened species were measured (i.e., length, height, and width). Whether specimens were live or recent dead was documented. In the Fall of 2014 three locations for each of the Texas Pigtoe and Louisiana Pigtoe and 1 location for the Triangle Pigtoe were designated for population analysis. These were chosen from sites with the highest abundance from previous surveys. The Texas Pigtoe sites were on the Sabine and Angelina Rivers and the Louisiana Pigtoe sites were in the Neches River. Only one location had sufficient Triangle

Pigtoes to mark and it was in the Angelina River. In the Fall of 2014, 0.25 meter quadrat surveys were conducted in each area to determine the highest density within these high density locations. An area of 25 square meters around the quadrat with the highest density of the threatened species was designated as the mark-recapture area. The total 25 m^2 area was then surveyed using 1-meter quadrats. All mussels were dug out to a depth of 10 cm. All threatened species were individually marked by gluing a numbered Queen Bee tag or a Passive Integrated Transponder (PIT) tag onto the shell. Mussels were then replaced within the 25 m^2 area. Flooding delayed recapture surveys until July 2015. A second recapture survey was conducted within 3 weeks after that sample. The sites were then sampled again at the end of the summer in 2016. During each sampling all marked animals were recorded and new animals marked. In the second and third years all marked animals found were measured again to examine growth. Any dead mussels were also measured. Although few juveniles were recaptured we did examine the growth rate for individuals that had multiple recaptures. We also grouped the data into size classes from each site to produce information on age structure, which was used to examine recruitment. There were very few mortalities in the study so age specific mortality rate was only examined from the size class data.

Mark-recapture models were created from the data for each location using Program MARK version 8.0 (White and Burnham, 1999). Program MARK is used to provide parameter estimates from organisms that are marked and reencountered, whether alive or dead, and can provide population size estimates within closed populations (White and Burnham, 1999). The POPAN model was chosen for this study because in addition to estimating capture probability, survival probability, and overall population size, this model has the capability to estimate the population size at each encounter (Arnason and Schwarz, 1995; Schwarz and Arnason, 1996). Models for each of the seven field sites and both real values and derived values were extracted from the top model(s) for each site. Real and derived estimates for φ_1 (survival probability between the initial visit and the 2nd visit), p_2 (probability of capture at 2nd visit), N_1 (initial population estimate), N_2 (population at 2nd visit), and Gross N (overall population estimate during course of study) were calculated for the top model(s) at each field site. These values are reflective of the population within this high density, 5 m x 5 m, mark-recapture area and not the entire field site.

Habitat Use and Modeling

The upper portion of the Sabine River was modeled in order to determine velocity and shear stress profiles throughout the river. These are two parameters that are expected to affect mussel survival as these are known to be the only factors consistently correlated to mussel habitat distribution. If we can model these physical characteristics we can correlate them to the occurrence of threatened species. There are many methods to calculate shear stress in a waterway; however, each method has its advantages and disadvantages. No one method is considered applicable to all stream and sediment types. The Reach Averaged Method is a popular method to study stream shear stress (Nikora, 2001). It is also the basis for

Shield's assessment of critical shear stress that has been used in previous mussel research (Vermont Agency of Natural Resources, 2009; Allen and Vaughn, 2010). Determining velocity and shear stress for large river reaches is typically done using a computer model, with the Army Corps of Engineers Hydraulic Engineering Center River Analysis System (HEC-RAS) model being a widely used and accepted model (Aggett and Wilson, 2009).

Thirty-five (35) miles of the Sabine River, from the Mineola USGS gauge through the Hawkins gauge to the Gladewater gauge, were modeled in HEC-RAS version 4.1.0. Elevation data were downloaded from the Texas Natural Resources Information System (TNRIS) as a digital elevation model (DEM) from the National Elevation Dataset 2013 and imported into ESRI ArcMap version 10.2.2. In addition, a Trimble hand held GeoHX global positioning system (GPS) was used to collect elevation, latitude, and longitude in the field in a small area and these were used to calibrate the HEC-RAS model. Once the data were imported into ArcMAP, the stream centerline and cross-sections were cut through the river. Cross-sections were cut through the river every 500 feet for the 35-mile model and each was 25,000 feet long. The long cross-sectional length is because the flood plain outside of the channel is very large (i.e., the land is fairly flat) and the entire floodplain should be represented in HEC-RAS. In addition a model of only 1-mile was made from the GPS collected points (which were augmented with DEM data when needed). The cross section at station number 22+57 (STA 22+57) on the small model is the same location as station number 779+14 (STA 779+14) in the large model. This information was used to calibrate the large model from the small model. The cross-sectional data were imported into HEC-RAS using the Army Corps. Of Engineers ArcMap add-in HEC-GeoRas. HEC-RAS modeling was conducted to determine velocity and shear stresses along the river. This detailed hydrological modeling could be extrapolated to all east Texas rivers.

In addition to hydrological modeling, we used landscape environmental layers in a Geographic Information System (GIS) to create habitat suitability maps (also known as ecological niche models) for the six state-threatened mussel species in east Texas. This study focused on the associations of environmental factors with mussel distributions at a relatively fine-grained resolution (100 m^2 x 100 m^2 resolution), where local habitat parameters including water velocity, depth, and substrate type are commonly thought to influence mussel abundance and distribution (Vannote and Minshall, 1982; Holland-Bartels, 1990; Strayer and Ralley, 1993; Strayer et al., 1994).

The ecological niche modeling software that we used, Maxent, produces a geographic model of habitat suitability by searching for the best solution that matches the distribution of the observed occurrences to the environmental variables (i.e., ArcGIS layers) (Phillips et al., 2006). It produces a map with a logistic score for each grid cell (corresponding to the resolution of the environmental data), which can be interpreted as the degree of suitability of a particular location for the species, given the environmental attributes of that location and their similarity to

other locations where the species is known to occur (Phillips and Dudik, 2008). These habitat suitabilities, covering every place along the rivers at the resolution of our analysis, range from 0 to 1 with 0 representing the least suitable habitat and 1 representing the most suitable habitat. The analysis was restricted primarily to the Trinity, Cypress, Sulphur, Sabine, Neches, and Angelina rivers and their associated watersheds. Habitat suitability models were built separately for each species. To minimize autocorrelation at 1km, we used the 'thin' function of the package spThin (Aiello-Lammens et al., 2014) in R version 3.2.2 (R Development Core Team 2015).

Nine continuous environmental variables were incorporated into the model for each species: available water content in the surrounding soil (in/in), bulk density of the surrounding soil (in g/cm^3), the percentage of the surrounding soil consisting of clay, the percentage (by weight) of the surrounding soil consisting of organic matter, the erodibility factor (k) of the surrounding soil from the Universal Soil Loss Equation (USLE; Ontario Ministry of Agriculture, Food, and Rural Affairs, 2015), slope of the map unit as a percentage, the mean annual ground water recharge of the stream/river at that location (mm/year), the velocity of the stream/river at that location (ft/s), and the flow volume of the stream/river at that location (ft^3/s). Soil characteristics were obtained from the State Soil Geographic (STATSG0) Data Base (United States Department of Agriculture, 1994), and the data processing steps used to make this dataset are described in Wolock (1997). The hydrology layers were obtained from the NHDFlowline dataset (USEPA and USGS, 2005). These environmental variables were chosen because we hypothesize that they are important for freshwater mussel distributions. Water velocity and substrate type are known to influence mussel distribution and abundance (Vannote and Minshall, 1982, Strayer and Ralley, 1993). Both flow volume and groundwater recharge are related to water velocity. Additionally, the percentage of surrounding soil consisting of clay provides information regarding substrate type. The percentage of surrounding soil consisting of organic matter is important because freshwater mussels filter organic matter from the water column (Strayer et al., 1999), and presumably organic matter in the soil is related to organic matter in the water column. We also included the presence of oil and/or gas wells to determine if these features influence the habitat suitability for mussels using the Oil and Gas Exploration and Production in the United States Shown as Quarter-Mile Cells layer from USGS (2008).

The environmental data was converted to raster format in ArcGIS for Desktop Basic version 10.1 (esri.com). All rasters were sampled to achieve a common resolution of 100 m x 100 m and all rasters were in the NAD 1983 UTM Zone 15N projection using a geographic (XY) coordinate system with meters as the unit. Environmental layers were clipped in order to constrain them to lotic habitats. We did this by adding a 100 m buffer around water features (ponds, streams, rivers, canals, and dams), delineated by the NHDFlowline dataset (USEPA and USGS, 2005), and clipping the environmental layers to match the lotic buffer. These maps were used to make some general recommendations of locations of critical habitat for each species.

Model validation

We subdivided our datasets into the training data, species occurrence locations used to develop the models, and test data, species occurrence locations that were not used in model development. For Louisiana Pigtoe, Sandbank Pocketbook, Texas Heelsplitter, and Texas Pigtoe, we validated the models by setting aside 20% of the location data for each species as test data. Due to the low sample sizes of Southern Hickorynut and Triangle Pigtoe, we used a "*n*-1" cross-validation method for those species, as previously described by Pearson et al., (2007): we set the number of folds for each species to equal the number of samples, so that each fold contained *n* - 1 observations, where *n* is the total sample size. Each fold had a single test data point, and each presence location was the test data point, in turn, for a separate fold. Model statistics were averaged across the *n* folds for each species.

We validated our models using the test AUC, or the area under the operator receiving curve. AUC measures the probability that a randomly chosen presence site will be ranked above a randomly chosen pseudoabsence site (Phillips and Dudik, 2008). The test AUCs represent the average percentage of the pseudoabsence data with lower habitat suitability scores than single "test" presence locations left out of the model building process for each model fold. Importantly, this model validation procedure is based on data points (test data) that were naïve to the model building process for each model fold, and therefore represent a form a ground-truthing of the models with independent data.

We measured model fit using the gain statistic. Gain is a likelihood (deviance) statistic that measures the model performance compared to a model that assigns equal habitat suitabilities to all areas of the landscape. Taking the exponent of the final gain gives the (mean) probability of the presence sample(s) compared to the pseudoabsences. For instance, a gain of 3 means that an average presence location has a habitat suitability of e^3 = 20.1 times higher than an average pseudoabsence site.

Genetic analysis

During timed surveys some individuals of each Pigtoe species and Texas Heelsplitters were collected for tissue samples. In addition, Pink Papershells from the Sulphur River were collected for comparison to the Texas Heelsplitter. Tissue samples were preserved in 95% ethanol and stored at -20 °C followed by DNA extraction prior to polymerase chain reactions (PCRs). PCR was used to selectively amplify the NADH dehydrogenase subunit 1 (ND1) gene, which has been used in previous genetic studies of bivalves (Grobler et al., 2011). The PCR product was then purified and stored at -20 °C. Purified DNA was sent to Eurofins MWG Operon for sequencing. Genetic data was used at two levels: 1) at the phylogenetic level to re-evaluate the taxonomy of the Pigtoes (Triangle Pigtoe (*Fusconaia lananensis*), Texas Pigtoe (*F. askewi*), and Louisiana Pigtoe (*Pleurobema ridellii*)), and 2) to obtain

genetic information for the Texas Heelsplitter (*Potamilus amphichaenus*) and Pink Papershell (*Potamilus ohiensis*) to allow us to distinguish between these two species where they were sympatric. Genetic divergence estimates were used to determine which species should be considered "good" and which may require reclassification. In addition, our data was compared with GenBank sequences (Benson et al., 2005) to further confirm their potential identity.

Results and Discussion

Distribution of state-threatened freshwater mussels in east Texas

Surveys were conducted, from Fall of 2014 to summer of 2016, at 21 sites in the Angelina River, 11 sites in the Attoyac Bayou, 125 sites in the Neches River, 76 sites in the Sabine River, 13 sites in the Cypress Creek basin, 17 sites in the Sulphur River, 3 sites in the San Jacinto River, and 7 sites in the Trinity River. These records were added to our previous survey data that we had started collecting in 2009 for a total of 31,130 individual mussels of 35 species collected (of which 26,371 were live specimens). This indicates that 15.3% overall were recent dead individuals. We recorded 1,853 live and 243 dead mussels of 22 species in the Angelina River and its tributary the Attoyac Bayou (Fig. 1). In ranking, the Texas Pigtoe was the 5th, the Triangle Pigtoe was the 8th, the Louisiana Pigtoe was the 13th and the Sandbank Pocketbook was the 15th most abundant species. The Neches River had the highest diversity with 10,122 live and 972 dead mussels of 28 species recorded (Fig. 2).   In the Neches River, Texas Pigtoe was the 4th most abundant, Louisiana Pigtoe was the 9th, Sandbank Pocketbook was the 18th, Triangle Pigtoe was the 19th, Southern Hickorynut was the 25th, and Texas Heelsplitter was the 28th most abundant species. We collected 460 live and 294 recently dead mussels of 19 species from the Cypress Creek basin (3 bayous, Fig. 3). In these rivers Louisiana Pigtoe was the 7th and Texas Pigtoe was the 14th most abundant species. We collected 2,215 live and 1,139 dead mussels of 19 species from the Sabine River (Fig. 4), where the Texas Pigtoe was the 2nd most abundant species, Sandbank Pocketbook was 12th, Texas Heelsplitter was 15th, and Louisiana Pigtoe was the 18th most abundant species. We recorded 940 live and 95 dead mussels of 21 species from the Sulphur River (Fig. 5). The Texas Pigtoe was the only threatened species encountered there and it was the 13th most abundant species if we combine it with the individuals we called Wabash Pigtoes (See genetics section). We found 1,124 live and 679 dead mussels of 16 species in the Trinity River (Fig. 6) where the Texas Pigtoe was ranked 11th although these too could be Wabash Pigtoes. The Louisiana Pigtoe was ranked 14th there and we found a single presumptive Sandbank Pocketbook. We only found 48 specimens in the San Jacinto River basin but of those, one live and one dead were Texas Pigtoes. If we exclude the San Jacinto surveys because of low sample size, the highest mortality rate occurred in the Sabine and Cypress Creek basins and the lowest mortality was recorded in the Neches and Sulphur Rivers.
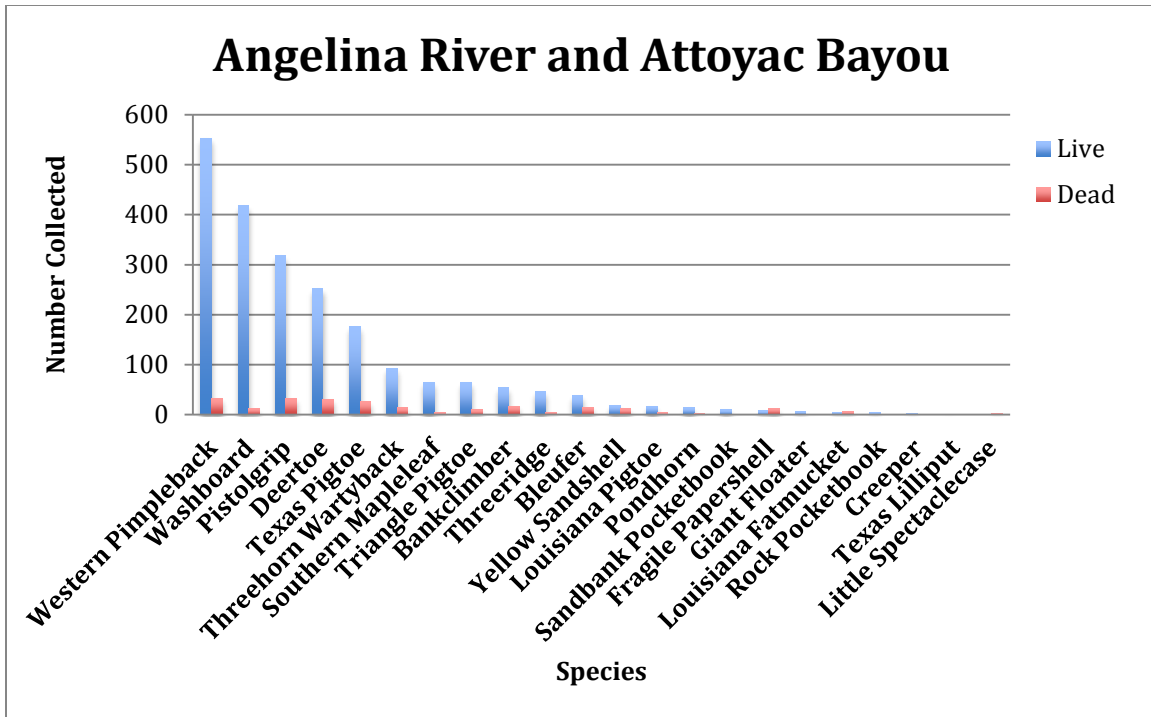
Fig. 1. Rank abundances of live and recent dead mussels collected in the Angelina River and its tributary, the Attoyac Bayou.
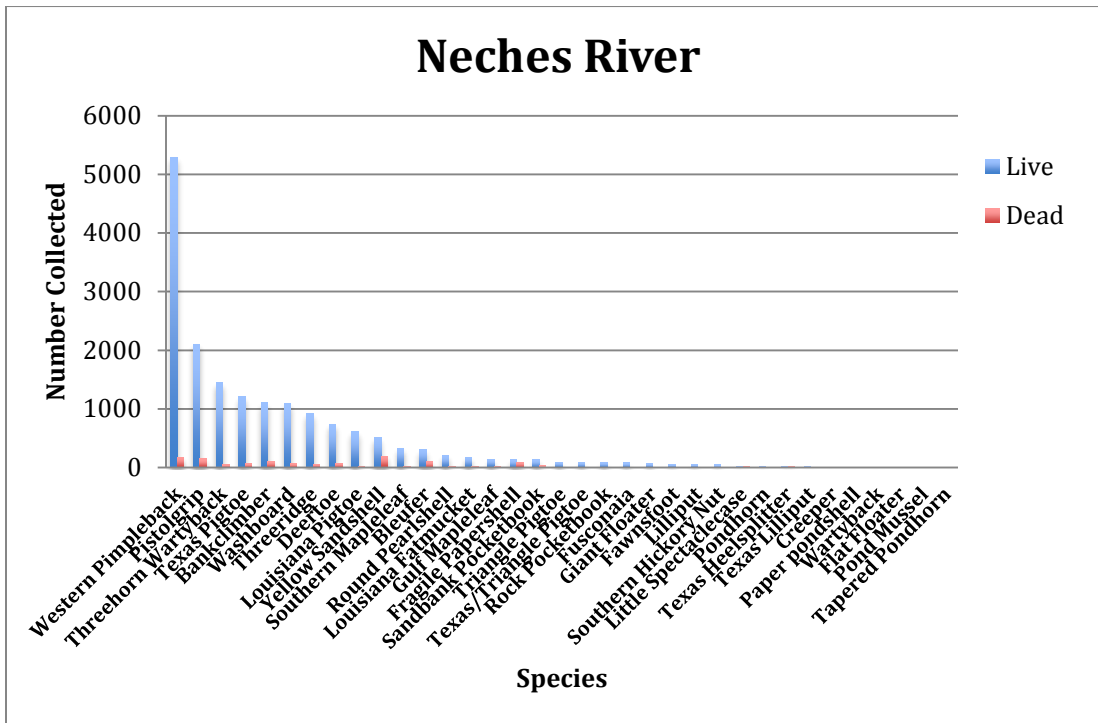

