

**FINAL REPORT**

**Endangered Species Research Projects for the  
Bluehead Shiner**

*Specific Study Title:*

**Current Status, Critical Habitat and General Ecology of the  
Bluehead Shiner (*Pteronotropis hubbsi*) in Texas**



*Submitted By:*

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## EXECUTIVE SUMMARY

Bluehead Shiner (*Pteronotropsis hubbsi*; hereafter BHS) at the time of this research had been petitioned for federal listing under the U.S. Endangered Species Act (ESA). During the course of this study, the species was removed from this review process. The limited distribution of the BHS across the United States and potential threats to the species' habitat catalyzed the federal action for potential listing. Therefore, because the range of BHS extends into northeast Texas, listing could have potentially impact several Texas counties and harmed several local economies and state-level gross domestic product.

To date, there have been few studies that have comprehensively studied the current status and ecology of the BHS throughout Texas (or the species entire range), and most documented occurrences of this species are at least 20 years old. Therefore, our goal herein was to evaluate the current status of this species in Texas, to investigate BHS habitat, ecology, life history, and evaluate the potential for captive rearing, and develop a model to predict BHS distribution in Texas.

We first documented the historical distribution of BHS in the U.S. and Texas by reviewing archived BHS records from museum databases. From this analysis we created a historical distribution map of BHS throughout Texas and the U.S. The records used to develop the historical distribution for BHS were several decades old, demonstrating a strong need for a contemporary study on the status of BHS throughout the U.S. and Texas. To document the current distribution, we resampled all 16 know historical localities in Texas and sampled an additional 27 localities within the drainage that could potentially support BHS. We documented the presence of BHS from 3 of the 43 localities. Two of the localities historically supported BHS and one locality was not identified from the historical records (i.e., a non-historical locality). Within two of the localities supporting BHS (Iron Ore Lake and Pruitt Lake), we analyzed population-level patterns of dispersion, density and habitat preference using fine-scale fish sampling and detailed habitat measurements. We found that densities in Iron Ore Lake (a historical locality) ranged from 0 fish/m<sup>2</sup> to 2.0 fish/m<sup>2</sup>, and in Pruitt Lake (a non-historical locality) densities ranged from 0 fish/m<sup>2</sup> to 1.0 fish/m<sup>2</sup>. In both localities, fish dispersion was clumped with fish densities correlating with abundance of aquatic vegetation and clay in the substrates. Habitat was expanded to the watershed by estimating regional and local abiotic parameters at each of the localities sampled. These data were compared across sample sites and correlated with BHS presence/absence. Based on the habitat analyses, we identified several microhabitat variables that were important for discerning presence/absence of BHS throughout this drainage. Bluehead Shiner was typically present in deeper localities that had an abundance of aquatic vegetation and soft substrates. Because vegetation was a predictor of BHS presence, we tested the importance of vegetation for reproductive success of BHS in an outdoor mesocosm experiment. Data from this rearing study suggested that BHS requires vegetation for successful reproduction. Finally, abiotic data collected at each sample locality was used to develop a logistic model that predicted the presence-absence of BHS across the drainage. Based on this model, we identified a number of localities throughout the drainage that have local abiotic parameters suitable for BHS. Therefore, our results suggest that the current BHS distribution in Texas is smaller than historically documented, and is smaller than the distribution predicted based on our logistic model. We believe this may indicate that isolated BSH populations are dynamic, changing over space and time, or, alternatively, these data may suggest that there are unidentified driving forces that have caused a range reduction of BHS in Texas.

## INTRODUCTION

*Global Distribution.* The Bluehead Shiner (*Pteronotopsis hubbsi*; hereafter BHS) occurs in the Red, Ouachita, White and Atchafalaya River systems within the Gulf Coastal Plain of southern Arkansas, northern Louisiana, southeastern Oklahoma and northeast Texas; a disjunct population also occurs in southwestern Illinois in and around Wolf Lake (Robison and Buchanan 1988; Burr and Warren 1986). Although distributed across 5 states, the BHS is rare throughout its range and documented occurrences are limited within each state. For example, BHS historically have been reported from only 3 localities in Illinois (pre-1974), and at least 10 localities in Arkansas, 2 localities in Louisiana, 2 localities in Oklahoma and 17 localities in Texas (Burr and Warren 1986; Robison and Buchanan 1988; Taylor and Norris 1992; Douglas and Jordan 2002). The 17 localities in Texas historically supporting BHS include 3 localities in Caddo Lake and 14 localities in Big Cypress Bayou and its tributaries upstream from Caddo Lake but downstream from Lake O' the Pines. Specifically, BHS has been collected in Big Cypress Bayou (4 localities), Kitchen Creek (1 locality), Haggerty Creek (2 localities), Little Cypress Bayou (3 localities), Black Cypress Bayou (3 localities), and Iron Ore Lake on Jim Bayou (1 locality; University of Texas Natural History Museum).

*Preferred Habitat.* Bluehead Shiner is a lowland species that inhabits quiet backwater areas of small to medium-sized sluggish, tannin-stained streams/bayous and oxbow lakes (Bailey and Robison 1978). It is often associated with submerged and emergent aquatic vegetation such as mermaid weed, swamp smartweed, and American Lotus (*Proserpinaca palustris*, *Polygonum hydropiperoides*, and *Nelumbo pentapetala*, respectively; Robison and Buchanan 1988). The fish schools within the watercolumn in areas of low-flow adjacent to dense vegetation. Bluehead Shiner typically are collected over substrates composed of mud or a mixture of mud and sand. Localities supporting BHS typically have intact riparian zones and are in watersheds with little anthropogenic disturbance (Burr and Warren 1986).

*General Ecology.* Few studies have evaluated the ecology of Bluehead Shiner in the Gulf Coastal Plain populations. When available, reports are largely anecdotal or limited to the northern most population in Illinois (Ranvestel and Burr 2002). Based on these reports, BHS is likely an opportunistic feeder, consuming a diversity of food items from the watercolumn, water surface, benthos and on vegetation. For example, adults consume an abundance of pelagic invertebrates such as chlodocerans, copepods, and rotifers (Fletcher and Burr 1992), as well as some benthic invertebrates such as chironomid larvae, nematodes and filamentous algae and diatoms (Burr and Heidinger 1987).

Reports on BHS life history also are limited, but literature indicates that BHS likely spawns from early May-July (Burr and Warren 1986; Robison and Buchanan 1988). Females are likely sexually mature at one year; whereas, dominant males mature at 2 years. Bluehead Shiner may migrate upstream to spawn, and, when in spawning habitat, males display courtship behaviors to attract females (Robison and Buchanan 1988). Males likely die after spawning. Bluehead Shiner may be a nest associate, spawning over nests of other species (e.g., centrarchids) that protect the nest and keep it swept clean of silt during hatching (Mayden and Simons 2002; Fletcher and Burr 1992; Ranvestel and Burr 2002).



*Need for Research.* We identified two reasons for an immediate need to study the current status and ecology of BHS populations in Texas. First, the limited number of populations coupled with relatively low densities of extant populations makes the BHS a species of concern throughout its range. For example, it was listed as endangered in Illinois in 1981 (Illinois Endangered Species Protection Board 1999), is listed as threatened in Texas (Texas Parks and Wildlife 2001), and of special concern in Arkansas (Arkansas Natural Heritage Commission 2001). It remains unlisted in Louisiana, as well as in Oklahoma; however, its distribution is limited in both states (Miller 1984, Lemmons et al. 1997). We expected that the limited global distribution and general rarity of this species would have affected the outcome of the 12-month review for federal listing by the USFWS under the Endangered Species Act.

Second, the limited distribution of BHS throughout its range likely is a result of specific habitat needs and limited availability of these habitats throughout the Gulf Coastal Plain (Fletcher and Burr 1992; Bailey and Robison 1978). Channelization, dredging, land clearing, and wetland draining continue to threaten lowland, swamp-type habitat throughout Bluehead shiner range (Fletcher and Burr 1992; Phillippi et al. 1986; Pfeleger 1997; Burr and Warren 1986; Robison and Buchanan 1988). Therefore, lowland habitat modification has the potential to further jeopardize extant populations of BHS. We expected that these threats also could have affected the outcome of the 12-month review, because critical habitat availability is an integral component to listing under the ESA.

*Objectives.* Although the USFWS elected to remove BHS from consideration as endangered, we continued to carry out the objectives of the study. Therefore, this study represents a thorough documentation of current status of BHS in Texas. Specifically, herein we report on six objectives designed to document the current status and ecology of BHS in Texas. These objectives include (1) document the historical distribution of BHS in Texas; (2) resample all known historical localities and sample additional localities throughout the potential BHS range in Texas; (3) quantify the dispersion patterns, local densities, and microhabitat associations of BHS in localities supporting this species in Texas; (4) quantify the regional, mesohabitat, and microhabitat in localities supporting and not supporting BHS; (5) examine food habits and reproductive requirements of BHS; and (6) develop a predictive model to demonstrate potential distribution of BHS throughout Texas.

## **TASK 1: Document Historical Distribution of Bluehead Shiner in the U.S. and Texas**

*Objective:* Survey museum databases for all known historical collections of Bluehead shiner in Texas and the U.S. Results for this objective are published (Hargrave & Gary, 2016).

*Question 1:* What was the documented historical distribution of Bluehead Shiner in the U.S?

*Question 2:* When were the historical records for Bluehead Shiner collected in the U.S?

*Question 3:* How many specimens of Bluehead Shiner were archived in the historical records?

*Question 4:* What was the documented historical distribution of Bluehead Shiner in Texas?

### **Methods**

To document the historical distribution of BHS, we first searched for *P. hubbsi* or *Notropis hubbsi* (earlier nomenclature) records on 6 internet databases: Fishnet2 (<http://www.fishnet2.net/>), FishBase (<http://www.fishbase.org/>), Global Biodiversity Information Facility (<http://www.gbif.org/>), Fishes of Texas (<http://www.fishesoftexas.org/home/>), University of Michigan Ichthyology Collection (<http://www.lsa.umich.edu/ummz/fishes/>), and University of Alabama Ichthyology Collection (<http://uaic.as.ua.edu/>). We then contacted 29 individuals, including curators from natural history museums, ichthyologists, and naturalists at public and private institutions of higher education, as well as biologists from state agencies that potentially held unpublished collection records of BHS. Individuals were identified and contacted via email and/or phone. We asked for individuals to search their museums and databases for *P. hubbsi* or *N. hubbsi*.

We used GeoLocate Version 3.22 (Rios & Bart 2010) to georeference any collection records that lacked geographical data. We used the locality string (name of water body, county and state information) and visual inspection of satellite imagery to best identify the coordinates of the collection locality. Of the 170 records collected from our museum search, we georeferenced 34 collection localities using the method described above. Following georeferencing, we examined all data for duplicate collection localities. Upon the removal of duplicate collection records, we analyzed patterns in BHS distribution using Geographic Information System (GIS).

### **Results and Discussion**

Of the 29 individuals associated with natural history museums, state agencies, and public and private universities, 28 individuals responded to our requests and, of those responses, 11 individuals had records of BHS in their collections (Table 1). This search resulted in a total of 100 independent collection records for BHS, representing 57 different collection localities (46 stream/bayous and 11 lake/oxbows), from 15 counties and 5 states (Fig1).