Fig. 2. Rank abundances of live and recent dead mussels collected in the Neches River.
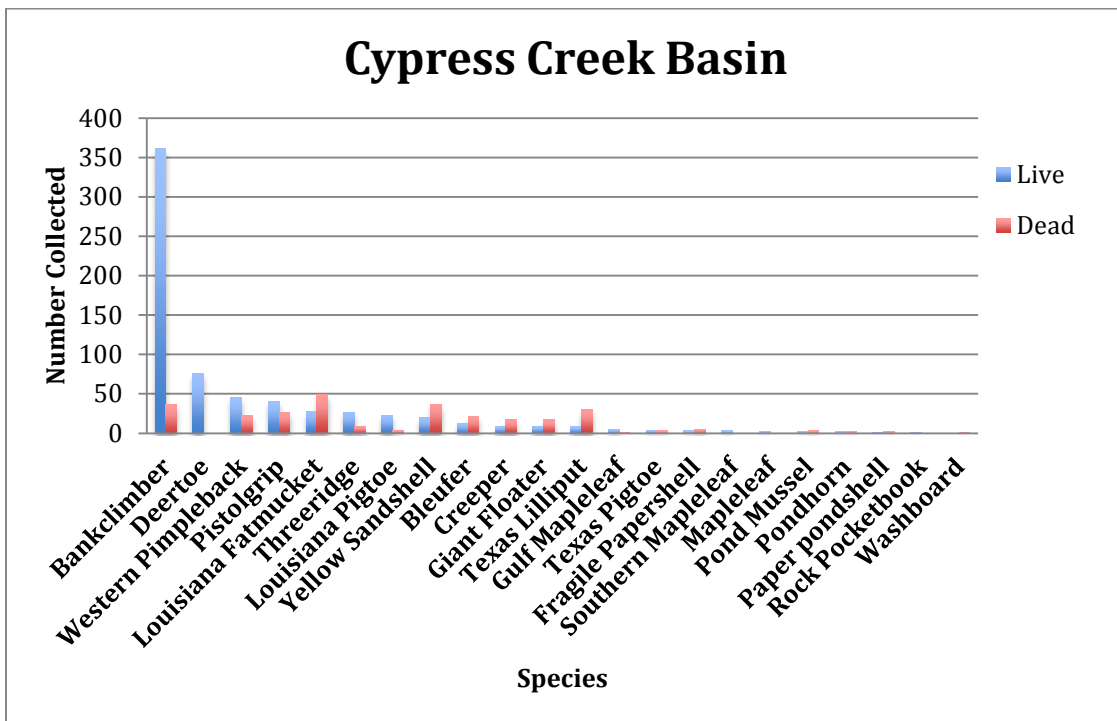
Fig. 3. Rank abundances of live and recent dead mussels collected in Cypress Creek basin.
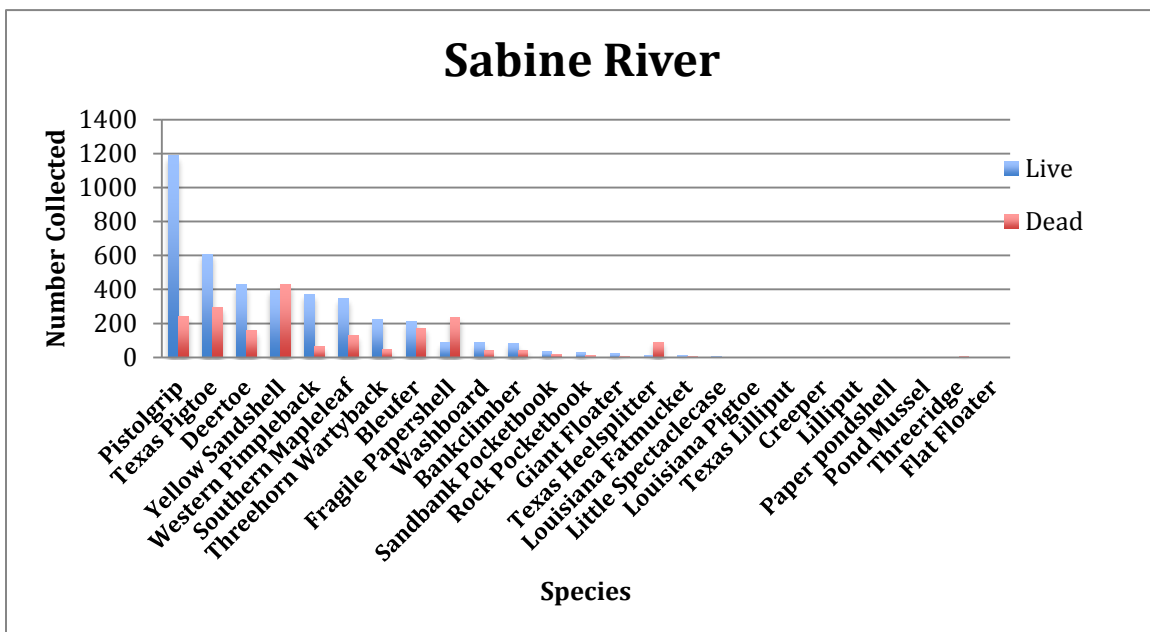

Fig. 4. Rank abundances of live and recent dead mussels collected in the Sabine River.
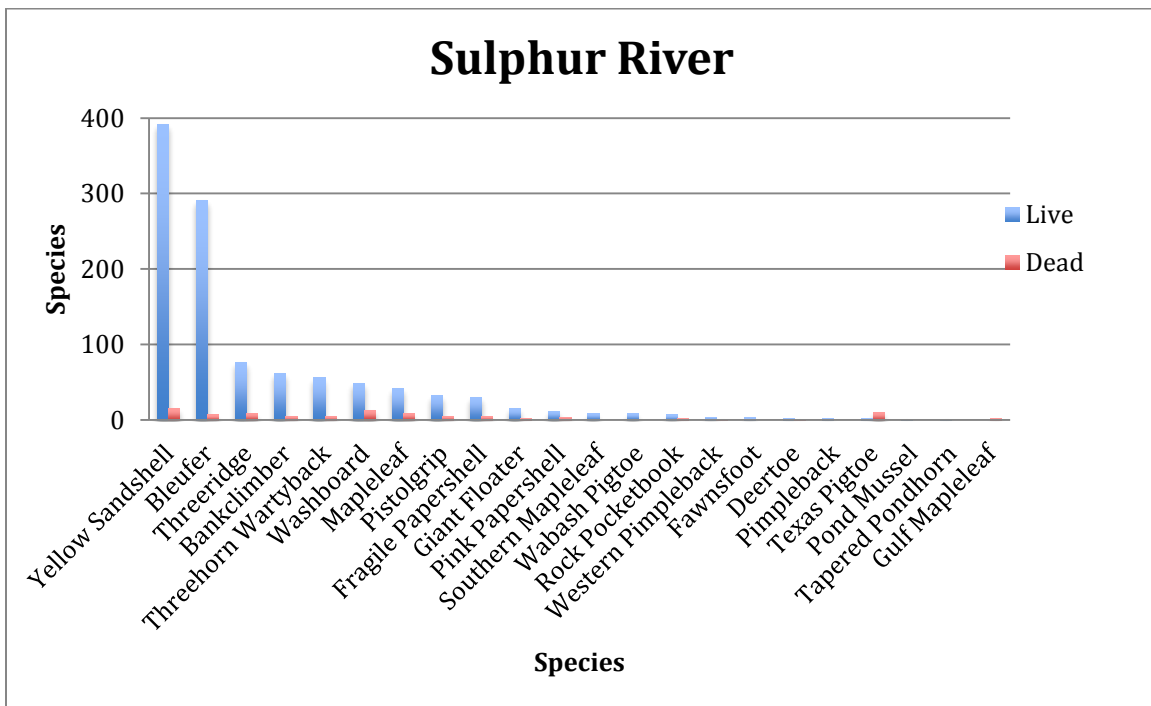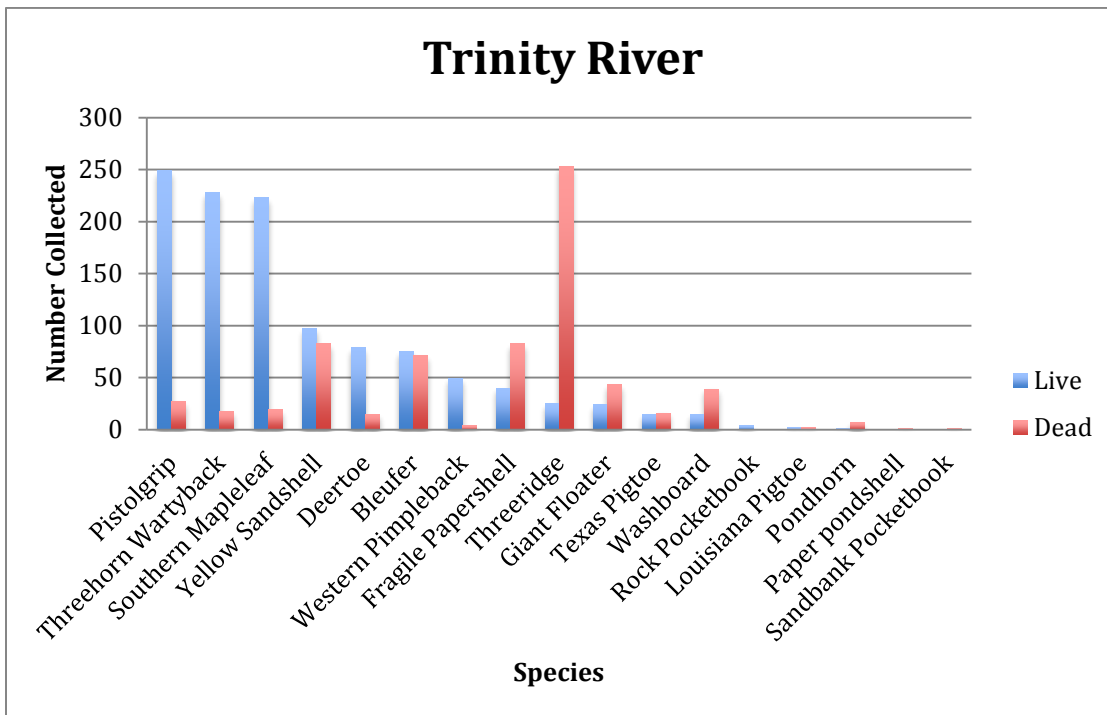
Fig. 5. Rank abundances of live and recent dead mussels collected in the Sulphur River.

Fig. 6. Rank abundances of live and recent dead mussels collected in the Trinity River.

Watershed distribution of east Texas State-threatened mussels

We found all six species of east Texas mussels listed as state-threatened. The highest mussel diversity for any river was 28 species and occurred within the upper Neches River. We compared the abundance of each species in each river basin by dividing numbers collected by the number of sites surveyed. The Texas Pigtoe occurred in all six river basins (Fig. 7) and in sites in the Neches River basin and the Sabine River it was in higher numbers per site than any of the other threatened species (Fig. 8). Texas Pigtoes were rare in the Cypress Creek drainage, the Sulphur River and the San Jacinto River (Fig. 8).   The Triangle Pigtoe was difficult to distinguish from the Texas Pigtoe but appeared to be only in the Angelina River, Attoyac Bayou, and the lower Neches River (Fig. 9). This was the only species that was not found in higher numbers per site in the Neches (Fig. 10). However, it was most abundant in the Angelina River and Attoyac Bayou both of which are tributaries of the Neches River (Fig. 9). The distribution of the Southern Hickorynut is restricted to only a very limited reach on the Neches River (Fig. 11). The species is also rare in sample locations where it is found (Fig. 12). The Louisiana Pigtoe was also much more abundant in the Neches River drainage but was also found in the Little Cypress Creek and Trinity River (Fig. 13 & 14). A very few specimens were found in the Sabine River (Fig. 13 & 14). The Sandbank Pocketbook was only found in the Neches River basin and the Sabine River (Fig. 15) and was rare in those drainages (Fig. 16). The Texas Heelsplitter was only found in the Neches and Sabine Rivers (Fig 17) and was rare in those rivers (Fig. 18).
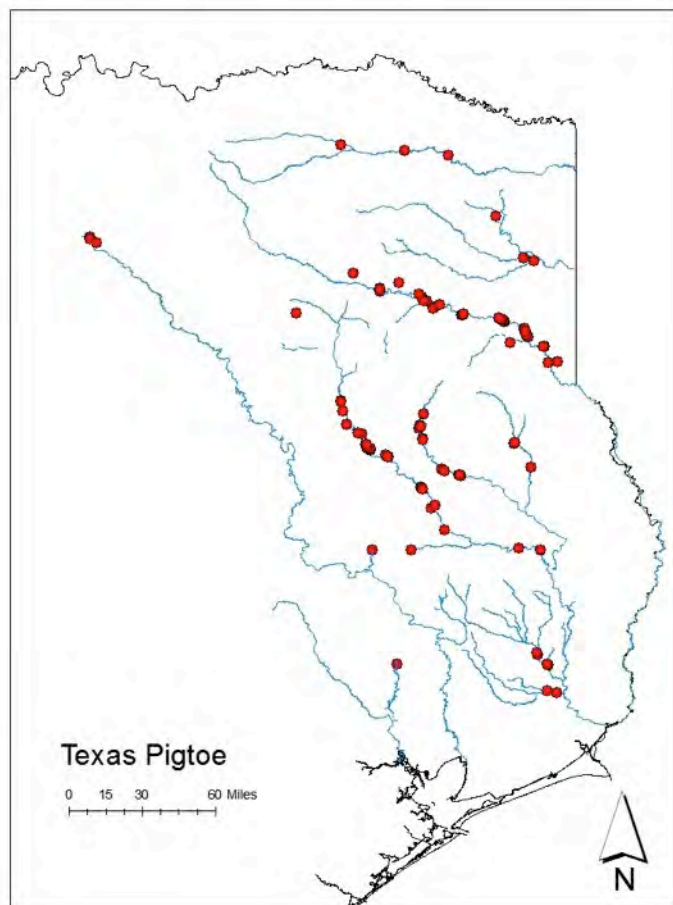
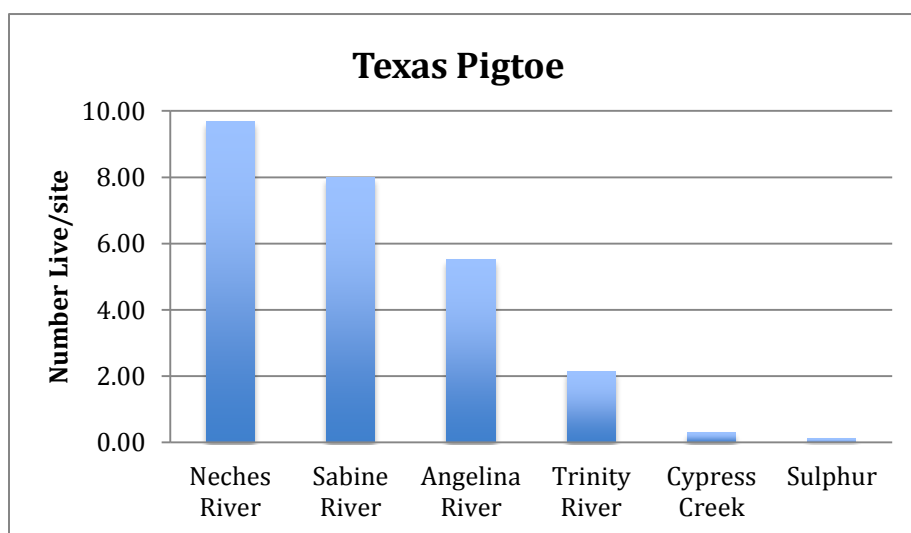Fig. 7. Sites where Texas Pigtoes were found during this study.

Fig. 8. The number of live Texas Pigtoes per site in different streams.
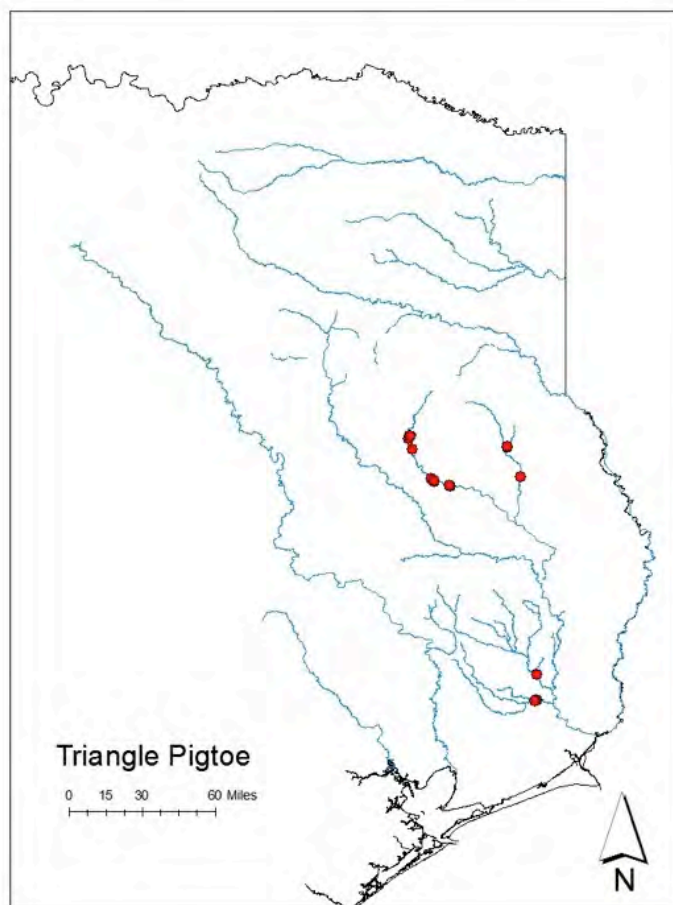
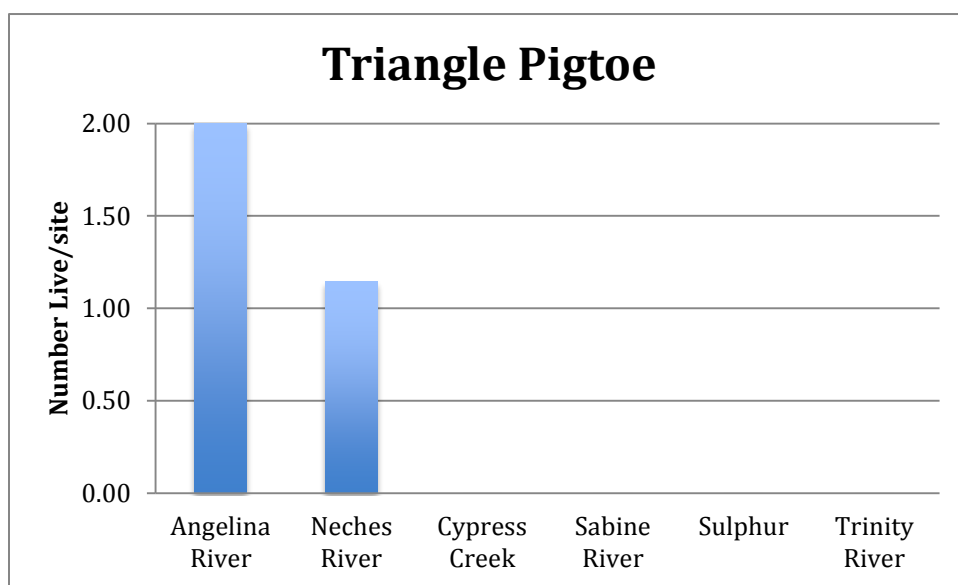Fig. 9. Sites where Triangle Pigtoes were found during this study.

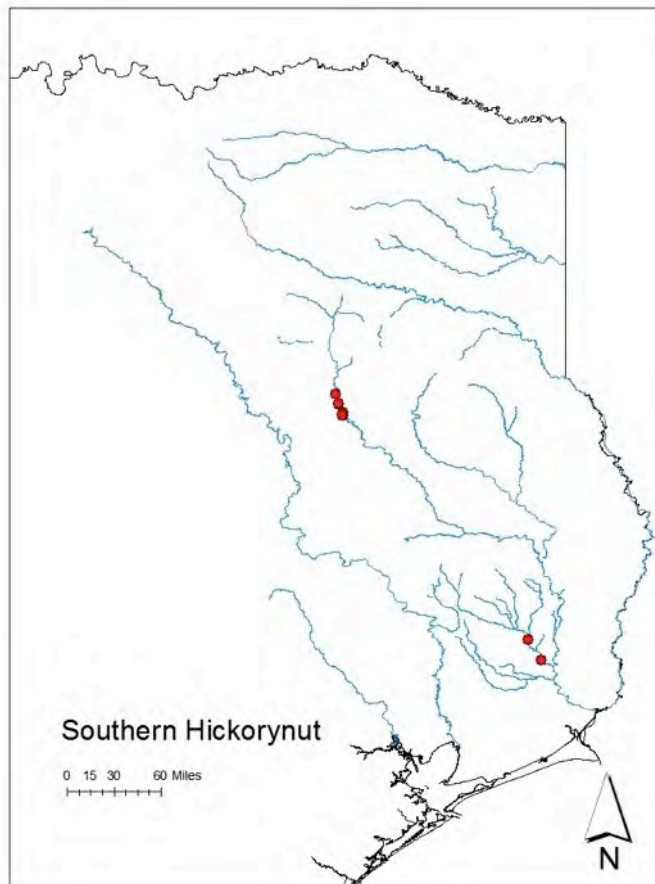Fig. 10. The number of live Triangle Pigtoes per site in different streams.

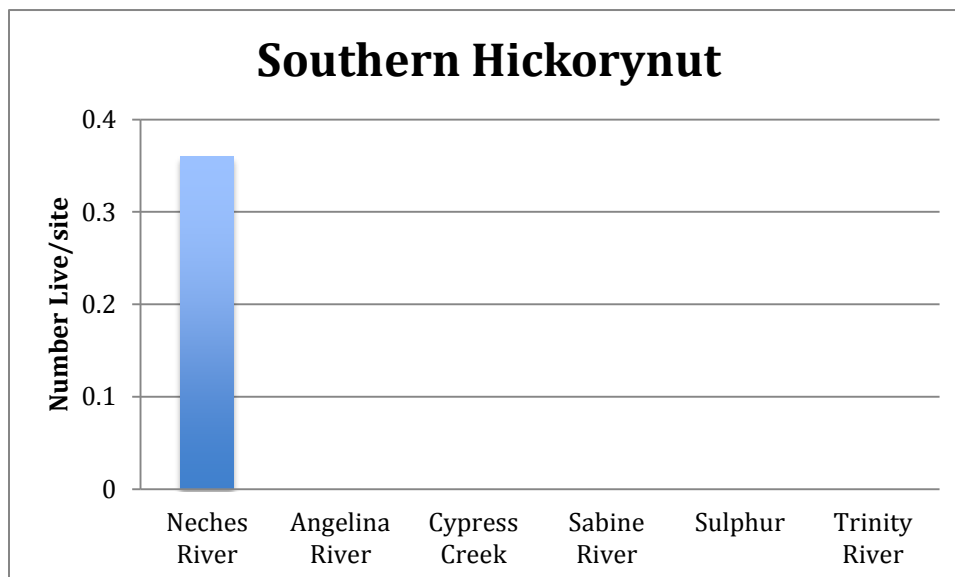Fig. 11. Sites where Southern Hickorynuts were found during this study.

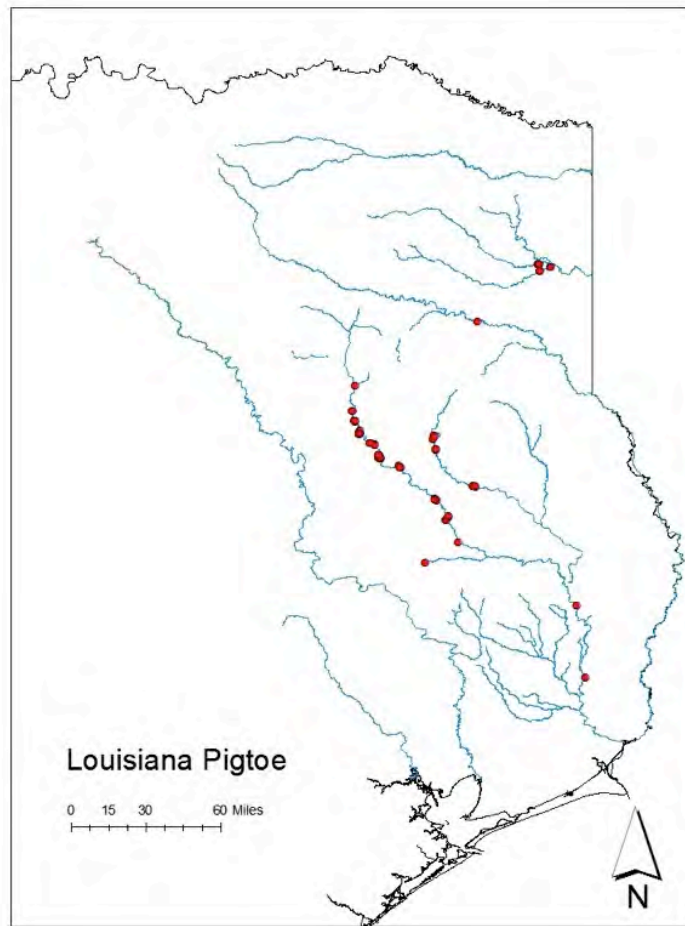Fig. 12. The number of live Southern Hickorynuts per site in different streams.

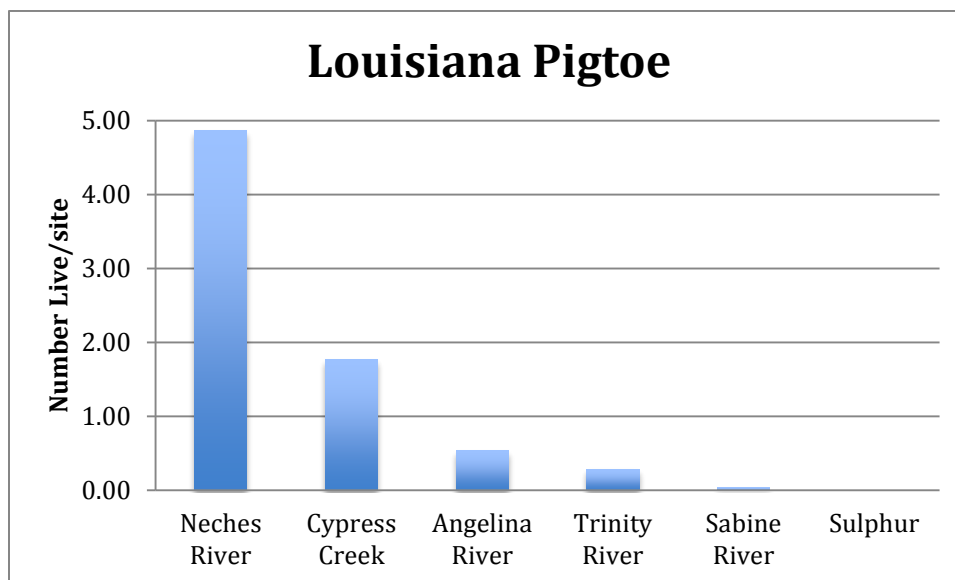Fig. 13. Sites where Louisiana Pigtoes were found during this study.

Fig. 14. The number of live Louisiana Pigtoes per site in different streams.
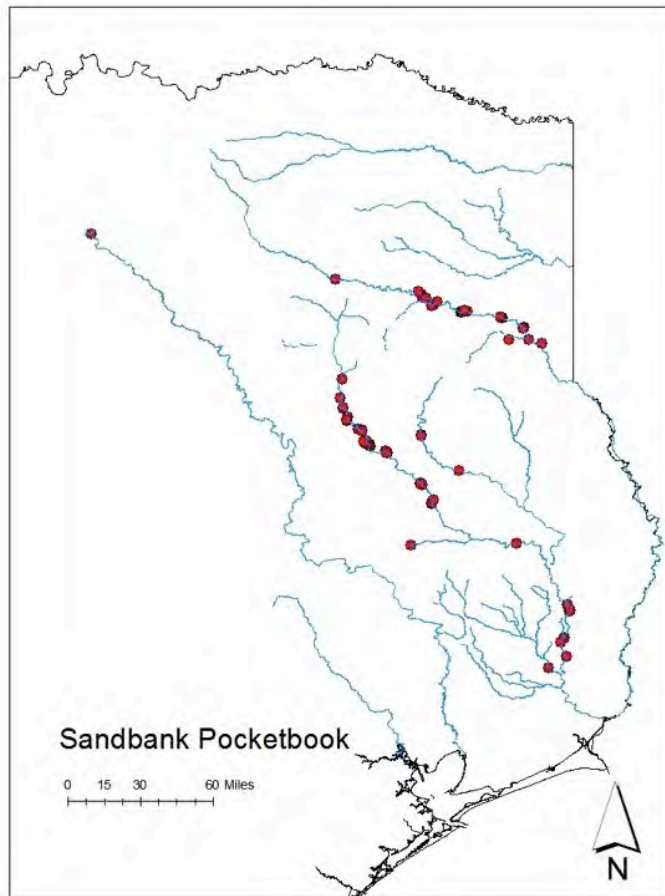
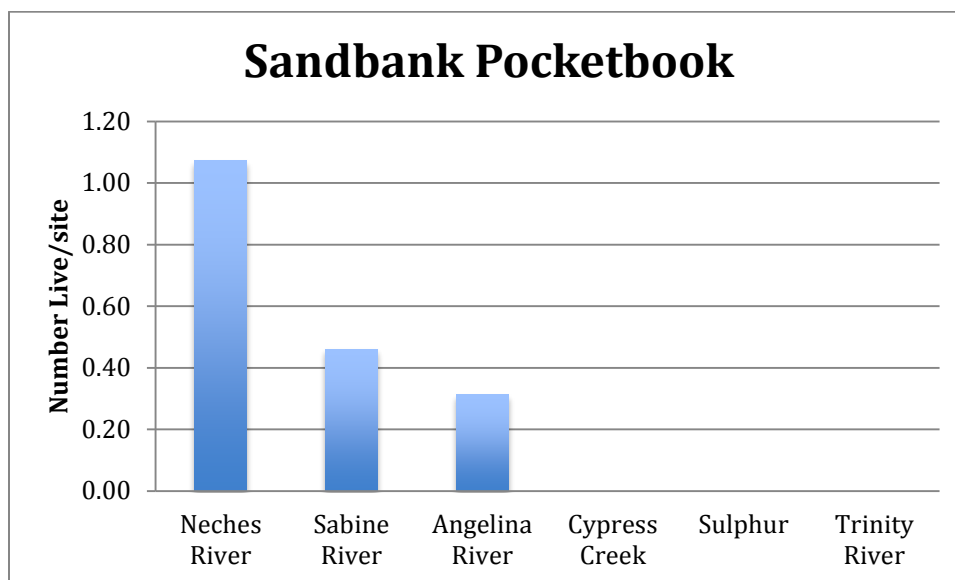Fig. 15. Sites where Sandbank Pocketbooks were found during this study.

Fig. 16. The number of live Sandbank Pocketbook per site in different streams.
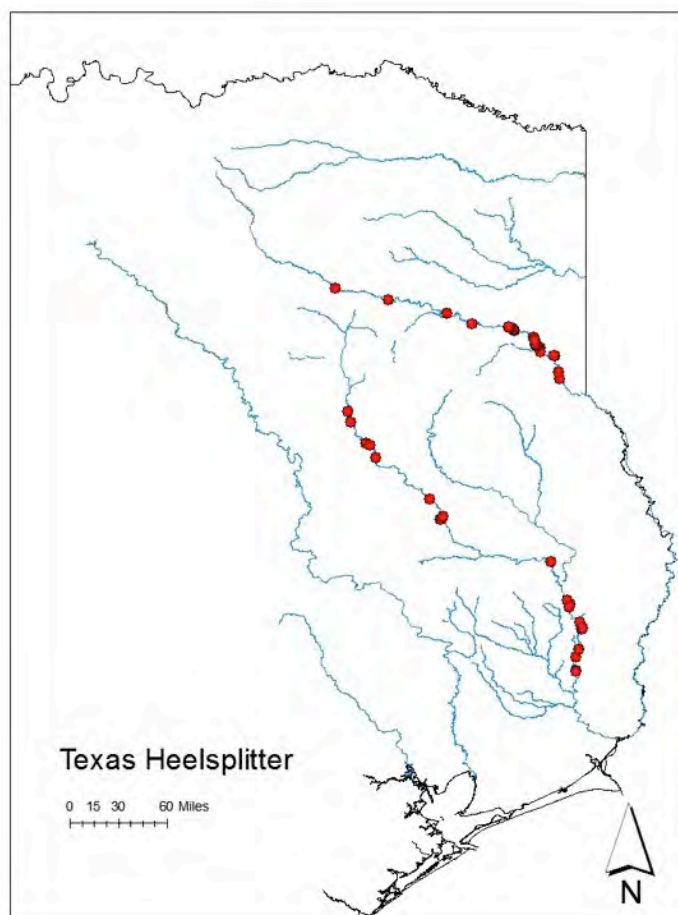
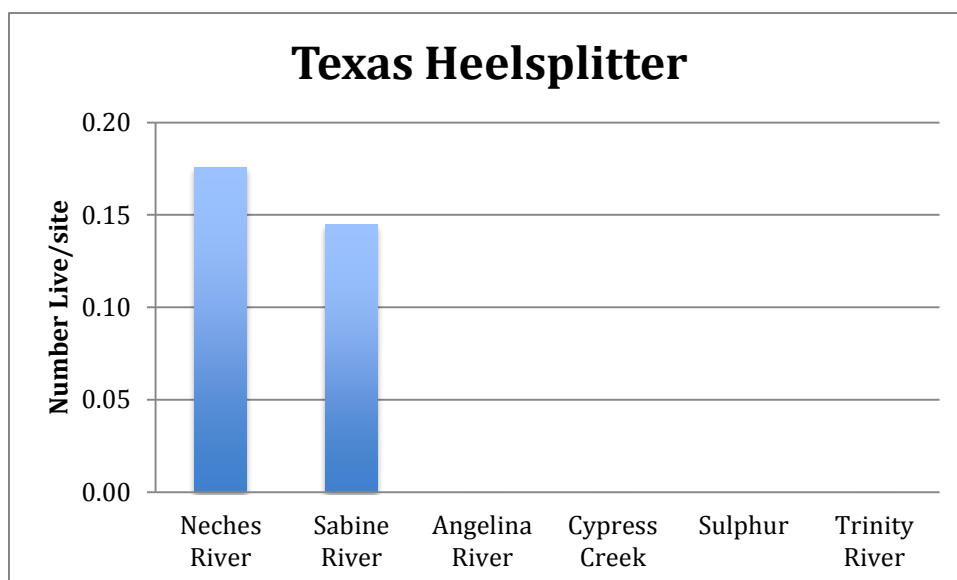Fig. 17. Sites where Texas Heelsplitters were found during this study.

Fig. 18. The number of live Texas Heelsplitter per site in different streams.

Density Studies

Reaches in the Sabine and Neches Rivers that had previously been found to have the highest densities of Pigtoe mussels were sampled using 0.25 m quadrats to determine the highest density sites to do mark-recapture studies of each species. Three sites in the Neches River were selected for Louisiana Pigtoes, 3 sites in the Sabine River for Texas Pigtoes and one site in the Angelina River for Triangle/Texas Pigtoes (Table 1). A 25 m^2 area around the quadrat with the highest number of each species was then surveyed in the Fall of 2014 and all Pigtoes marked with Queen Bee tags and PIT tags and measured for length. Louisiana Pigtoes were marked at three sites of high density on the Neches River, Texas Pigtoes were marked at three sites of high density on the Sabine River and Triangle Pigtoe was marked at one site on the Angelina River, which had the most Triangle Pigtoes. Texas Pigtoes were also marked there because it was difficult to distinguish the species in the field.

Table 1. Site names, locations, coordinates, and species of study for each field site.

Site Name	Location	Coordinates	Species
Neches 1	Upstream Hwy 294	N 31.643610, W-95.285900	Louisiana Pigtoe
Neches 2	Cherokee Hunt Club	N 31.715680, W-95.332570	Louisiana Pigtoe
Neches 3	Downstream Hwy 79	N 31.841370, W-94.425150	Louisiana Pigtoe
Sabine 1	Downstream Hwy 14	N 32.553450, W-95.200690	Texas Pigtoe
Sabine 2	Hwy 14 Bridge	N 32.557638, W-95.205906	Texas Pigtoe
Sabine 3	Upstream Hwy 43	N 32.377156, W-94.465937	Texas Pigtoe
Angelina 1	Upstream 343	N 31.753400, W-94.961610	Triangle Pigtoe

Densities for Texas Pigtoes were higher than for Lousiana Pigtoes (Table 2). Texas Pigtoes were higher in density in the Sabine River than in the Neches River and no Louisiana Pigtoes were found in the Sabine River in these studies. Texas Pigtoes had more recent dead individuals than did Louisiana Pigtoes, in fact, no dead Louisiana Pigtoes were found (Table 2). These results match with what had been noted with abundance surveys throughout east Texas. Densities were also calculated during the first mark-recapture survey by dividing the total number collected by 25 m (the total area surveyed was 25 m^2). These numbers were a little higher as might be expected since the recapture area was chosen from the best site of the quadrat survey. The mean density of Texas Pigtoes was about twice the density of the Louisiana Pigtoes (Table 3).

Table 2. Mean m² densities for Texas Pigtoes, *Fusconaia askewi,* and Louisiana Pigtoes, *Pleurobema riddellii,* from surveys at high density sites between 2014-2015.

	Texas Pigtoe		Louisiana Pigtoe	
Site	Mean m² density Live	Mean m² density Dead	Mean m² density Live	Mean m² density Dead
Neches 1	0.54 ± 1.45	0.15 ± 0.70	0.222 ± 0.51	0 ± 0
Neches 2	4.37 ± 7.58	0.07 ± 0.54	2.815 ± 7.34	0 ± 0
Neches 3	0.22 ± 1.21	0.22 ± 0.93	--	--
Sabine 1	5.63 ± 5.90	9.04 ± 10.26	--	--
Sabine 2	--	--	--	--
Sabine 3	0.89 ± 2.56	0 ± 0	--	--
Angelina 1	2.22 ± 9.35	0 ± 0	--	--

Table 3. Comparison of mean m² densities between quadrat surveys and those at our mark-recapture sites. Mean densities for mark-recapture sites were obtained by dividing the total captured by 25 m.

	Mean m² density	
Site	Quadrats in high density sites	Mark-Recapture sites
Louisiana Pigtoe		
Neches 1	0.22±0.51	2.52
Neches 2	2.82±7.34	1.12
Neches 3	0	2.32
	Mean for Louisiana Pigtoe	
	1.01	1.99
Texas Pigtoe		
Sabine 1	5.63±5.90	4.88
Sabine 2	0±0	2.52
Sabine 3	0.89±2.56	3.16
	Mean for Texas Pigtoe	
	2.17	3.52
Triangle Pigtoe		
Angelina 1	2.22±9.35	1.76

Mark-recapture population estimates

Model selection in MARK resulted in a total of 10 top models as three sites had two top models of equal values (Table 4). The largest population sizes were estimated for Texas Pigtoe populations (Table 4). Largest Gross N values were also estimated for Texas Pigtoe populations (Table 4). An increase in estimated population size was seen in most populations between 2014 and 2015 except at sites Sabine 2 and

Neches 3. Though the largest population estimate from the models was for Texas Pigtoes, there were no significant differences in estimated population sizes between the Neches River and the Sabine River within the 25 m² mark-recapture area. This can be seen both during 2014 and 2015. Though there was no significant difference in population sizes, the largest population size estimated for Texas Pigtoes was higher than the largest estimated population size for Louisiana Pigtoes. The largest population estimate for a Texas Pigtoe population was at site Sabine 1 with a population estimate of 302 ± 26.72 in 2015 while the largest population estimate for a Louisiana Pigtoe population was at site Neches 1 with a population estimate of 101 ± 4.99 in 2015 within the 25 m² mark-recapture area (Table 4). Both sites Neches 3 and Sabine 2 saw a decrease in estimated population sizes between 2014 and 2015 while all other sites saw an increase in estimated population size during this time (Table 4). Though site Neches 3 saw a small decrease in estimated population size in 2015, site Neches 3 had a larger Gross N value than site Neches 2. A value of 91 ± 2.33 was calculated for site Neches 3 and 71 ± 2.89 for site Neches 2 (Table 4). This larger Gross N value indicates that the model estimated that there were more individual mussels present in the 25 m² mark-recapture area over the course of the study, which can also be seen when including the number of individuals found during the final visit (Table 4).