All records from Illinois are from a single locality - Wolfe Lake, Union County. This disjunct population in Illinois was introduced and may no longer persist (Ranvestel and Burr 2004, Scharpf 2005). Thus, the native range of BHS (i.e., the distribution excluding the population in Illinois) includes Arkansas, Oklahoma, Louisiana, and Texas.

The native range of BHS spanned 51,956 km<sup>2</sup> and most collection localities were in tributaries and backwaters within the Red River drainage (Fig 1). In Arkansas, BHS was documented from 6 counties (Ashley, Bradley, Calhoun, Clark, Ouachita, and Union) within the Ouachita River drainage. The Arkansas records were from 15 different collection localities (14 stream/bayou, 1 lake/oxbow). These localities within the Arkansas spanned a geographic range of 2,100 km<sup>2</sup>. In Oklahoma, BHS was documented from 12 different localities (7 streams/bayous, 5 lakes/oxbows) within the Little River drainage, McCurtain County, and spanned a range of 140 km<sup>2</sup>. In Louisiana, BHS was documented from 2 parishes (Ouachita and Morehead parishes) in the Ouachita drainage and 2 parishes in the Red River drainage (La Salle and Rapides parishes). The Louisiana collections were from 11 different localities (10 streams/bayous, 1 lake/oxbow) and spanned a geographic range of 3,995 km<sup>2</sup>. In Texas, BHS was documented from 3 counties (Cass, Harrison and Marion). All collections from Texas were within Big Cypress-Sulphur Basin (including Caddo Lake) and represented 16 different localities (13 stream/bayou, 3 lake/oxbow) that spanned a geographic range of 673 km<sup>2</sup>. In Texas, one collection record was reported from Lake Texoma (Grayson Co., TX). Hargrave has extensively sampled Lake Texoma (see Gido et al 2002) and never collected BHS. Thus, we believe this record is suspect and would require verification of fish identification as well as locality based on field notes. As a result, we left this record out of the distribution map and did not include the locality in the summary above.

Three records did not have data identifying the date of the collection (1 collection from Arkansas and 2 collections from Texas); these records were excluded from the following discussion. Bluehead shiner collection records existed for the following decades: 1940s, 1 record; 1950s, 2 records; 1970s, 28 records; 1980s, 38 records; 1990s, 23 records; 2000s, 1 records; Fig. 2). In Illinois, all records were from 1973 and 1974. In Arkansas, 15 records were from the 1970's, 4 from 80's, and 4 records from 90's. Oklahoma collections were from the 1980's (21 records), 90's (7 records), and 2000's (1 record). Louisiana had 2 records from the 1970's, 5 from the 80's, 6 from the 90's, and 1 record from 2000s. Texas had records from 5 decades: 1 record from 1949, 2 records from the 1950's, 4 records from 1970's, 8 from 80's, and 8 from 90's. The majority of all BHS collection records (89; 92%) from the native range were from the 1970s, 80s, and 90s. We believe this may reflect a period of intensive sampling by field-active ichthyologists, namely: W. Matthews, A. Echelle (Oklahoma), H. Robison, T. Buchanan (Arkansas), R. Suttkus, R. Cashner, H. Bart (Louisiana), and Clark Hubbs (Texas). Since the majority of records were historical (20 to 40 years old), we argue there is a need for renewed sampling effort across this region.

The ability to use fish count data from museum collections to infer natural density is limited. Sampling efforts may not have been standardized across collections, and, in many cases, it is impossible to know whether archived collections represent all individuals collected or a subsample of individuals (e.g., voucher specimens). Although these count data may be biased or inaccurately identified, all regions had collections with high fish counts. For example, Oklahoma and Texas had 4 records where more than 25 individuals were archived. These records in Texas include the following localities: Marshal Pump Station and Iron Ore Lake (average BHS per collection record = 31 & 47, respectively).

Table 1. List of museums/institutions and individuals contacted that held archived records, and the number of records held of *Pteronotropis hubbsi* in their respective ichthyology collection.

<b>Museum/Institution</b>	<b>Contact</b>	<b>Records</b>
Arkansas Tech University	Dr. Charlie Gagen	2
Texas A & M University	Dr. Kevin Conway; Heather Prestridge	2
University of Arkansas – Fort Smith	Dr. Tom Buchanan	2
Louisiana State University Museum of Natural Science	Dr. Prosanta Chakrabarty	3
Oklahoma Department of Environmental Policy	Randy Parham	6
University of Oklahoma Sam Noble Museum	Sarah Cartwright	6
Illinois Natural History Survey	Dr. Chris Taylor; Chris Mayer	9
Tulane University Royal D. Suttkus Fish Collection	Dr. Hank Bart	13
Henderson State University	Dr. Renn Tumlison	16
University of Texas Biodiversity Collections	Dr. Dean Hendrickson; Adam Cohen	23
Oklahoma State University	Dr. Tony Echelle	24

Arkansas had 2 records with more than 25 individuals archived, and Louisiana had 1 record where more than 25 individuals were archived. We argue that this suggests there is currently no known area within the Red River drainage that is the epicenter of the native BHS distribution. Rather, we believe these data suggest that large populations exist on the periphery of a potential epicenter of this distribution. This may indicate that BHS distribution is more widely distributed across this region and collections may be lacking that document occurrence of BHS within the interior of this geographic distribution.

Our review of the museum records for BHS support the known distribution reported in state fish books (e.g., Robison and Buchanan 1988). However, because these state books often do not provide detailed locality data (see Douglas 1974, Miller and Robison 2004, Thomas et al. 2007) our study is important because it provides, to our knowledge, the most comprehensive list of all BHS records throughout its range to date. Although, we made a strong attempt to identify and contact all individuals within the region that could have held records of BHS, we acknowledge that we likely have missed records collected by individuals unknown to us. However, because a number of major field biologists, ichthyologists and associated museums were contacted throughout this region, we feel that any missed records likely would not change our interpretation of the results.