Table 4. Real and derived estimates with standard error values from top POPAN models for all seven mark-recapture sites. Neches 3, Sabine 2, and Angelina 1 had two top models with equal real and derived estimates. These values are reflective of the 25 m² mark-recapture high density site and not the entire river.

Site	Model*	Survival (φ_1)	Capture (p_2)	N_1 at 2014	N_2 at 2015	Gross N
Louisiana Pigtoe						
Neches 1	φ(t)pent(t)p(t)	0.92 ± 0.03	0.85 ± 0.06	64 ± 5.78	101 ± 4.99	150 ± 3.75
Neches 2	φ(t)pent(t)p(t)	0.98 ± 0.01	1 ± 0	29 ± 3.96	60 ± 3.12	71 ± 2.89
Neches 3	φ(.)pent(t)p(t) φ(.)pent(t)p(.)	0.98 ± 0.01	1 ± 0	58 ± 4.12	54 ± 4.21	91 ± 2.33
Texas Pigtoe						
Sabine 1	φ(.)pent(t)p(.)	0.92 ± 0.01	0.44 ± 0.04	280 ± 34.56	302 ± 26.72	909 ± 97.03
Sabine 2	φ(t)pent(t)p(t) φ(t)pent(t)p(.)	0.02 ± 0.07	1 ± 0	90 ± 6.12	64 ± 6.12	381 ± 63.95
Sabine 3	φ(.)pent(t)p(.)	0.97 ± 0.01	0.44 ± 0.05	116 ± 20.19	169 ± 18.61	293 ± 32.75
Triangle Pigtoe						
Angelina 1	φ(t)pent(t)p(.) φ(.)pent(t)p(t)	0.77 ± 0.04	1 ± 0	42 ± 4.3	95 ± 4.71	181 ± 13.49

* φ, survival probability; p, capture probability; pent, recapture probability; N, population estimates; (.), constancy; (t), temporal variation

Population dynamics

Recapture rates were quite low for Texas Pigtoes (Table 5), which accounted for the large calculated population sizes of approximately 12 to 36 per square meter. There were large flood pulses during the spring of 2015, which may have displaced animals and deposited others. The recapture rates were much higher for Louisiana Pigtoes in the Neches River in the same time periods. That river does have a more connected floodplain, which may have reduced the shear stresses produced by the floods (Troia et al., 2015). High shear stress is known to scour out mussels from beds. Because of the poor recapture rate for the Texas Pigtoes, the Sabine River sites were not sampled in 2016. Recapture rates were quite high for Louisiana Pigtoes in the Neches River in 2016 and no mortality was noted.

Table 5. Recapture Rates (RR) for all seven mark-recapture sites within the 25 m² sites. Visit 1 (not included in the table) was the initial visit where we first collected and marked individuals. At each subsequent visit we searched for marked individuals and marked new individuals.

Site	RR Visit 2	RR Visit 3	RR Visit 4
Neches 1 (Louisiana)	0.49	1.00	0.89
Neches 2 (Louisiana)	0.61	1.00	0.94
Neches 3 (Louisiana)	0.55	1.00	0.88
Sabine 1 (Texas)	0.07	0.49	
Sabine 2 (Texas)	0.01	0.02	
Sabine 3 (Texas)	0.14	0.53	
Angelina 1 (Triangle)	0.33	0.94	0.86

The largest population sizes calculated from the mark-recapture data were found in the Sabine and Angelina Rivers where Texas Pigtoe populations were monitored (Table 5). In addition, the Texas Pigtoes at Sabine site 1 had the largest gross N value of 909 ± 97.03 for the 25 m² (Table 5). Our results and previous research suggests that populations of Texas Pigtoes are found in much higher densities than Louisiana Pigtoes (Burlakova et al., 2010, Ford et al., 2012, Ford et al., 2014). We found no difference between the mean density of Texas Pigtoes in the Neches River and the mean densities of Texas Pigtoes in the Sabine River. In previous studies Texas Pigtoes were reported in higher densities in the Sabine River (Ford et al., 2012). More juvenile Texas Pigtoes were detected during the study suggesting high recruitment of this species.

Average density: comparisons to other studies

Mean m² densities calculated for Louisiana Pigtoes averaged 1.99 per m² and mean densities for Texas Pigtoe averaged 3.52/m². The state threatened Texas Hornshell, *Popenaias popeii*, had densities between 0-0.186 individuals per m² (Karatayev et al., 2015). Densities for a locally rare species *Quadrula pustulosa*, the Pimpleback, were approximately 0.25 individuals per m² (Sethi et al., 2004). In comparison, common mussel species had values of up to 1.25 individuals per m² in the same area (Sethi et al., 2004). Surveys for the critically imperiled *Epioblasma torulosa rangiana*, the Northern Riffleshell, produced mean densities ranging from 0.01 – 6.67 m² (Crabtree and Smith, 2009). When comparing the densities of the Texas Hornshell, Pimpleback, and Northern Riffleshell to both Louisiana Pigtoe and Texas Pigtoe densities, the Pigtoe species had densities more comparable to common mussel species. However, it is important to note that higher density values should be expected as this study was conducted at sites with high suitable habitat that historically had recorded higher densities of these species. Quadrat surveys for these species at random site locations produced densities similar to those found in other studies involving rare species (see below).

Heavy flooding occurred between the 2014 and 2015 sampling events. Both the Sabine River and the Neches River experienced higher water levels during the winter of 2014-2015 as opposed to the winter of 2013-2014. Flooding has been found to have negative effects on mussel populations (Strayer, 1999; Hastie et al., 2001); however, the numbers of Texas Pigtoes found in the Sabine River in 2015 were similar to the numbers found before the flooding events. There were few recaptures and most were new adult animals. Potentially, the flooding dislocated the marked individuals and replacements resettled from upstream.

Density surveys in random sites within suitable habitats

We used ecological niche models for the Neches and Sabine Rivers to select 30 random quadrat survey locations. Within each site thirty randomly placed 0.25 m² quadrats were excavated and all species of mussels recorded. Mean densities from the total 900 quadrats were determined for all state listed species found (Table 6).

Table 6. Mean density of mussels from 30 quadrats from 30 random sites (900 total) in the Sabine and Neches Rivers.

	Neches River	Sabine River
Species	Density (individuals/m²)	Density (individuals/m²)
Louisiana Pigtoe	0.55	0.001
Southern Hickorynut	0.253	0
Sandbank Pocketbook	0.039	0.013
Texas Pigtoe	0.989	0.24
Texas Heelsplitter	0	0.007

Although these surveys were conducted in sites with suitable habitat the densities were much lower than those from the mark recapture sites with known high densities. The values are in general more comparable to other studies of rare or endangered mussels (see above). Texas Pigtoes had the highest densities and Texas Heelsplitters had the lowest densities (Table 6). The quadrats were randomized within each site; thus the values represent the average densities among all the mesohabitats (riffles, runs, pools, etc). These densities could be used to approximate the total number of individuals in the areas of suitable habitat obtained from side scanning sonar and LiDAR maps in future studies.

Size classes, growth, recruitment and mortality in Louisiana and Texas Pigtoes

Louisiana Pigtoes collected throughout the Neches River were nearly all adults with little evidence of recruitment of the smallest size classes (Fig. 19). Increases in size classes between 2014 and 2016 suggest Louisiana Pigtoes were growing. Maturity is likely close to 40 mm in length. There was little evidence of mortality in this species in our abundance surveys and in the mark-recapture studies. Few shells were found and only one marked individual was found dead in one year. Conditions for adults appear to be adequate for survival and growth. Recruitment in mussels does not occur every year; therefore, it is unknown whether the lack of recruitment observed was due to recent weather/flooding events or a more pervasive, long-term conservation concern.
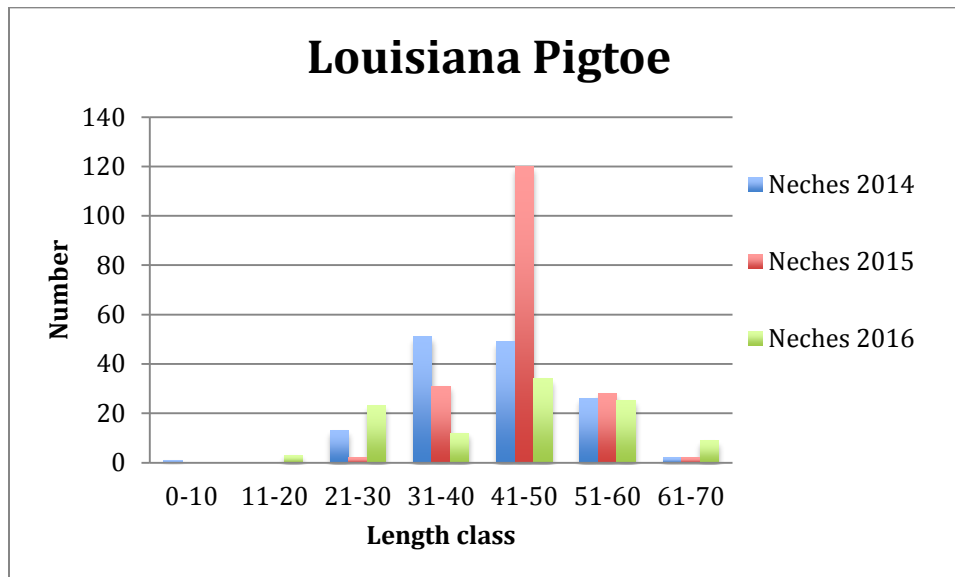

Fig. 19. Size classes of Louisiana Pigtoes from the Neches River in 3 different years.

Very small individuals of Texas Pigtoes were collected in the Sabine River in 2014 suggesting reproduction was successful that year (Fig. 20). Individuals in larger size classes were collected in the next two years also indicating that earlier years

had recruitment of juveniles. A number of dead Texas Pigtoes were collected during each survey. Juveniles were found during every visit; thus, the species appears to have high turnover each year. We did sample Texas Pigtoes in the Neches River in 2016 but there was less evidence of recruitment there (Fig. 21). The adult Texas Pigtoes in the Neches River were smaller in size and fewer dead individuals were found than in the Sabine River.
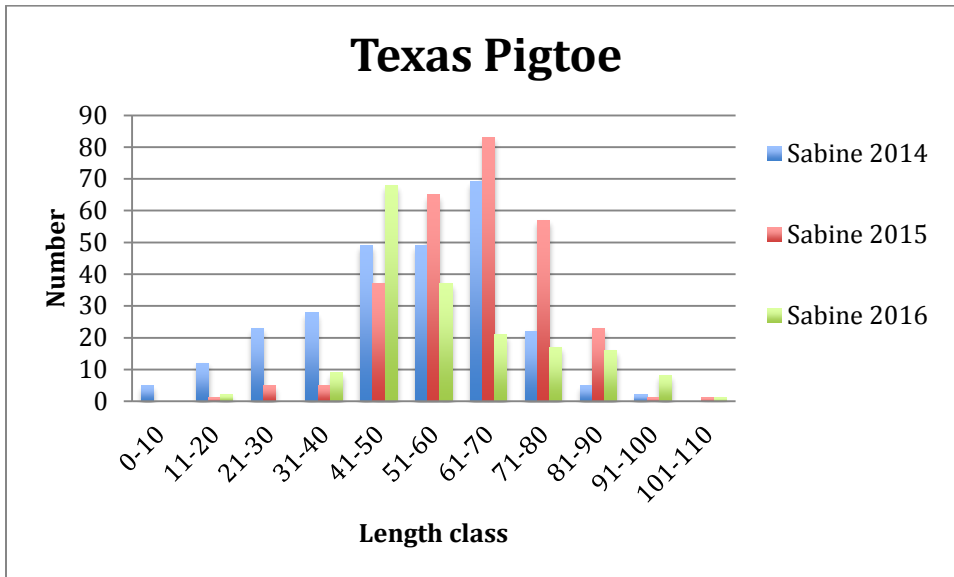

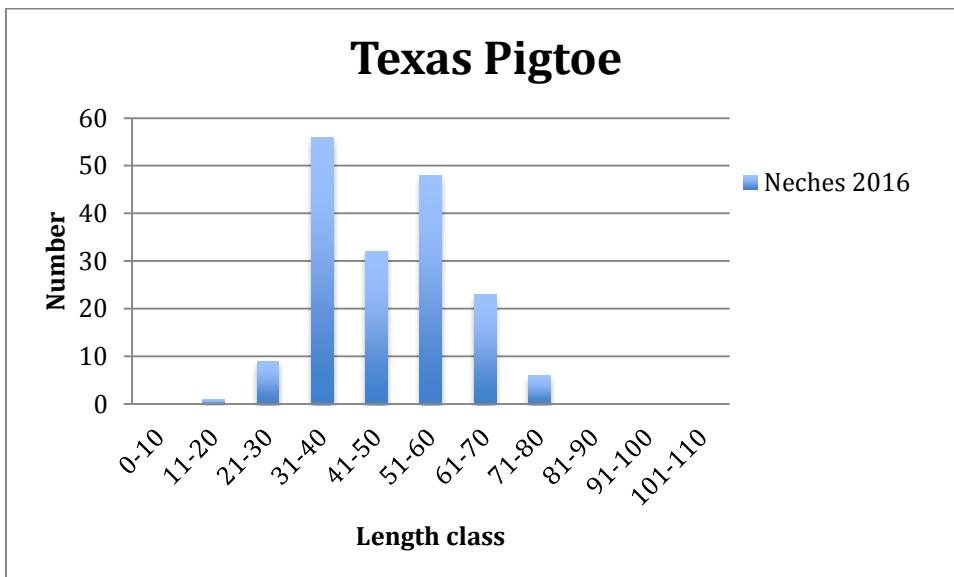Fig. 20. Size classes of Texas Pigtoes collected in the Sabine River in 3 years.

Fig. 21. Size classes of Texas Pigtoes from the Neches River in 2016.

Growth of marked mussels

Louisiana Pigtoes grew about 2.5 mm in length per year after reaching maturity (mature adults are estimated to be about 40 mm in length; Fig. 22). The smallest individual found was 2 mm in length. The only small individual marked that was recaptured was only recaptured once but it grew 15 mm in that one year. If all young specimens grow at the same rate they should reach 35 mm within 3 years.
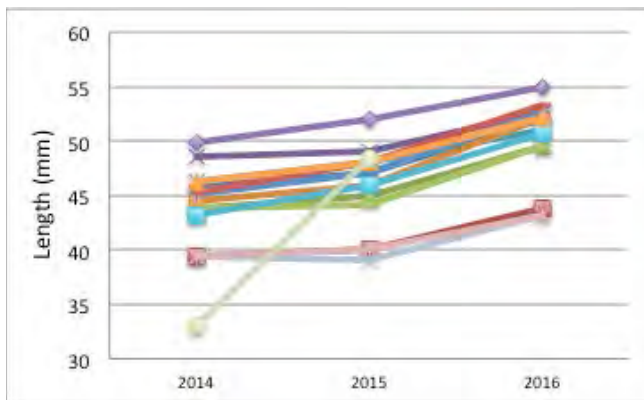

Fig. 22. Growth of Louisiana Pigtoes marked in 2014 and recaptured in either 2015 and 2016 from the Neches site 1.

Texas Pigtoe growth in the Angelina River was dependent on the initial size of the individual (Fig. 23). The largest Texas Pigtoes grew about 2.5 mm in length per year. Those at 35 mm in length grew 5 mm a year. The smallest individual recaptured was 30 mm in the first period and grew approximately 7 mm in the next year. Texas Pigtoes showed an initial age class of 30 – 40 mm, which could take about 3 or 4 years to achieve (Fig. 23). Examining the change in size classes from year to year suggests that individuals were growing into the next size class (approximately 10 mm larger) within a year.
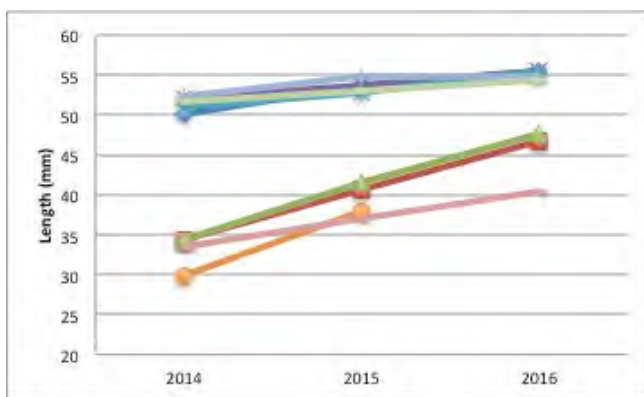

Fig. 23. Growth of Texas Pigtoes marked in 2014 and recaptured in either 2015 and 2016 from the Angelina site.

The Triangle Pigtoe was not found in enough numbers to place into length classes so conclusions as to recruitment and growth for this species are limited. We found no individuals in the smallest size classes. Most larger individuals had eroded shells and appeared to be older specimens. We also recorded several dead Triangle Pigtoes each year.

Nine Sandbank Pocketbooks were measured in both the Sabine and Neches Rivers. One small individual (less than 50 mm in length) was recorded in each and the rest varied in size up to 130 mm. Although rare it does seem to be recruiting juveniles within both the Sabine and Neches Rivers.

Seven Texas Heelsplitters from the Sabine River were measured, and two of the seven were small individuals, presumably juveniles (60 mm). This species is very rarely found alive, but young were present so recruitment does occur. Dead individuals are commonly found along the shore suggesting high mortality probably from raccoons (Walters and Ford, 2013).

Nineteen Southern Hickorynuts were measured with only one small individual (19 mm) recorded. This species is small, and juveniles are difficult to find although we were using a screen size that would have recorded much smaller individuals. This species is very restricted in its distribution; thus, if it is not recruiting in this area it could decline rapidly.

Hydraulic Components of the upper Sabine River

The hydraulic model was calibrated by running the flowrates taken from the USGS gages on the day the field data were collected; the depth recorded at the gage was compared to the two model data. It was found that both models calibrated with field depth and modeled depth differences ranging from 2.7% to 16%. The models were deemed applicable for larger analyses. It was found that at bridge crossing the data were not all that accurate as the LiDAR elevation data recorded the bridge height and not the land height, thus skewing the data. If depth, velocity, or shear stress data were needed at a bridge crossing then additional information would be needed, for example taking field surveys at the bridges.

In order to further calibrate the model the large 35-mile model was compared to the smaller model where field data were collected. It was found that channel profile was very similar between field data and stream channel creation from large-scale elevation data (DEM from LiDAR) (Fig. 24). The larger model is much longer across the channel because more data were available. During the field survey, points were taken from bank top to bank top, not for the full floodplain.
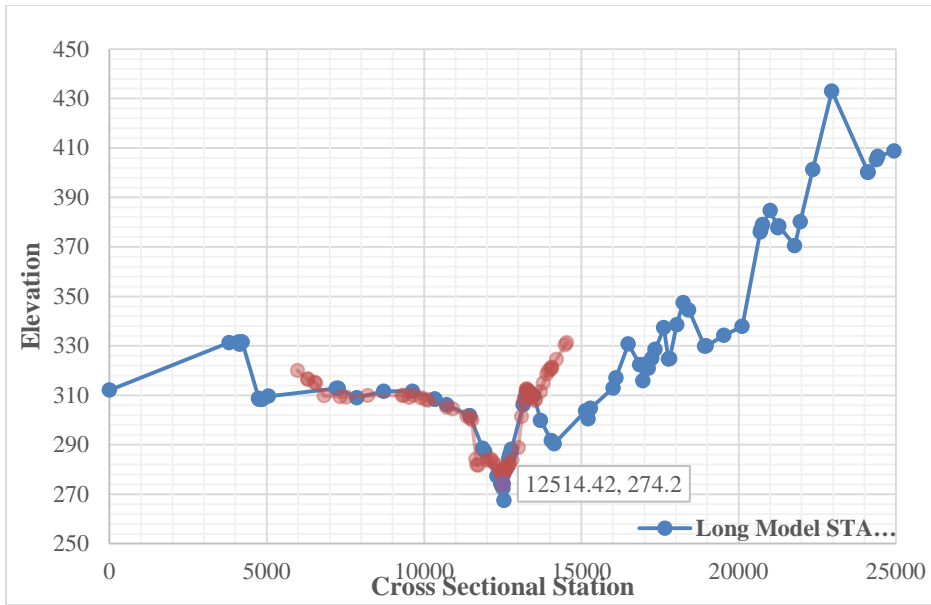
Fig. 24. Cross Section Stream Channel Comparison

The model was run at different flowrates associated with different flood return periods. This information was calculated from USGS gauge data. The higher the flowrate, the higher the channel velocity and shear stress. The shear stress at STA 779+14 was only 0.1 lb/sq ft at the 2-year flow, 0.25 lb/sq ft at the 10-year flow, and 0.4 lb/sq ft at the 100-year flow. The velocity at STA 779+14 was only 2.37 ft/s at the 2-year flow, 3.95 ft/s at the 10-year flow, and 5.37 ft/s at the 100-year flow. The shear stress and velocity did not increase linearly with downstream distance (Fig. 25). The more important factor in increasing shear stress was the channel sinuosity (i.e., bends in the channel). In addition, when tributaries enter the Sabine River, the channel width widens slowing the velocity and lowering the shear stress.
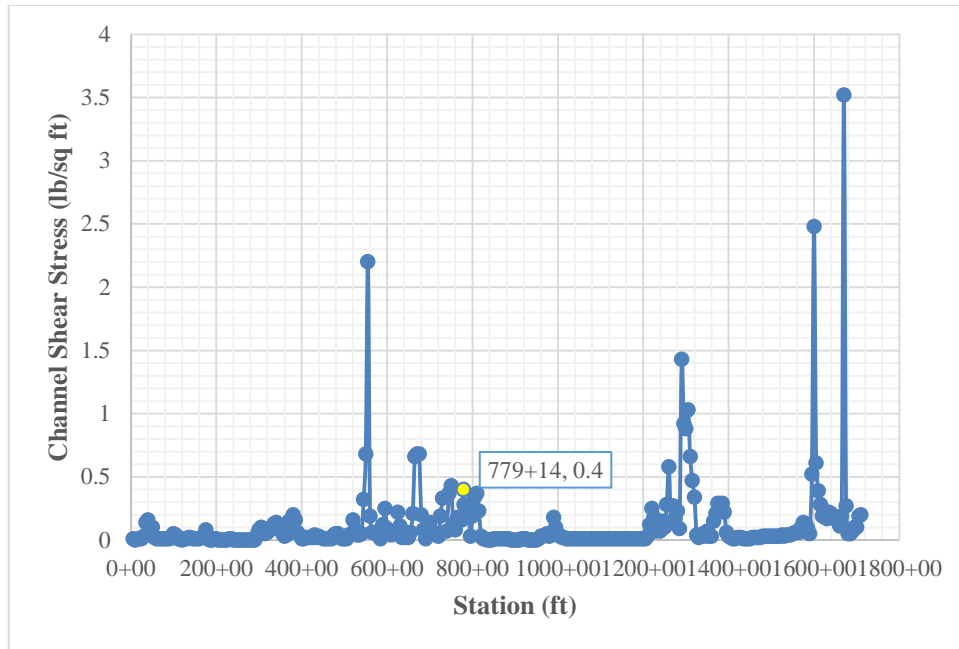
Fig. 25. 100-year Channel Shear Stress

The shear stress and velocity are related (Fig. 26), which correspond to results found by Allen and Vaughn (2010) who used field collected data to calculate shear stress. It is important to note that the shear stress and velocity are more directly related at lower shear stresses. Once the velocity and shear stress increase there are other factors that affect shear stress such as sediment type and size.
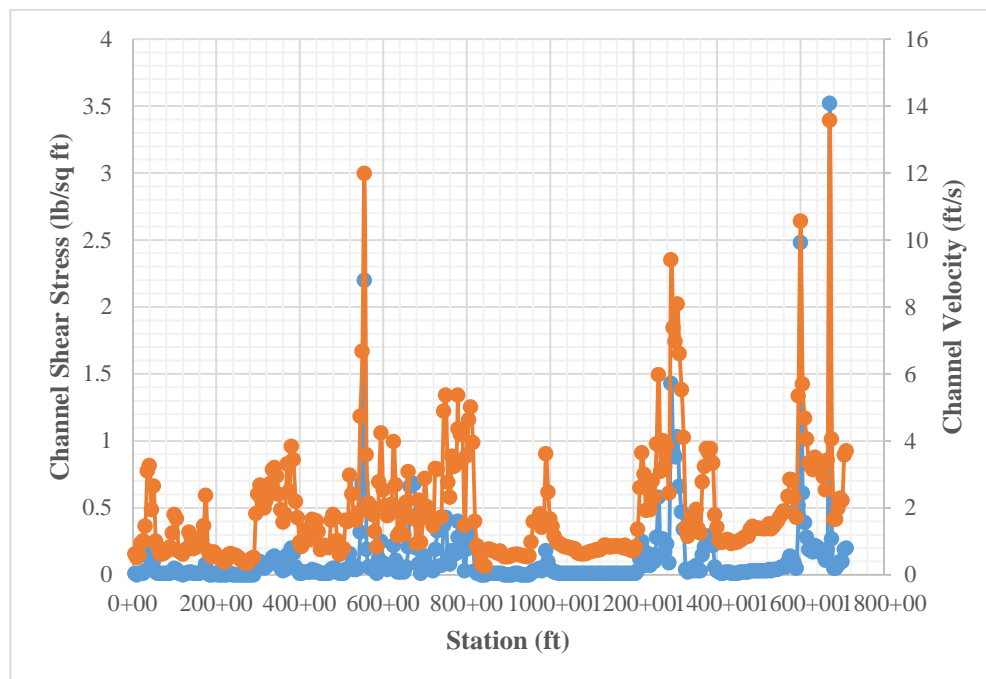

Fig. 26. 100 year Channel Shear Stress and Velocity Comparison

The HEC-RAS data were imported into ArcMAP using HEC-GeoRAS. The following maps are the 100-year storm shear stress and velocity profiles for the upper Sabine River. It is important to note that the floodplain is represented in both figures. The floodplain is modeled in HEC-RAS and can be seen as having a shear stress of 0. The red areas are typically in areas of bends that cause increases in shear stress (Fig. 27). The same can be seen with velocity where the velocity outside of the channel is near 0 and is higher in the channel. Also there are areas of higher velocity (red areas) that coincide with bends or obstructions in the river (Fig. 28). The smaller model is shown for comparison and for detail that is not available in the larger figures (Fig. 29). The smaller area has many bends and therefore higher shear stress. It can be seen that floodplains are represented as mainly zero for both shear stress and velocity. The 100-year flow is a higher storm event, where flooding would be expected, and can be seen where there some is shading outside of the river, particularly at bends.
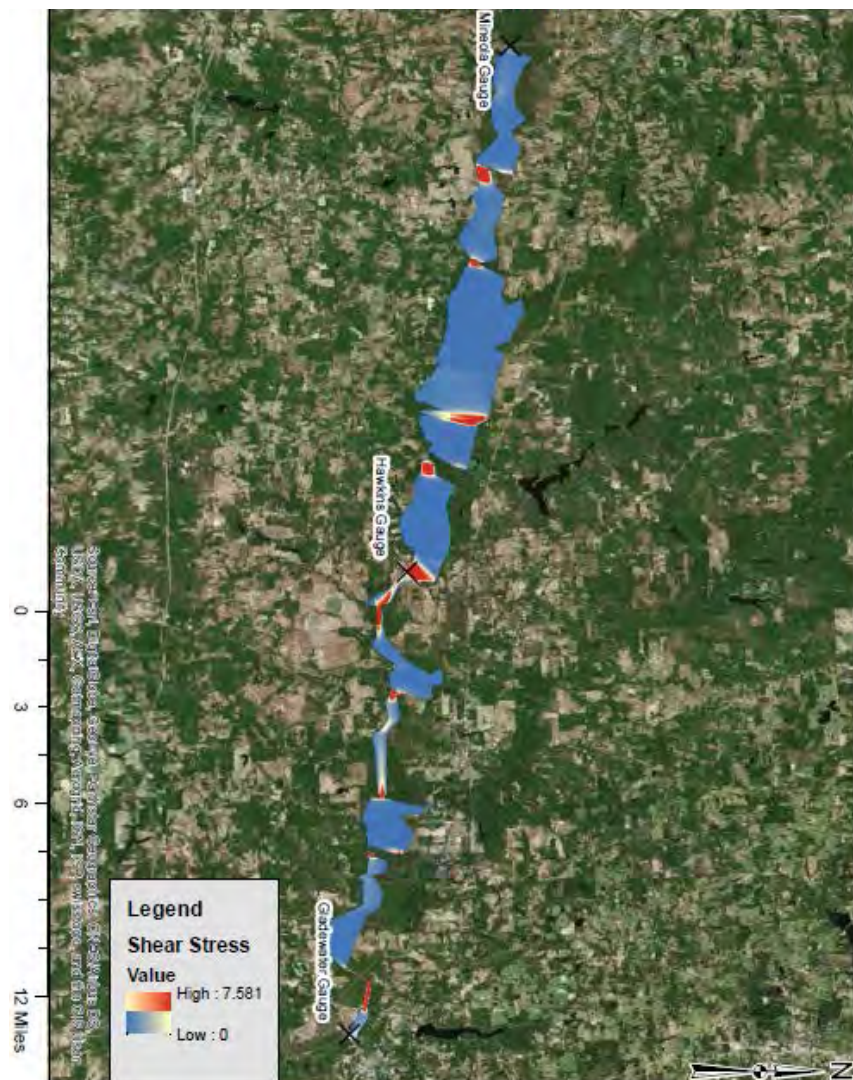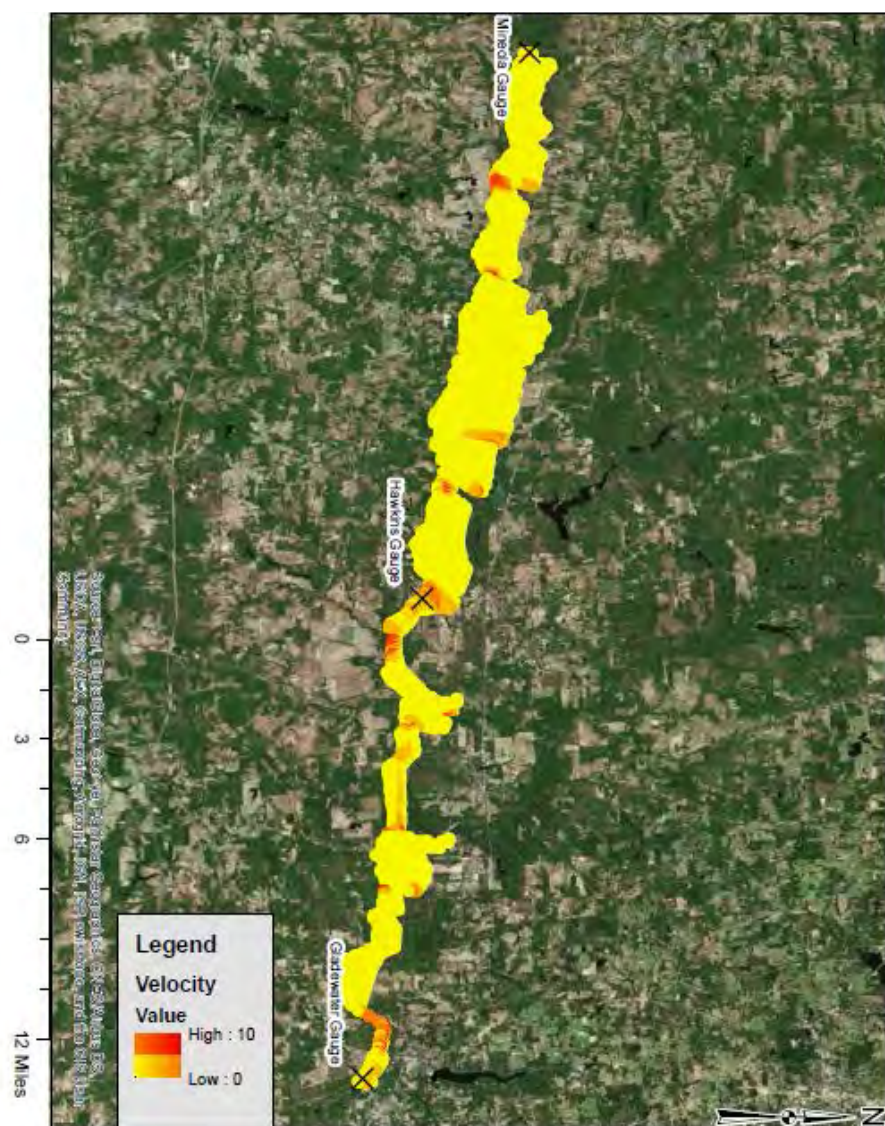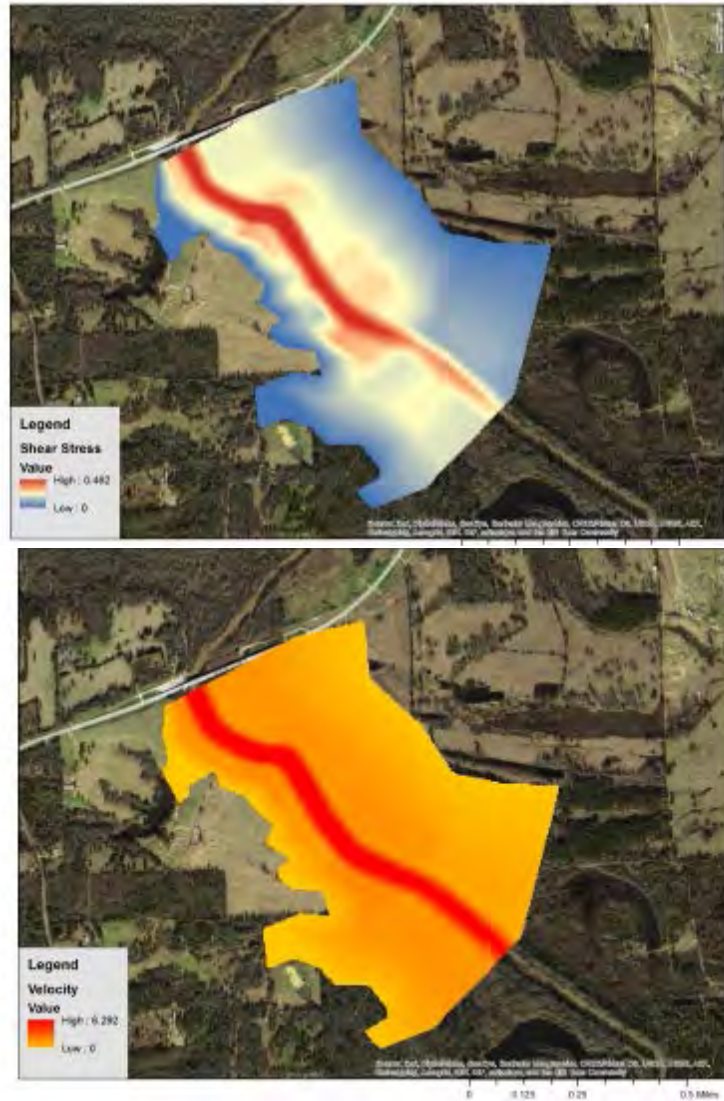

Fig. 27. 100-year shear stress

Fig. 28. 100-year velocity

Fig. 29. 100-year shear stress and velocity in the smaller model

Hydrologic Components of the Sabine River

Streamflow is the volume of water per time in a stream that passes a location (i.e., a gage station). Streamflow plays a direct role in aquatic ecosystems and geomorphology (USGS, 2003). In the stretch of the Sabine River upon which the hydraulic model was performed there are three United States Geological Survey (USGS) stream gages. The farthest upstream gage is Near Mineola, TX and is USGS site number 08018500; one gage is near the FM 14 in Hawkins, TX and is USGS site number 08019200; and the furthest gage downstream used in this study was near Gladewater, TX and is USGS site number 08020000. The USGS operates the National Water Information System (NWIS), which contains current and historical data for streams collected at USGS gaging stations (http://nwis.waterdata.usgs.gov/nwis), including the peak streamflow which was used in this study. Information pertaining to the three gage stations was collected from the aforementioned USGS NWIS

website (Table 6). All years are water years that begin on October 1 and end September 30 of the water year (i.e., 3 months are from the calendar year prior to the recorded water year).

Table 6. Information on Sabine River USGS gage stations.

USGS Gage Station Number	08018500	08019200	08020000
Site	Near Mineola, TX	Near Hawkins, TX	Near Gladewater, TX
County	Wood	Wood	Gregg
Hydrologic unit code	12010001	12010002	12010002
Latitude (NAD27)	32°36'49"	32°33'35"	32°31'37"
Longitude (NAD27)	95°29'08"	95°12'23"	94°57'36"
Drainage area, miles^2	1,357	2,259	2,791
Gage datum, ft (NGVD29)	304.16	266.59	243.85

Recurrence Intervals

Recurrence intervals, also called return periods or the frequency, is the probability of that event occurring in a given year. Recurrence intervals are calculated by plotting stream flow data versus the plotting position. Streamflow data are ordered greatest to lowest value and a rank is assigned to each value based on its position (i.e., the largest streamflow is assigned the rank, m, of 1). The Recurrence interval was determined using the Weibull method shown in Equation 1 (Viessman and Lewis, 2003). The return period was determined using an annual series in which the maximum value for each year was used (Viessman and Lewis, 2003).

$$T = \frac{n+1}{m} \hspace{3cm} \text{Equation 1}$$

Where: T = Recurrence interval
             n = Number of values (i.e. number of years studied)
             m = Rank of descending values, with largest equal to 1

The data were plotted as the T on the x-axis and stream flow, cfs on the y-axis. This was done in Microsoft Excel and a best-fit line was used to determine an equation for the streamflow in terms of recurrence interval. This equation was further used to calculate streamflow rates at specific frequencies of interest: 2, 10, and 100-year storms. The fits were logarithmic functions (Table 7).

Table 7. Recurrence Interval by Stream Gage in the Sabine River.

USGS Gage Station Number	08018500	08019200	08020000
Site	Near Mineola, TX	Near Hawkins, TX	Near Gladewater, TX
Equation*	y = 16726ln(x) + 230.38	y = 11237ln(x) + 2047.7	y = 20898ln(x) - 1039.4
2-year storm, cfs	11,824	9,837	13,446
10-year storm, cfs	38,743	27,922	47,080
100-year storm, cfs	77,256	53,796	95,199
Data years used (total number of years)	1938-2015+ (69)	1998-2015 (18)	1933-2015 (83)

*Where y is the stream flow in cubic feet/second and x is the recurrence interval in years.
+does not include year 1939, 1960-1967 as the data were unavailable

7-day low flows

The 7-day low flow of a stream is defined as the minimum average discharge for 7-consecutive days. The 7-day low flow with a recurrence interval of 10 years (7q10) is used to determine contamination inputs in Texas and the 7-day low flow with a recurrence of 2 years (7q2) is typically used to allocate wastewater discharge permits (Rifai et al., 2000). The 7q10 is also used as an indicator criterion to protect aquatic life during droughts and as a chronic criteria for aquatic life (Pryce, 2004). Low flow stream properties can be calculated directly from daily mean discharge data if the stream is monitored via a gage station. It is recommended to use at least ten consecutive years of data (Raines and Asquith, 1997). The return period can be calculated in the same manner as the recurrence intervals for stream flowrates.