We argue that this spatial and temporal analysis of historical museum records for BHS result in two general conclusions. First, we believe that our results illustrate that there are 4 known population centers of BHS throughout its native range. These populations exist on the periphery of the geographic range of BHS, and, thus, there is a large geographic area within this boundary with no records. It is possible that there exists localities within the center of this boundary that may support BHS. Therefore, we feel this illustrates a great need to explore and sample suitable habitat within this region. Second, our results show that the majority of documented records are well between 20 to 40 years old – the typical length of an active field career. Thus, this temporal pattern may represent intensive sampling from a few individuals throughout the 70s, 80s, and 90s. Unfortunately, the current status of BHS from historical localities is unknown. Since human population and habitat alteration continue to progress throughout this region, this illustrates a great need to revisit known localities.

There was interest in listing Bluehead shiner as federally endangered. This interest was likely driven by the perceived rarity of the species across its range, the lack of current distribution data, the species affinity toward lowland aquatic habitat, and the continued threat to such habitat for agriculture, oil and gas development, and urbanization. Our study, which provides a summary of historical distribution of BHS, supports the impetus to consider conservation action for this species. However, our summary also illustrates a great need to invest in sampling efforts that will illuminate the current status of BHS throughout its native geographic range in the Gulf Coastal Slope of the Southeastern United States.

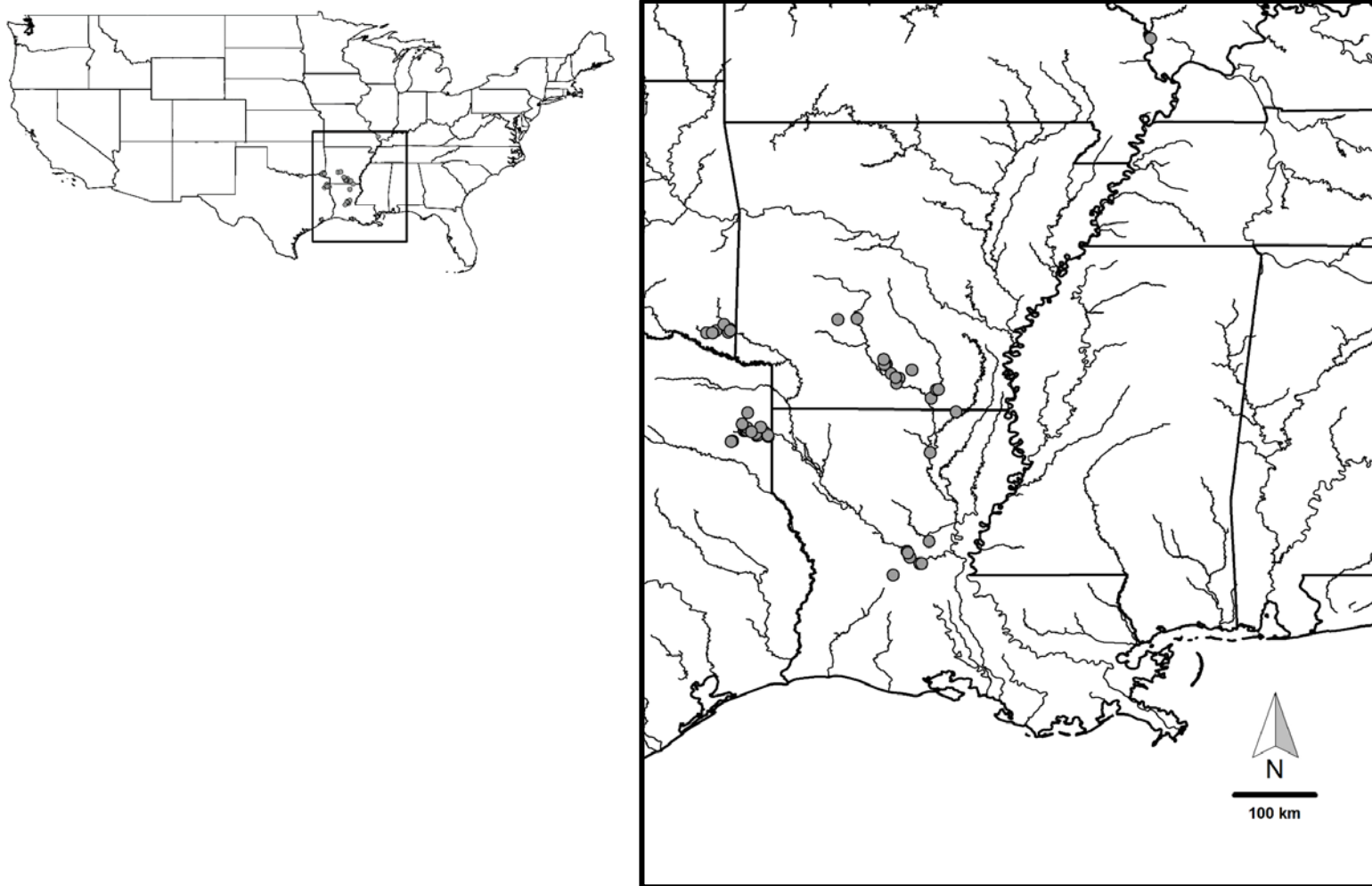


Figure 1. Map showing the historical distribution of BHS based on 100 known archived records.