The data used in this analysis were obtained from the same USGS website previously described, however not as many years were available for daily average streamflow as compared to the peak annual streamflow (Table 8).

Table 8. 7-day low flows for three sites on the Sabine River.

USGS Gage Station Number	08018500	08019200	08020000
Site	Near Mineola	Near Hawkins	Near Gladewater
Equation*	y=-3.694(lnx) +9.3015	y=-27.68(lnx) +74.603	y=-23.05(lnx) +76.76
7q2, cfs	6.74	55.42	60.78
7q10, cfs	0.79	10.87	23.68
Data years used (total #)	1996-2015 (20)	1998-2015 (18)	1996-2015 (20)

*Where y is the stream flow in cubic feet per second and x is the recurrence interval in years

Particle size distribution

Sediment samples were collected from the Sabine River in plastic zip-closure bags and returned to the laboratory for a sieve analysis. The samples were an aggregate of sediment in the area. Sieve sizes used for characterization of sediments were: 0.5 inch (12.7 mm); #4 (4.75 mm); #40 (0.43 mm); #100 (0.15 mm); #200 (0.075 mm); and a pan (<#200). The samples were oven dried at 110 degree Celsius for at least 24 hours prior to analysis. Five samples were collected: 2 downstream of Highway 31; 2 upstream of Highway 42; 1 above Highway 79.

The D50 for the Highway 31 samples were 0.33 mm and 0.29 mm. The D50 for the Highway 42 sites was 1.6 mm and 4.5 mm. The D50 at Highway 79 was 1.2 mm. These results show good correlation in samples taken in relatively close proximity. The results also show that the sediment particles appear to be increasing in size with downstream progression.

Water Quality

Water samples were taken on September 2, 2016 and analyzed in the field. Samples were taken at locations near public boat ramps on Highway 14, Highway 271 and Highway 43. The USGS gauges located in this region of the Sabine River do not collect water quality data; therefore, there is no water quality information readily available.

Table 9. Water quality values at three sites on the Sabine River.

Parameter	Highway 14	Highway 271	Highway 43
Turbidity, NTU	38.4	36.9	34.4
Nitrate, mg/L	0.6	0.0	0.0
Phosphate, mg/L	0.27	0.21	0.18
pH	8.88	8.66	8.97
Conductivity, µS	360	262	404
ORP, mV	146	243	278
Temperature, °C	29.2	32	32.4

These results show that in general water quality is increasing with downstream progression as the first three parameters in the above table decreased at downstream locations (Table 9). These are interesting results as it is expected that there would be more fertilizer indicators (nitrate and phosphate) as more land area drains into the river, but that was not found.

These results need to be ground-truthed with field surveys of mussels before conclusions about the applicability to identifying suitable habitat. The method also need to be tested in other reaches.

Ecological Niche Modeling

For the individual models of the four species, the test AUC values (based on the set-aside test points used for verification) ranged from 0.9598 – 0.9949, which is considered sufficiently useful to identify suitable habitats for these species (Elith, 2002).
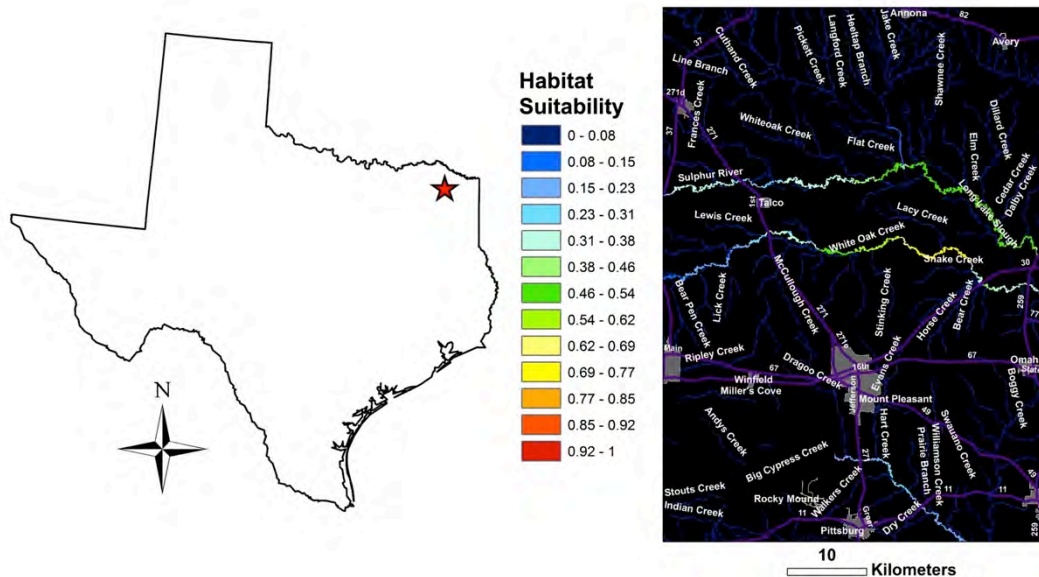

Fig. 30. Habitat suitability scores for Texas Pigtoe, *Fusconaia askewii,* at the indicated location. Municipalities are indicated in grey, and roads are indicated in purple. Rivers are indicated by a range of colors corresponding to the habitat suitability at that location. Refer to the legend in the figure.

Fusconaia askewii. See the "*Fusconaia askewii* Maxent results" layer of the ArcGIS Map Package "Comptroller results.mpk" to interactively view habitat suitabilities of the streams and rivers for this species and see how they intersect with major roads and municipalities. Areas of high suitability were found on the middle stretches of the Neches, Angelina, Attoyac, and Sabine Rivers and their tributaries, which is not surprising, considering that sampling was concentrated in those areas. More surprising is that the Trinity River was not particularly suitable habitat for Texas Pigtoe, even though we found some specimens there. There were areas of relatively high habitat suitability predicted for Texas Pigtoes in areas that were not sampled along the Sulphur River's tributary, White Oak Creek, near Mt. Pleasant, TX (Fig. 30), as well as the area near the confluence of Black Cypress Bayou and Big Cypress Bayou near Jefferson, TX. There is also a small area of relatively high habitat suitability along Pine Island Bayou, a tributary of the lower Neches River, along the
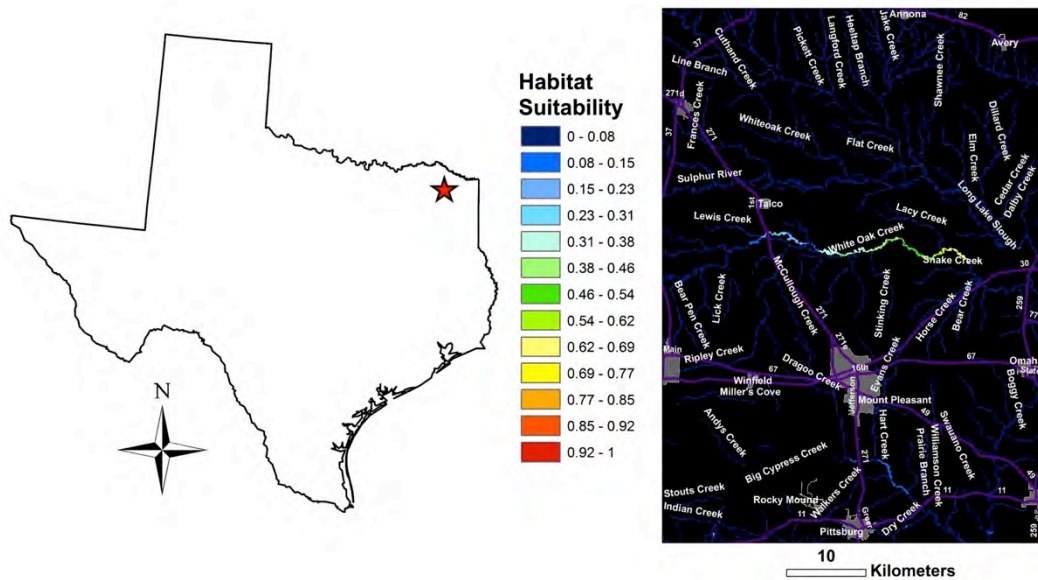
border with Beaumont, TX.

Fig. 31. Habitat suitability scores for *Lampsilis satura* at the indicated location. Municipalities are indicated in grey, and roads are indicated in purple. Rivers are indicated by a range of colors corresponding to the habitat suitability at that location. Refer to the legend in the figure.

Lampsilis satura. See the "*Lampsilis satura* Maxent results" layer of the ArcGIS Map Package "Comptroller results.mpk" to interactively view habitat suitabilities of the streams and rivers for this species and see how they intersect with major roads and municipalities. Like *P. amphichaenus*, high habitat suitabilities for *L. satura* are generally relegated to larger rivers/tributaries with greater flow volumes. These relatively high habitat suitabilities are found in the middle and lower Neches River, as well as the Sabine River. The same section of White Oak Creek mentioned above (Fig. 31) is also suggested as relatively highly suitable habitat for *L. satura*, even though we did not sample there.
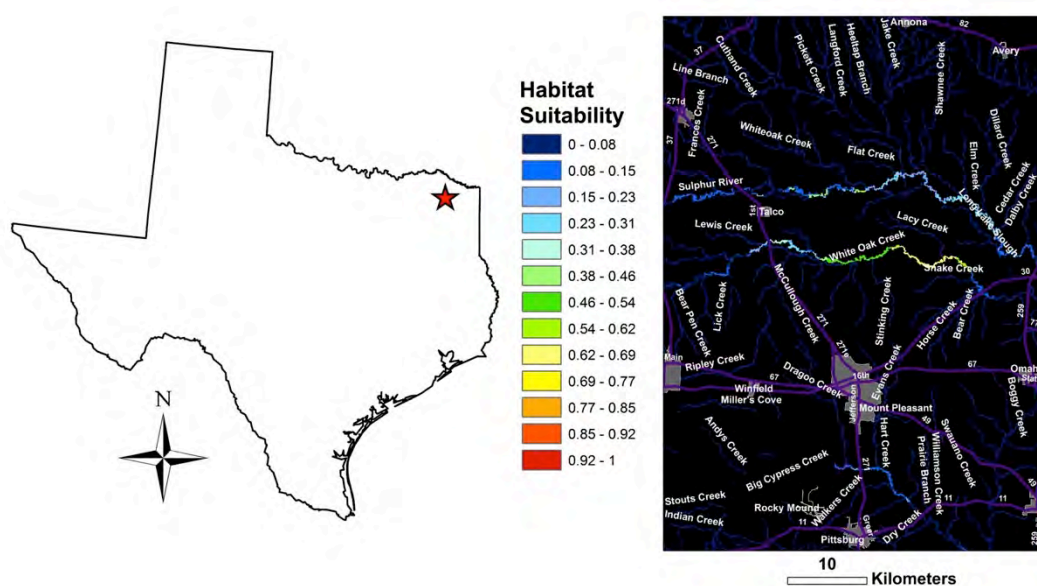
Fig. 32. Habitat suitability scores for *Potamilus amphicaenus* at the indicated location. Municipalities are indicated in grey, and roads are indicated in purple. Rivers are indicated by a range of colors corresponding to the habitat suitability at that location. Refer to the legend in the figure.

Potamilus amphichaenus. See the "*Potamilus amphicaenus* Maxent results" layer of the ArcGIS Map Package "Comptroller results.mpk" to interactively view habitat suitabilities of the streams and rivers for this species and see how they intersect with major roads and municipalities. High habitat suitabilities for *P. amphichaenus* are relegated to larger rivers/tributaries with greater flow volumes. These relatively high habitat suitabilities are found in the middle and lower Neches River, as well as the Sabine River. The same section of White Oak Creek mentioned above is also suggested as relatively highly suitable habitat for *P. amphichaenus* (Fig. 32), even though we did not sample there, as well as Twelvemile Bayou on the Louisiana side of the border (not shown).

Pleurobema riddellii. See the "*Pleurobema riddellii* Maxent results" layer of the ArcGIS Map Package "Comptroller results.mpk" to interactively view habitat suitabilities of the streams and rivers for this species and see how they intersect with major roads and municipalities. *P. riddellii* seems to prefer areas with intermediate flow volume, and does not appear to be as tolerant of higher flow volumes as compared to *L. satura* or *P. amphichaenus*. *P. riddellii*'s highest habitat suitabilities are on the middle sections of the Neches and Angelina Rivers, as well as the Attouyac River.

Obovaria arkansasensis. See the "*Obovaria arkansasensis* Maxent results" layer of the ArcGIS Map Package "Comptroller results.mpk" to interactively view habitat

suitabilities of the streams and rivers for this species and see how they intersect with major roads and municipalities. *O. arkansasensis* seems to have narrower environmental tolerances as compared to the other species. Areas of relatively high habitat suitability are scattered within the middle Neches and Angelina Rivers, as well as Big Cow Creek, a tributary of the lower Sabine River. There are also spots of high habitat suitability on the lower Trinity River near Coldspring, TX and near Goodrich, TX. There is also one spot of relatively high habitat suitability on the Attoyac River near Chireno, TX. Interestingly, many of these pockets of highly suitable habitat were found in rivers where we have not yet found this species. We have only recorded it so far in the middle Neches River and in a tributary of the lower Neches River, Village Creek, located near Silsbee, TX.

Fusconaia lananensis. See the "*Fusconaia lananensis* Maxent results" layer of the ArcGIS Map Package "Comptroller results.mpk" to interactively view habitat suitabilities of the streams and rivers for this species and see how they intersect with major roads and municipalities. The highest habitat suitabilities for this species were concentrated in southeast Texas, although areas of relatively highly suitable habitat were also found along certain reaches of the middle Neches, Angelina, and Attouyac Rivers. The highest habitat suitabilities were found in southeast Texas at the following locations: (1) the east fork of the San Jacinto River and its tributaries; (2) the lower Trinity River and its tributaries; (3) Pine Island Bayou and its tributary, Little Pine Island Bayou, both of which feed into the lower Neches River; (3) Village Creek, a big tributary of the lower Neches River, and its tributaries; (4) a few segments of the lower Neches River itself; and (5) two tributaries of the lower Sabine River, Cypress Creek and Big Cow Creek, but not the lower Sabine River itself. *F. lananensis* seems to prefer larger rivers with greater flow volume, as compared to its close relative *F. askewii*. Interestingly, many of these areas of highly suitable habitat were found in rivers where we have not yet found this species. We have only recorded it so far in the middle Angelina River, the Attoyac River, Village Creek (a tributary of the lower Neches River), and Pine Island Bayou.

Both the hydraulic analysis and the niche models can be used to define areas where future surveys for particular species should be concentrated.

Threats **- Oil and gas development**. The landscape level layer for oil and gas development was not associated with the habitat suitability of the mussels we modeled in this study. Figures 33-38 illustrate that the ecological niche models for each of the mussel species were unaffected by whether a layer representing oil and gas wells were included in the model or not. This means that oil and gas development over a landscape is not negatively associated with the distribution of mussels included in our study. However, this does not mean that at the local scale there is no impact. That would require associating sampling with individual wells.
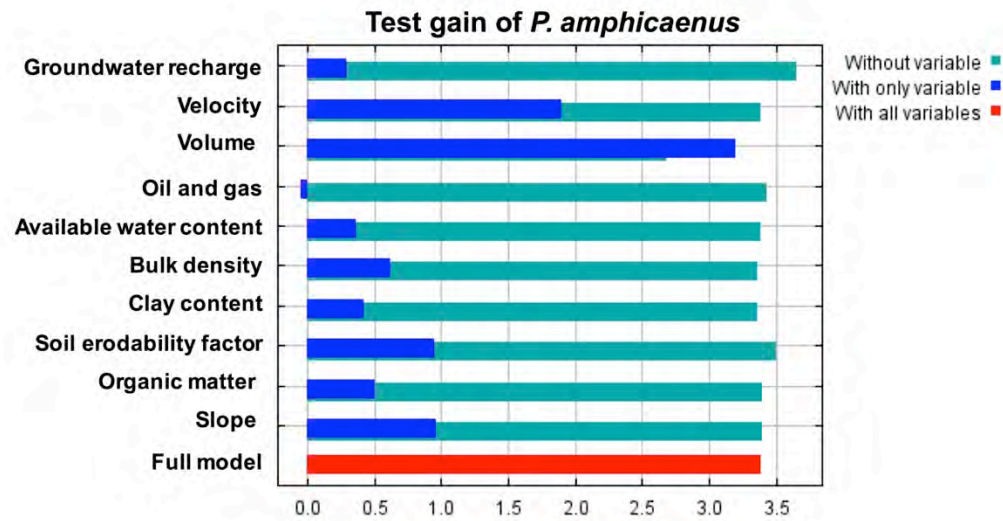
Fig. 33. Test gain of the full model for *Potamilus amphicaenus* (red) as well as when modeled with only the indicated variable (dark blue) or when modeled without the indicated variable (light blue). The closer the test gain of the model with only the indicated variable to the full model, the more that variable contributes to the habitat suitability model.
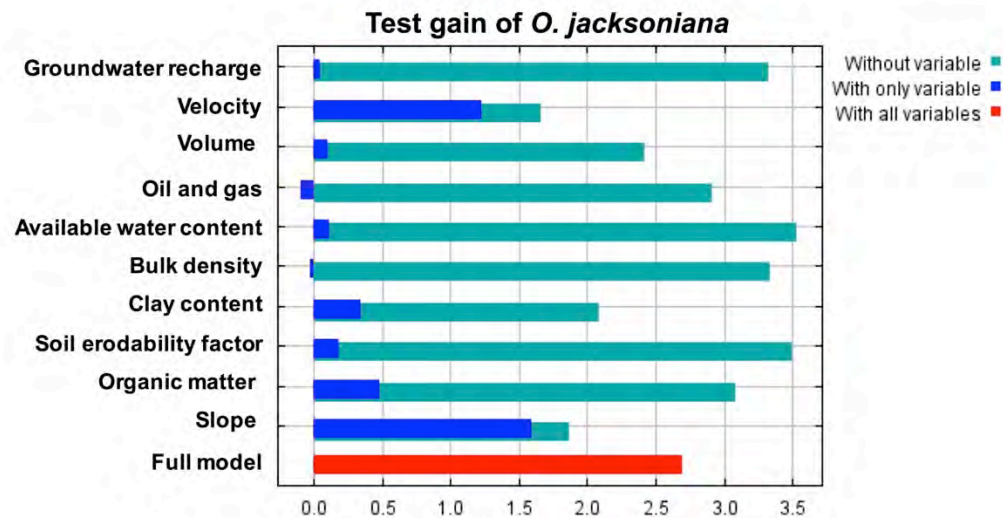

Fig. 34. Test gain of the full model for *Obovaria arkansasensis* (red) as well as when modeled with only the indicated variable (dark blue) or when modeled without the indicated variable (light blue). The closer the test gain of the model with only the indicated variable to the full model, the more that variable contributes to the habitat suitability model.
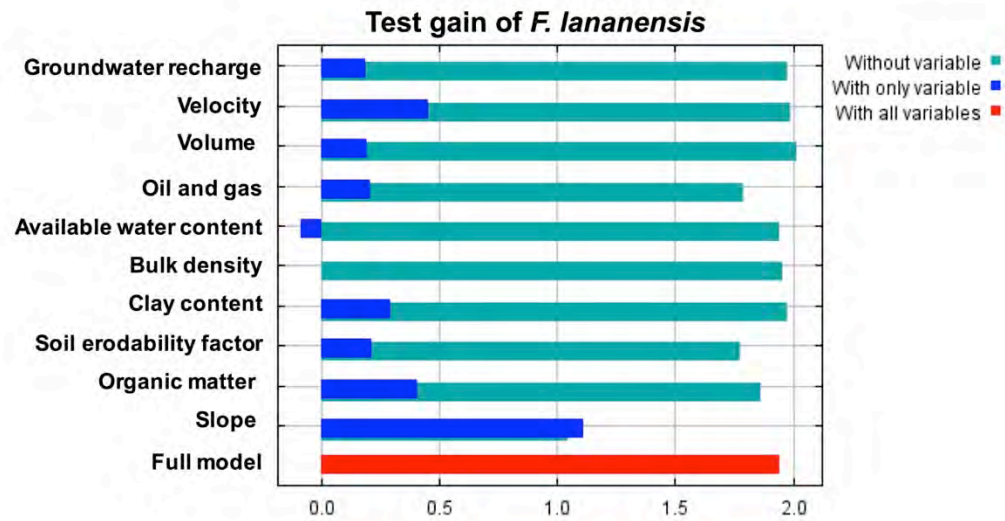
Fig. 35. Test gain of the full model for *Fusconaia lananensis* (red) as well as when modeled with only the indicated variable (dark blue) or when modeled without the indicated variable (light blue). The closer the test gain of the model with only the indicated variable to the full model, the more that variable contributes to the habitat suitability model.

Fig. 36. Test gain of the full model for *Lampsilis satura* (red) as well as when modeled with only the indicated variable (dark blue) or when modeled without the indicated variable (light blue). The closer the test gain of the model with only the indicated variable to the full model, the more that variable contributes to the habitat suitability model.

Fig. 37. Test gain of the full model for *Lampsilis satura* (red) as well as when modeled with only the indicated variable (dark blue) or when modeled without the indicated variable (light blue). The closer the test gain of the model with only the indicated variable to the full model, the more that variable contributes to the habitat suitability model.
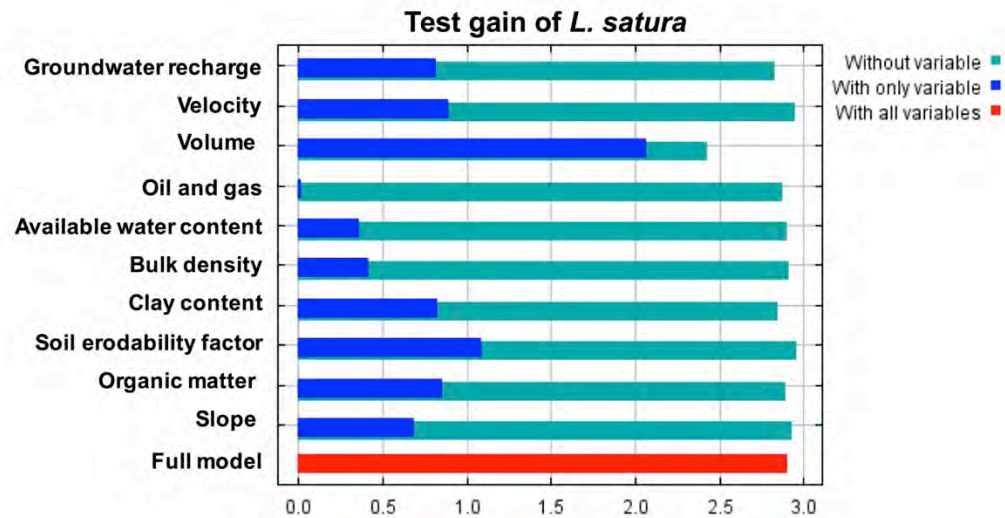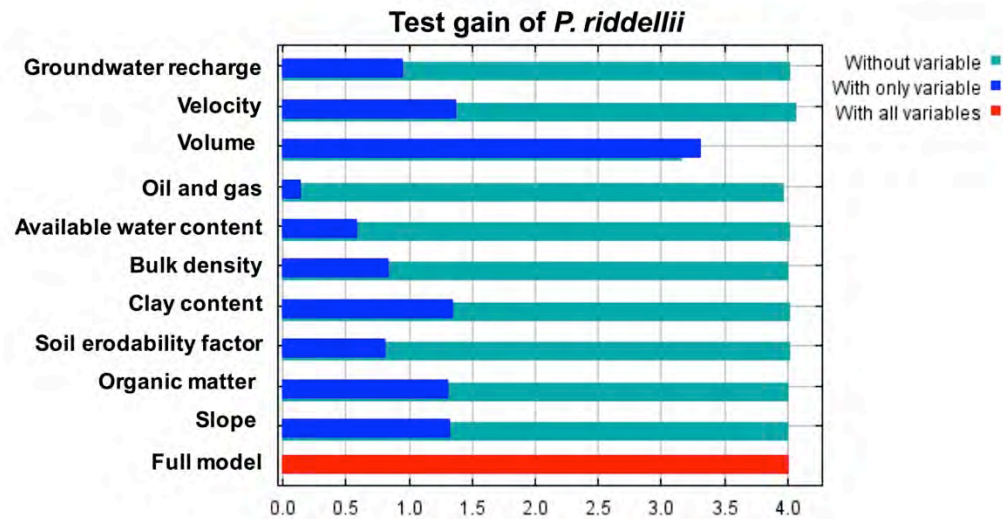

Fig. 38. Test gain of the full model for *Fusconaia askewii* (red) as well as when modeled with only the indicated variable (dark blue) or when modeled without the indicated variable (light blue). The closer the test gain of the model with only the indicated variable to the full model, the more that variable contributes to the habitat suitability model.

Threats – **Dams**
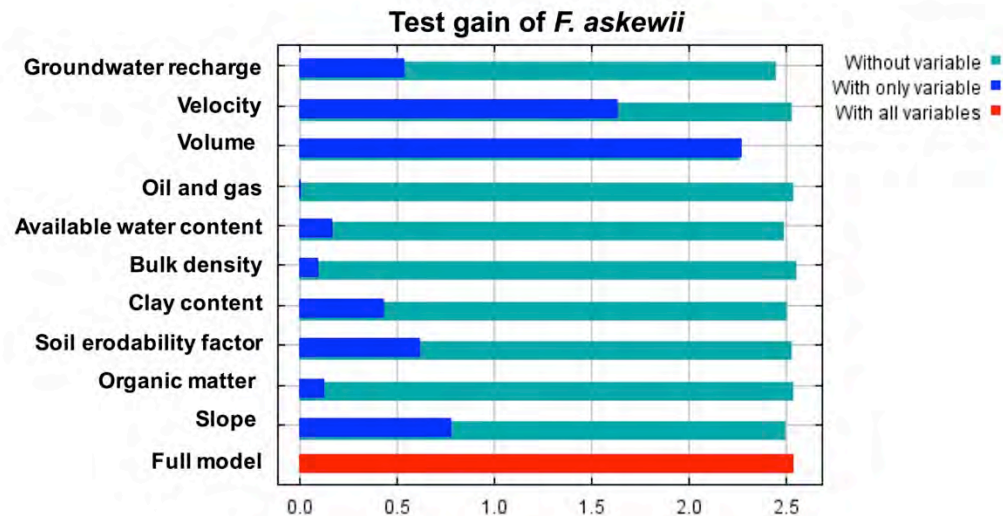
Mussels are known to be impacted downstream of reservoirs (Ranklev, *et al.*, 2015). In the two drainages where we sampled the most extensively below the reservoirs and along the mainstems (Neches and Sabine Rivers) we measured the distance

from the dam (river kilometers) where we began to collect live specimens of each of the threatened species (Table 10). It does appear that dams have an impact on the threatened mussel species with populations being extirpated several kilometers below the dam. The Sandbank Pocketbook seems to be most sensitive to this type of disturbance (>8.5 river km from the dam). In addition, Southern Hickorynuts and Texas Pigtoes may be sensitive to the presence of dams, in particular hydroelectric dams (B.A. Steinhagen), because they are found a considerable distance below Lake Palestine (5.0 and 7.7 river km, respectively) and are not found in the mainstem below Lake B.A. Steinhagen. Texas Heelsplitters were found a considerable distance below Lake Palestine (7.4 km); however, they appear to be able to handle the softer sediments accumulated below Lake B.A. Steinhagen as they are found within 3.2 river km of the dam.

Table 10. Hand digitized estimates of distance from dams in river kilometers of each state threatened species in the Neches and Sabine River watersheds.

Species	Distance below Lake Tawakoni (Sabine River)	Distance Below Lake Palestine (Neches River)	Distance Below Lake B.A. Steinhagen (Neches River)
Louisiana Pigtoe		2.3 km	6.7 km
Sandbank Pocketbook	65 km	8.5 km	26.3 km
Southern Hickorynut		5.0 km	
Texas Heelsplitter	60 km	7.4 km	3.2 km
Texas Pigtoe	80 km	7.7 km	

Threats – **Exotic Species.**

Reports of zebra mussels have been confirmed in the lakes and rivers of the upper Trinity River and Red River watersheds in Texas. A confirmed sighting of zebra mussels in Lake Livingston on the lower Trinity River also occurred in 2016. There has been one verified report of a zebra mussel found in Lake Fork on the headwaters of the Sabine River in Texas (Figure 39; TPWD, 2016; USGS 2016). During our roughly 10 years of sampling east Texas rivers for threatened mussel species we have not encountered physical evidence of zebra mussels in east Texas rivers (including our recent surveys below Lake Fork on Lake Fork Creek).

A more extensive monitoring program using the presence of zebra mussel DNA in water samples may be necessary to indicate if zebra mussels are beginning to spread to east Texas rivers where they have been previously unreported. This type of monitoring was beyond the scope of this project.
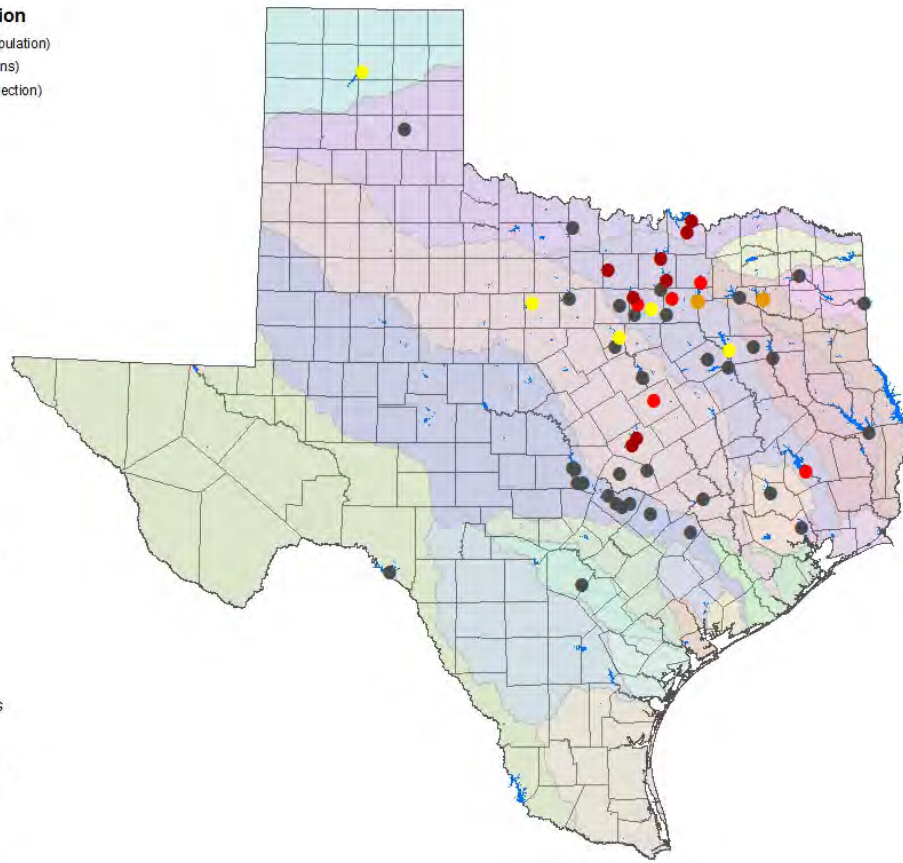
Figure 39: This graphic was borrowed from the website of Texas Parks and Wildlife Department (TPWD, 2016) to show the status of zebra mussels in Texas as reported in August of 2016.

Genetics

Texas Heelsplitter and Pink Papershell Genetics

DNA from 6 Texas Heelsplitters from the Neches and Sabine Rivers, 6 Pink Papershells from the Sulphur River, and 1 possible Texas Heelsplitter/Pink Papershell hybrid from Lake Lewisville were sequenced for the mitochondrial gene *ND1*. The Pink Papershell and Texas Heelsplitter are not known to co-occur in the Neches, Sabine, or Sulphur Rivers providing "pure" sources of each; however, a putative hybrid was collected in an area where the two are sympatric. Upon sequencing of its DNA, the suspected hybrid exhibited a mix of Texas Heelsplitter and Pink Papershell characteristics preventing us from positively identifying it as one of the two species in question. Its morphology also was a mixture of characteristics of the two species.

Eight hundred and twenty seven (827) basepairs (bps) were generated for each individual and showed a 6.17% divergence between Texas Heelsplitters and Pink Papershells, which is definitive in supporting that the two are well-diverged unique species (in most species divergence estimates of 3-4% are typically used as the minimum amount of divergence needed to support that two taxa are considered different species (e.g., Campbell and Lydeard, 2012). Given the substantial amount of diversity between these two species at this gene, an individual collected in the field will easily be able to be classified as either a Texas Heelsplitter or a Pink Papershell by amplifying this single gene. In fact, *ND1* typically acts as the barcoding gene in freshwater mussel studies, as the gene traditionally used for barcoding in animals does not express enough variation to be of use for unionids (Boyer et al. 2011). The cost of barcoding an individual for this gene to positively identify a specimen as one of these two species will be ~ $15 making this method cost effective in areas where the species are known to be sympatric.

Our hybrid specimen generally expressed Texas Heelsplitter characteristics, but shared four fixed differences with Pink Papershells indicating a mixed *ND1* haplotype, which supports that the individual in question is very likely a hybrid between the two species. Typically, mtDNA data is not as clearcut in determining hybridization, as mtDNA is maternally inherited in most animals meaning that the offspring of any hybridization event will have the same haplotype as the female in the cross. Mixed genetics resulting from hybridization, therefore, is not the norm in mtDNA data; however, freshwater mussels are extremely unique in having both paternal and maternal inheritance of mtDNA (Breton et al. 2007) permitting for some of the mitochondria to have paternally derived mtDNA and others to have maternally derived mtDNA resulting in haplotypes that appear to be a mix of the two. Because our 1 hybrid specimen does provide evidence that the two species can successfully hybridize and that only the Texas Heelsplitter is a candidate for Federal listing, the consequences of such hybridization events needs to be taken into account by conservation managers. Additional sampling to determine the extent of such hybridization is also called for to pinpoint Texas Heelsplitter populations that may already be compromised by Pink Papershell genetics versus those that may still be considered genetically "pure" Texas Heelsplitters.

Texas and Triangle Pigtoe Genetics

DNA from eight Triangle Pigtoes from the Angelina River and five Texas Pigtoes from the Neches River were sequenced for three mtDNA genes (*ND1*, *COX1*, *16S*) and one nuclear gene (*ITS1*) by one of our Graduate Research Assistants in partial fulfillment of her M.S. in Biology (Plants-Paris, 2016). We also had access to over two-dozen Triangle and Texas Pigtoe *ND1* sequences from the Neches River from a previous study conducted by our group (Marshall, 2014) and at least half a dozen GenBank sequences were available for comparison.

The *ND1* gene was successfully sequenced for a total of 14 samples (Table 11). The

other genes were less successfully sequenced with the *COX1* gene successfully sequenced for 7 individuals, the *16S* gene successfully sequenced for 10 individuals, and the *ITS1* gene successfully sequenced for 12 individuals (Table 11). The *COX1* gene could not be sequenced from the Texas Pigtoe samples obtained for this study. For this gene, two Texas Pigtoe *COX1* sequences were obtained from available GenBank sequences (Benson et al., 2005) and compared to our Triangle Pigtoe sequences for analysis. *ND1* sequences were trimmed to a length of 764 bases long and showed 0.39% divergence between Triangle and Texas Pigtoe sequences (Table 11). *COX1* sequences were trimmed to a length of 604 bases long and showed 0.99% divergence between the two (Table 11). The 16S sequences were trimmed to a length of 444 bases long and showed 1.35% divergence between Texas and Triangle Pigtoes (Table 11). Finally, *ITS1* sequences were trimmed to a length of 507 bases long and showed 0.80% divergence between the two Pigtoes in question (Table 11).

Table 11. Sample sizes and percent divergence values between Texas and Triangle Pigtoes for three mtDNA (*ND1*, *COX1*, *16S*) and one nuclear gene (*ITS1*).

	ND1	COX1	16S	ITS1
Number of Texas Pigtoes	5	2*	4	5
Number of Triangle Pigtoes	7	6	5	7
	Percent Divergence			
Texas Pigtoe/Triangle Pigtoe	0.39	0.99	1.35	0.80

Our data indicate that, based on this multi-gene sequence analysis, there is no support that Triangle and Texas Pigtoes are genetically diverged species. Typical minimum divergence estimates that would support two species as being distinct species would range between 3-4% (Campbell and Lydeard,, 2012). Comparing the data collected for this study and previous work done in our research group with a greater number of Pigtoes for only the *ND1* gene (Marshall, 2014), we found the same pattern. Even when increasing the sample size to over 2-dozen individuals, no significant difference in genetics can be detected between Triangle and Texas Pigtoes. In addition, preliminary data from other parts of the range of these two species is revealing no additional genetic variation. Therefore, our data suggest that these two Pigtoe species could be lumped together as one species; however, recent taxonomic revisions based solely on multi-gene sequence data have been criticized given the current availability of whole genome sequencing (e.g., Morrison, 2014). The logical next step, to definitively answer the question as to whether or not Triangle and Texas Pigtoes are genetically distinct species or not, will be to conduct a whole genome study.

Current Status of East Texas State Listed Mussels

Fusconaia lananensis, the Triangle Pigtoe, is no longer present in its type locality of

Lanana Creek. We found it only in a few sites in the Angelina River and the lower Neches. It was difficult to distinguish from the Texas Pigtoe, *F. askewi*. In the Angelina River both occurred together. This makes the evaluation of the current records difficult (including our MaxEnt niche model), as misidentifications of this species are very likely. It was rare in our surveys of both the Angelina River and Atoyac bayou. Most of the specimens were old and highly eroded. Historic land use in the area of its range has resulted in loss of vegetative cover and soils have increased siltation in the rivers. Overgrazing with cattle having direct access to the streams has also impacted this species habitat. Lumbering and general urbanization have cleared forests to the rivers edges in many locations. As many of the streams in which this species occurs are small, degradation of environmental conditions will have an increased impact relative to species in larger rivers. Our genetic results support other recent studies suggesting this species is not distinct from the Texas Pigtoe.

Fusconaia askewi, the Texas Pigtoe, was found in all the east Texas Rivers surveyed but was common only in the Sabine and Neches Rivers where it sometimes was abundant. Specimens from Cypress creek and the Sulphur and Trinity Rivers differed in morphology from the *F. askewi* collected from the Sabine and Neches Rivers and we are genetically testing to see if those are a separate species. The habitat in which most Texas Pigtoes have been found is shallow rocky riffles. Erosion of riverbanks is problematic in the Sabine River, and sand and silt have covered many mussel beds and large numbers of dead individuals were found at many sites. The Neches River is more connected to its floodplain and erosion is less problematic but feral hogs have become a serious issue. Hogs damage many miles of the banks of the Upper Neches River regularly. With no vegetation, organic matter and soil continually fills the streams at those locations. The organic matter decomposes and lowers oxygen levels which can kill mussels. The upper Neches has been proposed as a site for a reservoir in the recent past but plans have been stopped for its construction at the moment. We suggest this is the most important reach of any east Texas River to conserve for mussel habitat.