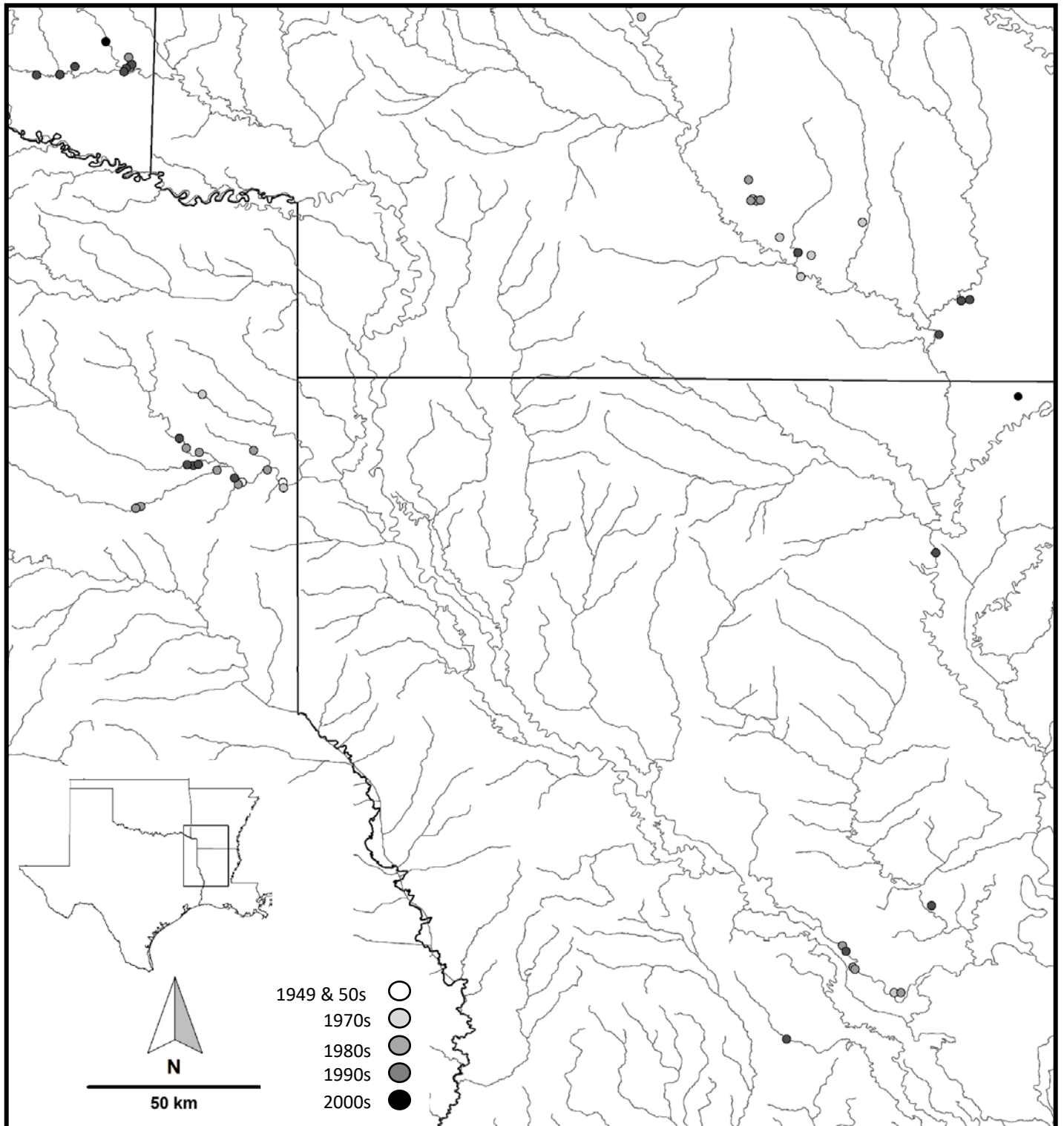


Figure 2. Map showing the number of known archived records of BHS by decade for Arkansas, Louisiana, Oklahoma and Texas.

## **TASK 2: Document Current Distribution of Bluehead Shiner and Distribution of Fish Assemblages in the Big Cypress/Sulphur Basin in Texas**

*Objective 1:* Document BHS presence/absence from all historical localities and non-historical localities throughout Big Cypress/Sulphur Basin in Texas (potential Bluehead Shiner range).

*Question 1.1:* What is the current distribution of BHS in Texas?

*Question 1.2:* How many historical localities currently support BHS?

*Question 1.3:* How many new localities currently support BHS?

*Objective 2:* Document spatial variation in fish assemblage structure across the Big Cypress/Sulphur Basin in Texas (potential BHS range).

*Question 2.1:* Do fish assemblages vary across the Big Cypress/Sulphur Basin in Texas?

*Question 2.2:* Does BHS significantly co-vary with other fish species Big Cypress/Sulphur Basin in Texas?

### **Methods**

Over a 3-year (2015-2017) period, we sampled a total of 43 localities within the potential range of Bluehead Shiner in Texas, i.e., across 6 watersheds within the Big Cypress-Sulphur Basin in Texas (Fig. 3). All localities were located at road crossings or on the navigable waters Big Cypress Bayou or Caddo Lake, and, therefore, all localities were accessible by car or boat, respectively (Fig. 4). Of the 43 localities sampled, 16 were historical localities (we omitted the Lake Texoma locality from this survey for reasons stated in the previous section) and 27 were localities lacking any historically reported presence of BHS (i.e., hereafter non-historical localities; Fig. 5).

We sampled localities on mainstem and tributary streams for most watersheds (Fig. 5). For example, we sampled 4 localities (all historical localities) in Little Cypress Bayou. We sampled 3 localities (2 historical, 1 non-historical) in upper Big Cypress Bayou. We sampled 7 localities (1 historical, 6 non-historical) in Black Cypress Bayou and its direct tributary streams. We sampled 3 localities in Jim's Bayou (1 historical, 2 non-historical) and 2 non-historical localities in Frazier Creek. We sampled 4 non-historical localities on Black Bayou or its tributaries. We sampled 1 historical locality and 1 non-historical locality in Kitchen Creek. We sampled 10 localities (5 historical, 5 non-historical) in lower Big Cypress Bayou above Caddo Lake. We sampled 8 localities (3 historical, 5 non-historical) in the body of Caddo Lake.

We used a range of gear types including mini-Fyke nets (i.e., minnow traps), electrofishing, and seining at the beginning of the study to evaluate collecting efficiency among these different methodologies. We compared these the number of individual fish and number of species collected by each methodology to determine the most appropriate method for surveying the fishes across this system. We collected the greatest number of individuals and number of species using seine netting compared to the other methods. Despite high degree of habitat complexity in some localities, we determined that seine netting was by far the most effective and non-



discriminative methodology for collecting fishes across localities. Therefore, we sampled all localities in 2016 and 2017 using seine nets.

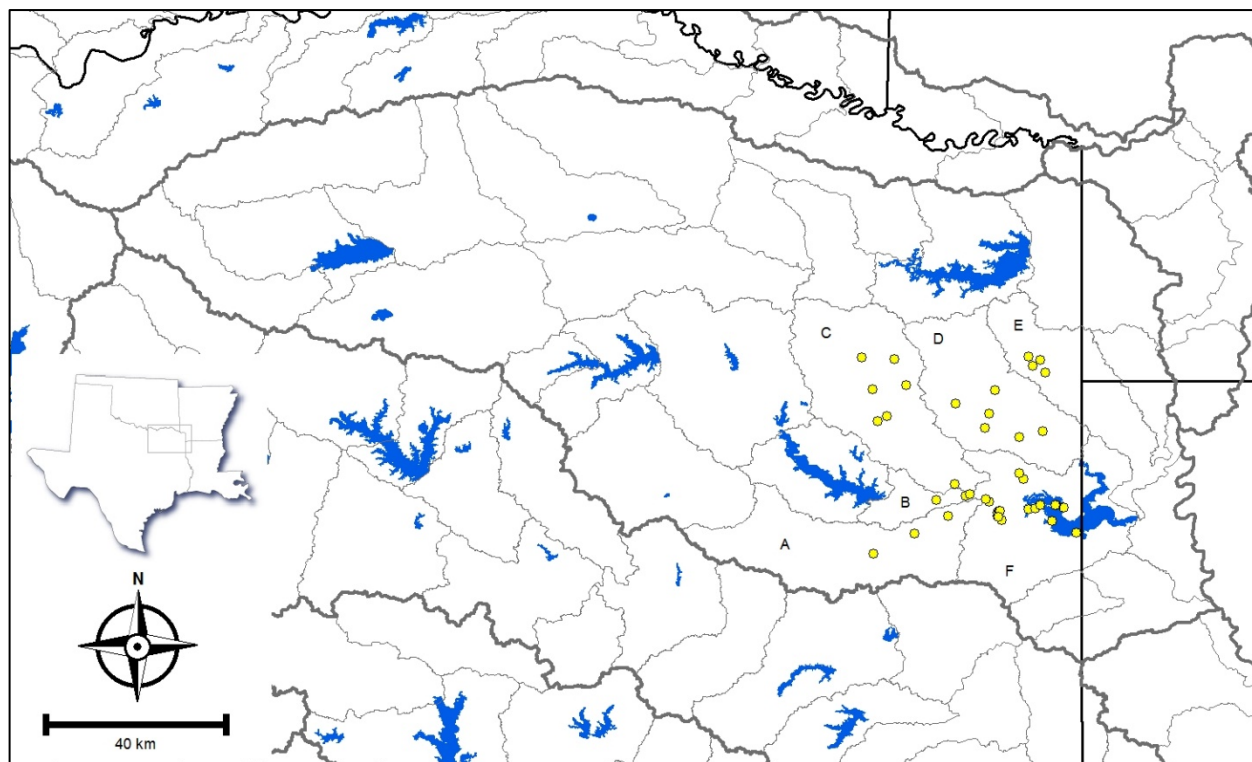


Figure 3. Forty-three sample localities and respective watersheds within the Big Cypress/Sulphur Basin. (A: Little Cypress; B: upper Big; C: Black Cypress; D: Jim's Bayou; E: Black Bayou; F: Lower Big Cypress and Kitchen Creek).

The size of the seine used to sample fishes varied in length, depending on complexity of the habitat. For example, we used smaller, 3.04 m (3.2 mm mesh) nets in small habitats with much structure (cypress knees and root wads), 4.57 m, (3.2 mm) nets were used in larger stream localities and lake localities that contained less structures, and 9.14 m (3.2 mm) nets were used in the most open lake and stream localities. We sampled fishes for about 1.5 hrs in each locality. To account for sampling effort, we recorded the duration of each sampling event, counted the number of seine hauls and measured the total distance sampled at each locality. We used this to standardize sampling events across localities and across sample years. Because of the difficulty in making accurate field identifications, we preserved most fishes (except large individuals and easily identifiable fishes) in 10% formalin. We returned these fishes to the laboratory where all individuals were sorted, identified to species, and counted. These fishes are in permanent archival storage in the Sam Houston State University Natural History Museum.