Lampsilis satura, the Sandbank Pocketbook, was found in all the larger east Texas streams. It appeared most common in sand substrates and as it was relatively mobile it seems to be dealing with the increasing siltation of our larger rivers. Although widely distributed it was uncommon everywhere and there appear to be no locations that exhibit significant populations. They were often the species with the lowest rank abundance. We did not find any in the Trinity and San Jacinto Rivers nor in the Sulphur River. It does seem to be susceptible to rapid water level changes after flooding events as we often found them stranded when waters came down quickly. The same anthropogenic effects that impact other species likely affect Sandbank Pocketbooks (i.e., pollution, erosion, and invasive species).

We found *Obovaria arkansasensis,* the Southern Hickorynut, only in a short reach in the Neches River and in Village Creek near Beaumont. This area is different from other areas of the Neches River in that it appears to be a habitat that is connected

with the floodplain (i.e., rivers with low banks). Most rivers of east Texas are being downcut by waters from dams and so are developing steeper banks. In addition, the cutting of trees and vegetation up to the edge of rivers then allows banks to fall into the river producing severe siltation. Mussels, which cannot move easily, are covered by siltation and die from lack of oxygen. We found several beds of mussels in which all were covered by silt and dead.

Pleurobema riddelli, the Louisiana Pigtoe, was very uncommon in all drainages with only the upper Neches River recording more than just a few specimens. In that area it can be as abundant as other species. However, in other rivers *P. riddellii* is so rare in all localities it may have been missed in passing surveys. For example, in our extensive surveying of the Sabine River, we have found 4 locations that had just one individual among many other mussels. Louisiana Pigtoe was found in a variety of habitats in the upper Neches River but most are associated with riffles of cobble and rocks. This species seems to be most susceptible to all the anthropogenic impacts of east Texas since the upper Neches is the most natural of all the streams.

The Texas Heelsplitter, *Potamilus amphichaenus*, was primarily found in bigger rivers such as the Sabine and Neches Rivers. It was also found in lakes in Dallas/Fort Worth. It was by far the rarest of the state-listed East Texas mussels. Its largest numbers were in the upper Sabine but nearly all of the specimens found were dead. It appears to live in sand in slow moving waters and so is exposed to predation by raccoons particularly because it is thin-shelled (Walters and Ford, 2013). Overgrazing, reduction in bank vegetation, scouring floods, and various pollution issues are the impacts that likely affect this species.

Conclusions and Additional Research Needs

As in most other states, mussels in east Texas are being impacted by factors that modify aquatic habitats, in particular, our rivers. The six state listed species for east Texas exemplify the problems of mussels throughout the state: a lack of data, issues with identification and little public understanding of their importance. Our 6 years of surveying mussels, along with our work trying to understand what factors are involved in their occurrence and survival, have improved our knowledge of how to conserve these important organisms. We suggest in particular that the following additional issues be addressed:

a. Because of their rarity and limited distributions we recommend that the Louisiana Pigtoe and Texas Heelsplitter be listed as Threatened by USFWF.

b. Because of the rarity of the Southern Hickorynut and Sandbank Pocketbook, additional research is needed on their distribution and abundance. They should remain State-Listed until these data is obtained.

c. Texas Pigtoe populations seem to be stable in several basins and probably does not need to be listed by the state; however, its relationship to the Triangle Pigtoe

and other *Fusconaia* in the state is still problematic. Genome-wide sequencing is the currently accepted method to validate species, and we recommend its use to determine if, as we suspect, the Triangle Pigtoe is not a separate species from the Texas Pigtoe.

d. To designate critical habitat for these mussels we need finer scale information than what MaxEnt produces. It is only useful for determination of large scale suitable habitat. The critical habitat for each species within those areas could be determined using side-scanning sonar combined with LiDAR analysis. It would also require ground-truthing with field surveys of mussels.

Literature Cited

Aiello-Lammens, M. E., Boria, R. A., Radosavljevic, A., Vilela, B. & Anderson, R. P. 2015. spThin: an R package for spatial thinning of species occurrence records for use in ecological niche models. *Ecography*, 38, 541-545.

Allen, Daniel C.; Vaughn, Caryn C. 2010. Complex hydraulic and substrate variables limit freshwater mussel species richness and abundance. Journal of the North American Benthological Society, 29 (2), 383-394.

Aggett, G.; Wilson, J. 2009. Creating and coupling high-resolution DTM with a 1-D hydraulic model in a GIS for scenario-based assessment of avulsion hazard in a gravel-bed river. Geomorphology, 113 (1-2), 21–34.

Arnason, A.N. and C.J. Schwarz. 1995. POPAN-4: enhancements to a system for the analysis of mark-recapture data from open populations. Journal of Applied Statistics, 22: 785-800.

Benson, D.A., I. Karsch-Mizrachi, D.J. Lipman, J. Ostell, and D.L. Wheeler. 2005. GenBank. Nucleic Acids Research 33 (Database Issue): D34-D38.

Bordelon, V. L. and R. Harrel. 2004. Freshwater mussels (bivalvia: unionidae) of the village creek drainage basin in southeast Texas. Texas Journal of Science. 56(1): 63-72

Boyer, S. L., A. A. Howe, N. W. Juergens, and M. C. Hove. 2011. A DNA-barcoding approach to identifying juvenile freshwater mussels (Bivalvia: Unionidae) recovered from naturally infested fishes. Journal of the North American Benthological Society 30:182-194.

Breton, S., H. D. Beaupre, D. T. Stewart, W. R. Hoeh, and P. U. Blier. 2007. The unusual system of doubly uniparental inheritance of mtDNA: isn't one enough? Trends in Genetics 23:465-474.

Burlakova L. E., D. Campbell, A. Y. Karatayev, and D. Barclay 2012. Distribution, genetic analysis and conservation priorities for rare Texas freshwater molluscs in the genera Fusconaia and Pleurobema (Bivalvia: Unionidae). *Aquatic Biosystems* 8(1):12

Burlakova, L. E., A. Y. Karatayev, V. A. Karatayev, M. E. May, D. L. Bennett, and M. J. Cook. 2011. Biogeography and conservation of freshwater mussels (Bivalvia: Unionidae) in Texas: patterns of diversity and threats. Diversity and Distributions: 17:393-407.

Campbell, D. C. and C. Lydeard. 2012. Molecular systematics of Fusconaia (Bivalvia: Unionidae Ambleminae. American Malacological Bulletin 30:1-17.

Crabtree, D.L. and T.A. Smith. 2009. Population attributes of an endangered mussel, *Epioblasma torulosa rangiana* (Northern Rifleshell), in French Creek and implications for its recovery. Northeastern Naturalist, 16: 339-354.

Dudik, M., S. J. Phillips, and R. E. Schapire. 2010. Maxent Version 3.3.3. http://www.cs.princeton.edu/~schapire/maxent/. Accessed November 20, 2012.

Dudgeon D., Arthington A., Gessner M., Kawabata, Z, Knowler, D., Leveque, C., Naiman, R., Prieur-Richard, A., Soto, D., Stiassny, M. and Sullivan, C. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81: 163–182.

Dunithan, A. 2012. Using ecological niche modeling to predict occurrence of rare fish and unionid mussels in East Texas. MS Thesis, UT Tyler.

Elith, J. 2002. Quantitative methods for modeling species habitat: Comparative performance and an application to Australian plants. In S. Ferson and M. Burgman (*Eds.*) *Quantitative methods for conservation biology* 39-58. New York: Springer-Verlag.

Ford, D. 2013. Ground truthing Maxent in East Texas rivers. MS Thesis, UT Tyler

Ford, N.B., L.R. Williams, and M.G. Williams. 2012. Surveys for threatened and endangered mussels and fishes in rivers of Northeastern Texas. Section 6 Grant, U.S. Fish and Wildlife Service and Texas Parks and Wildlife.

Ford, N. B., K. Heffentrager, D. F. Ford, A. Walters and N. Marshall. 2014. Significant recent records of Unionid mussels in Northeast Texas Rivers. Walkerana.

Grobler, P. J., J. W. Jones, N. A. Johnson, R. J. Neves, and E. M. Hallerman. 2011. Homogeneity at nuclear microsatellite loci masks mitochondrial haplotype diversity in the endangered fanshell pearlymussel (*Cyprogenia stegaria*). Journal of Heredity 102:196–206.

Hastie, L.C., Boon, P.J., Young, M.R. and S. Way. 2001. The effects of a major flood on an endangered freshwater mussel population. Biological Conservation, 98: 107–115.

Heffentrager, K. 2013. Utilizing Maxent to improve and explain a species distribution model for freshwater mussel species in East Texas. MS Thesis, UT Tyler.

Holland-Bartels LE. 1990. Physical factors and their influence on the mussel fauna of a main channel border habitat of the upper Mississipppi River. *Journal of the North American Benthological Society* **9**: 327-335.

Howells, R. G. 1997. Status of freshwater mussels (Bivalvia: Unionidae) of the Big Thicket Region of Eastern Texas. Tex. J. Sci. 49:21-34.

Howells, R.G. 2006. *Statewide Freshwater Mussel Survey: Final Report.* Report on file with Texas Parks and Wildlife Department, Austin, Texas.

Howells, R.G. 2010. Biological opinion: conservation status of selected freshwater mussels in Texas. 24 pp.

Howells, R.G. 2014. Field Guide to Texas Freshwater Mussels. 2nd edition. Biostudies, Kerrville.141 pp.

Howells, R.G., Randklev, C.R., and N.B. Ford . 2012. Taxonomic Status of Pigtoe

Unionids in Texas. Ellipsaria, 14: 11-15.

Jones, J. W., R. J. Neves and E. M. Hallerman. 2012. Population performance criteria to evaluate reintroduction and recovery of two endangered mussel species, *Epioblasma brevidens* and *Epioblasma capsaeformis* (Bivalvia: Unionidae). Walkerana 15: 27-44.

Karatayev, A.Y., and L.E. Burlakova. 2007. East Texas Mussel Survey. State Wildlife Grant submitted to Texas Parks and Wildlife Department, Austin.

Karatayev, A.Y., Burlakova, L.E., Miller, T.D., and M.F. Perrelli. 2015. Reconstructing historical range and population size of an endangered mollusc: long-term decline of *Popenaias popeii* in the Rio Grande, Texas. Hydrobiologia, 1: 1-17.

Marshall, N. T. 2014. Identification of potential fish hosts from wild populations of state-threatened East Texa freshwater mussels using a molecular Identification dataset. M.S. Thesis. Department of Biology, University of Texas at Tyler.

McCormack, J. E., S. M. Hird, A. J. Zellmer, B. C. Carstens, and R. T. Brumfield. 2013. Applications of next-generation sequencing to phylogeography and phylogenetics. Molecular Phylogenetics and Evolution 66:526-538.

Morrison, D. A. 2014. Next generation sequencing and phylogenetic networks. EMBnet.journal 20, e760. http://dx.doi.org/10.14806/ej.20.0.760

Nikora, V., & Goring, D. 2001. Spatially averaged open-channel flow over rough bed. Journal Of Hydraulic Engineering, 127(2), 123.

Ontario Ministry of Agriculture, Food, and Rural Affairs. 2015. Universal Soil Loss Equation (USLE). http://www.omafra.gov.on.ca/english/engineer/facts/12-051.htm (accessed December 12th, 2016).

Pearson, R. G., Raxworthy, C. J., Nakamura, M. and Peterson, A. T. 2007. Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography*, 34, 102-117.

Phillips, S. 2006. *A brief tutorial on Maxent*. AT and T Research. http://www.cs.princeton.edu/~schapire/maxent/tutorial/tutorial.doc (accessed August 7th, 2016).

Phillips, S. J., Dudik, M. 2008. Modeling of species distributions with e: new extensions and a comprehensive evaluation. *Ecography*, 31, 161-175.

Plants-Paris, E. D. 2016. Population dynamics and genetic differentiation of the threatened freshwater mussel species Fusconaia askewi, Fusconaia lananensis, and Pluerobema riddellii. M.S. Thesis. Department of Biology, University of Texas at Tyler.

Pryce, Rich. 2004. Hydrological low flow indices and their uses. WSC Report No. 04-2004, Watershed Science Center, Ontario, Canada.

R Development Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org (accessed August 7th, 2016).

Raines, Timothy H.; Asquith, William H. 1997. Analysis of minimum 7-day discharges and estimation of minimum 7-day, 2-year discharges for streamflow-gaging stations in the Brazos River Basin, Texas. United States Geological Survey Report 97-4117.

Randklev, C.R., B.J. Lundeen, J. Skorupski, J.H. Kennedy, and S. Wolverton. 2011. Toledo Bend relicensing project: lower Sabine River mussel study. Report on file with the Sabine River Authority, Orange, Texas.

Randklev, C.R., N. Ford, S. Wolverton, J.H. Kennedy, C. Robertson, K. Mayes, and D. Ford. 2015. The influence of stream discontinuity and life history strategy on mussel community structure: a case study from the Sabine River, Texas. *Hydrobiologia* 770: 279-292.

Rifai, Hanadi S.; Brock, Suzanne M.; Ensor, Katherine B.; Bedient, Philip B. 2000. Determination of low-flow characteristics for Texas streams. Journal of Water Resources Planning and Management, 126 (5), 310-319.

Schwarz, C.J. and A.N. Arnason. 1996. A general methodology for the analysis of capture-recapture experiments in open populations. Biometrics, 52: 860-873.

Sethi, S.A., Selle, A.R., Doyle, M.W., Stanley, E.H. and H.E. Kitchel. 2004. Response of unionid mussels to dam removal in Koshkonong Creek, Wisconsin (USA). Hydrobiologia 525: 157-165.

Sequencher® version 5.4.1 sequence analysis software, Gene Codes Corporation, Ann Arbor, MI USA. http://www.genecodes.com

Strayer, D. L., S. Claypool, and S. J. Sprague, Assessing unionid populations with quadrats and timed searches in *K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo (eds.). Conservation and Management of Freshwater Mussels II: Initiatives for the Future. Proceedings of a UMRCC Symposium, 16-18 October 1995, St. Louis, Missouri*, 1997, p. 163-169.Strayer, D.L. 1999. Use of Flow Refugees by Unionid Mussels in Rivers. Journal of the North American Benthological Society, 18: 468-476.

Strayer, D. L., Caraco, N. F., Cole, J. J., Findlay, S. E. G. and Pace, M. L. 1999. Transformation of freshwater ecosystems by bivalves: a case study of zebra mussels in the Hudson River. *BioScience*, 49.

Strayer, D. L., Hunter, D. C., Smith, L. C. and Bourg, C. K. 1994. Distribution, abundance, and roles of freshwater clams (Bivalvia, Unionidae) in the freshwater tidal Hudson River. *Freshwater Biology*, 31, 239-248.

Strayer, D. L. and Ralley, J. 1993. Microhabitat use by an assemblage of stream-dwelling unionaceans (Bivalvia), including two rare species of Alasmidonta. *Journal of the North American Benthological Society*, 2, 247-258.

Strayer, D. L. and L. C. Smith, 2003. A guide to sampling freshwater mussel populations. Am. Fish. Soc. 110 pp.

Strayer, D.L., Downing, J. A., Haag, W. R., King, T. L., Layzer, J. B., Newton, T. J. and S. J. Nichols. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. BioScience 54: 429-439.

Symonds, D. 2015. Importance of Tributary data to Rare Mussel Niches Models and Community Structure. Masters Thesis, Univ. Texas at Tyler.

Texas Parks and Wildlife Department (TPWD). 2016. The Zebra Mussel Threat. http://tpwd.texas.gov/huntwild/wild/species/exotic/zebramusselmap.phtml (accessed November 2016).

Texas Register 35. 2010. Threatened and endangered nongame species. Chapter 65. Wildlife subchapter G. 31 TAC 65.175. Adopted rules. Jan. 8, 2010:249-251.

Troia, M., L.R. Williams, N. Ford, and M.G. Williams. 2010. Hydrological and geomorphic controls on fish, mussel, and riparian vegetation communities in the Upper Neches River watershed. Final Report – Texas Parks and Wildlife Department.

Troia, M.J., L.R. Williams, M.G. Williams, and N.B. Ford. 2015. The process domains process as a framework for fish and mussel habitat in a coastal plain river of southeastern North America. Ecological Engineering 75:484-496.

U.S. Department of Agriculture (USDA). 1994. State Soil Geographic (STATSG0) Data Base: Data use information. Miscellaneous Publication Number 1492. http://www.fsl.orst.edu/pnwerc/wrb/metadata/soils/statsgo.pdf (accessed September 15th, 2015).

U.S. Environmental Protection Agency (USEPA) and the U.S. Geological Survey (USGS). 2005. *National Hydrography Dataset Plus. Horizon Systems Corporation*. www.horizon-systems.com/NHDPlus (accessed August 7th, 2016).

United States Fish and Wildlife Service (USFWS). 2009. Endangered and threatened wildlife and plants: 90-day finding on petitions to list nine species of mussels from Texas threatened or endangered with critical habitat. Federal Register 74:66260-66271.

United States Geologic Survey(USGS). 2003. Applying indicators of hydrologic alteration to Texas streams—Overview of methods with examples from the Trinity River Basin. USGS Fact Sheet 128-03.

United States Geological Survey (USGS). 2008. Oil and Gas Exploration and Production in the United States Shown as Quarter-Mile Cells. IHS Energy, Inc., and the U.S. Geological Survey. https://catalog.data.gov/dataset/oil-and-gas-exploration-and-production-in-the-united-states-shown-as-quarter-mile-cells (accessed September 28, 2016).

Vaughn, C.C., C.M. Taylor and K.J. Eberhard. 1997. A comparison of the effectiveness of timed searches vs. quadrat sampling in mussel surveys. Pages 157-162, *in* Cummings, K.S., A.C. Buchanan, C.A. Mayer and T.J. Naimo (eds)., Conservation and Management of Freshwater Mussels II: Initiatives for the Future. Proceedings of a UMRCC symposium, 16-18 October 1995, St. Louis, Missouri.

Vermont Agency of Natural Resources. 2009, May 1. Vermont Stream Geomorphic Assessment Appendix O. Retrieved November 1, 2014, from http://www.vtwaterquality.org/rivers/docs/assessmenthandbooks/rv_apxoshearstress.pdf

Vannote, R. L. and Minshall, G. W. 1982. Fluvial processes and local lithology controlling abundance, structure, and composition of mussel beds. *Proceedings of the National Academy of Sciences,* 79, 4103-4107.

Vidrine, M.F. 1993. *The historical distributions of freshwater nussels in Louisiana, Gail Q.* Vidrine Collectibles, Eunice, L.A.

Viessman, Warren Jr.; Lewis, Gary L. Introduction to Hydrology, 5th Edition. Pearson Education, Upper Saddle River, NJ, 2003. Pages 53-57.

Villella, R. F.; Smith, D. R.; Lemarie, D. P. 2004. Estimating Survival and Recruitment in a Freshwater Mussel Population Using Mark-recapture Techniques. American Midland Naturalist 151: 114-133.

Walters A. D. and N. B. Ford. 2013. Impact of drought on predation of a state-threatened mussel, *Potamilus amphichaenus*. Southwestern Naturalist. 58:479-481.

White, G.C. and K. P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. Bird Study 46 Supplement, 120-138.

Williams, J.D., A.E. Bogan, and J.T. Garner. 2008. *Freshwater mussels of Alabama and the Mobile Basin in Georgia, Mississippi, and Tennessee*. The University of Alabama Press, Tuscaloosa.

Williams, L.R., A. Dunithan, D. Ford, M.G. Williams, and N.B. Ford. 2013. Using ecological niche modeling to predict the probability of occurrence of rare fish and mussel species in East Texas. Final Report – U.S. Fish and Wildlife Service.

Williams, J.D., Warren M. L., Cummings K. S., Harris J. L. and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries, 18: 6–22

Wolock, D. M. 1997. STATSGO soil characteristics for the conterminous United States. U.S. Geological Survey. http://water.usgs.gov/GIS/metadata/usgswrd/XML/muid.xml (accessed December 12th, 2016).