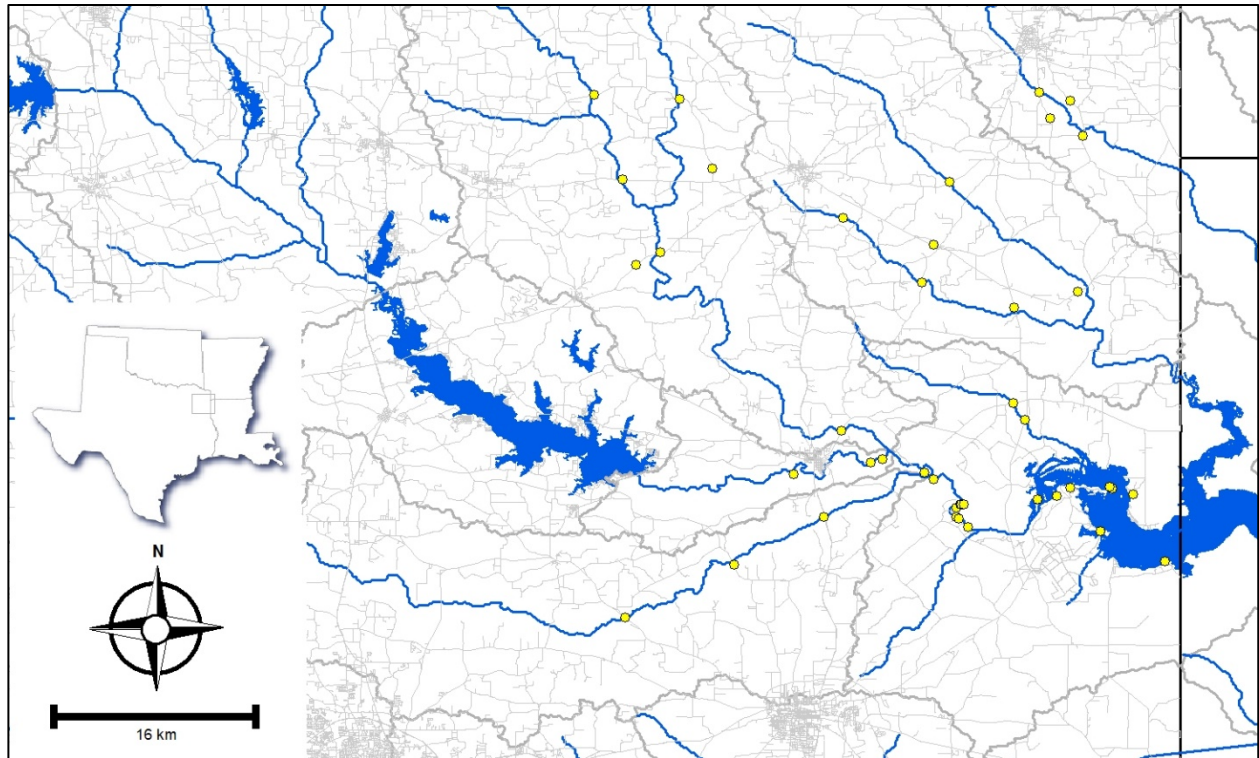


Figure 4. Forty-three sample localities at road crossings or water-accessible sites.

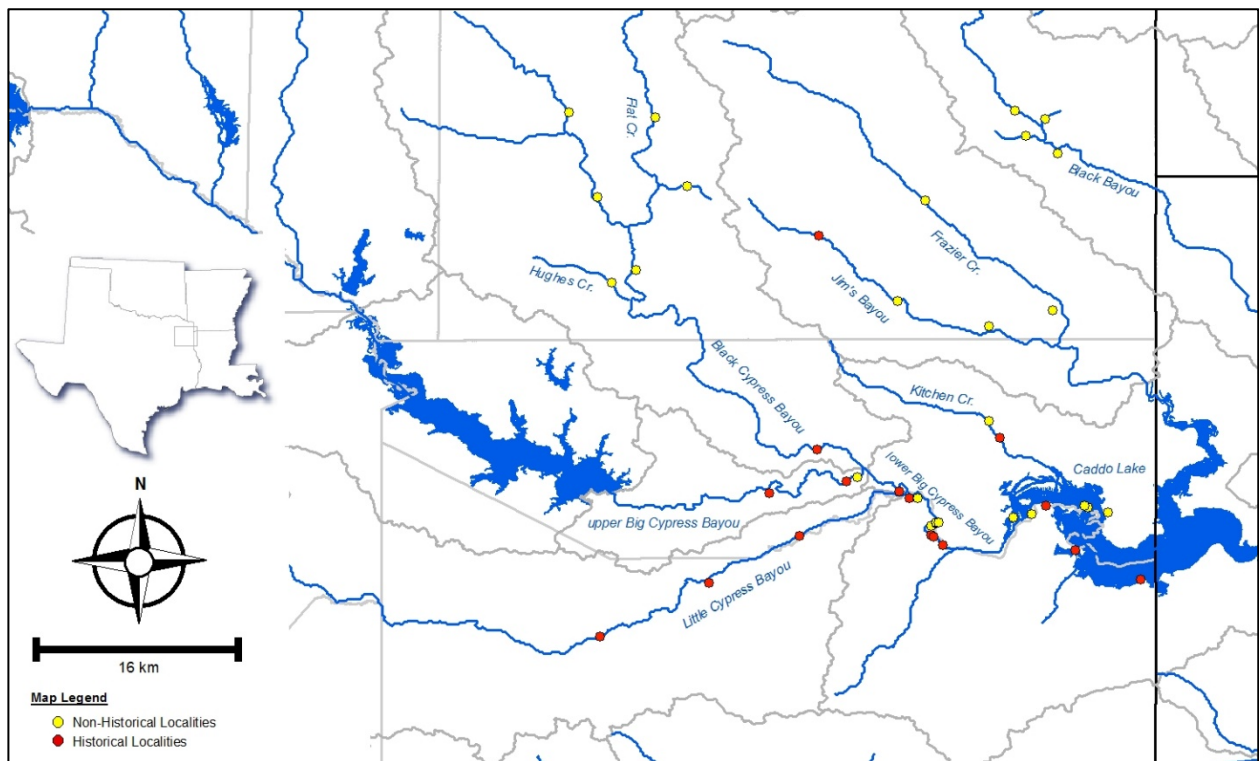


Figure 5. Sixteen historical and 27 non-historical sample localities.

## Results and Discussion

*Objective 1* - We collected 572 Bluehead Shiner from 3 localities during this study. Two of localities supporting BHS were historical localities (Big Cypress Bayou at Marshal Pump Station/Municipal Water Intake Facility and Iron Ore Lake on Jim's Bayou), and one of the localities supporting BHS was a non-historical locality (Black Cypress Bayou at Pruitt Lake; Fig. 6). Bluehead Shiner is present in 2 Texas counties (Cass and Harrison), from 3 watersheds (lower Big Cypress Bayou, Black Cypress Bayou, and Jim's Bayou), and from 3 unique waterbodies (Big Cypress Bayou, Black Cypress Bayou, and Jim's Bayou). The spatial distribution of BHS from our collections spanned a geographic range of 185 km<sup>2</sup>.

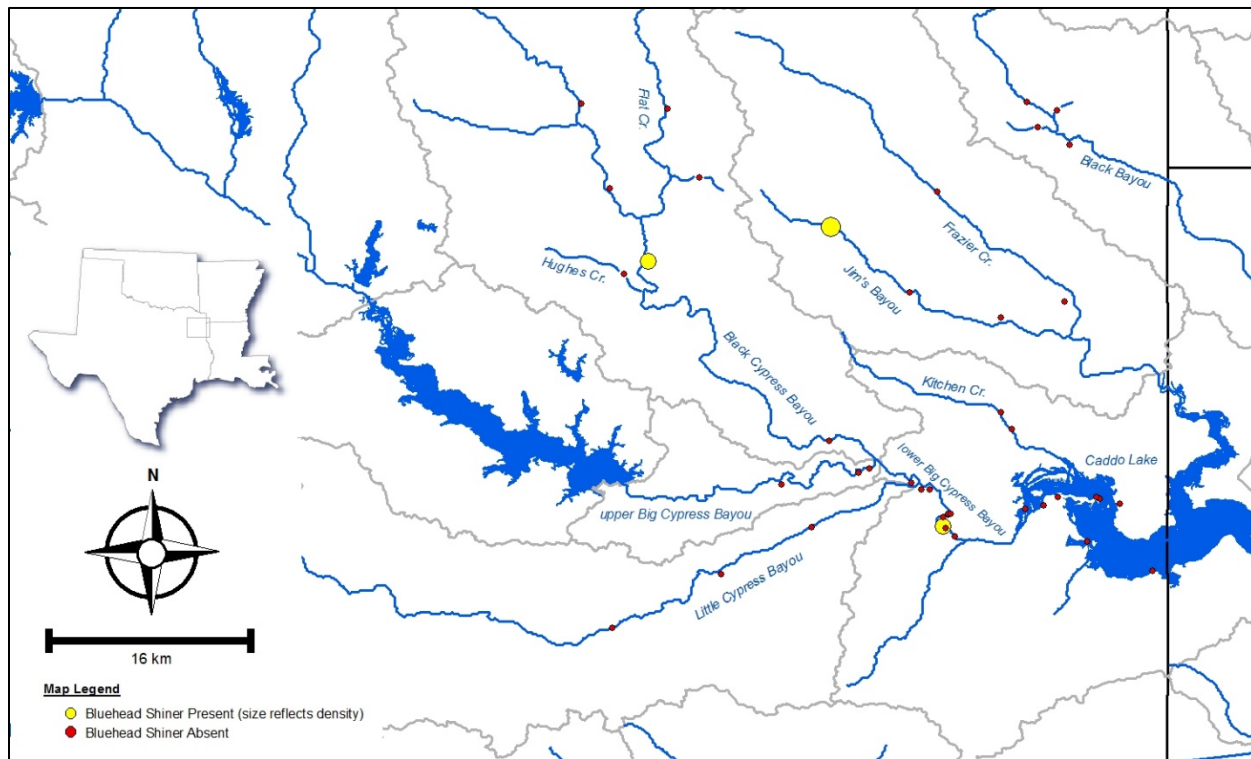


Figure 6. Current distribution (presence/absence) of Bluehead Shiner in Texas.

Our results suggest that the current distribution of BHS is smaller than the distribution based on historical records. Historically, BHS was documented from 3 counties (Cass, Marion, and Harrison), 6 watersheds (Little Cypress Bayou, upper Big Cypress Bayou, Black Cypress Bayou, Jim's Bayou, Kitchen Creek, and lower Big Cypress Bayou), and 7 unique water bodies (i.e., Little Cypress Bayou, Big Cypress Bayou, Black Cypress Bayou, Jim's Bayou, Kitchen Creek, Haggerty Creek, and the main body of Caddo Lake). The historical geographic range of BHS was 673 km<sup>2</sup>, which is ~30% greater than currently documented geography range.

Bluehead Shiner abundance collected in the field from our current study corresponded to the abundance of BHS in historical museum records. For example, we collected 523 BHS in Iron Ore Lake on Jim's Bayou in 2015, 2016 and 2017 combined (Fig. 6). This historical locality had the greatest number of specimens preserved in archival storage (average of 47 fish per collection). Furthermore, we collected 33 BHS at the City of Marshal Municipal Water Intake

Facility on the mainstem of the lower Big Cypress Bayou in 2017 (Fig. 6). This historical locality had the second greatest number of specimens preserved in archival storage (average of 31 fish per collection). Finally, we collected 16 BHS in from Pruitt Lake on Black Cypress Bayou in 2016 (Fig. 6). This locality was not represented in historical records, so fish numbers cannot be compared with historical records.

*Objective 2* – We collected a total of 22,602 individual fish and 69 different fish species over the course of this field study (Appendix 1). Based on rarefaction, we determined our sampling effort was adequate to capture the diversity present in the system (Fig. 7). Total number of fishes collected within each locality ranged from 81- 2,251 individuals and species richness ranged from 7-28 species. The most common and abundant species collected were *Labidesthes sicculus* (Brook Silverside; from 42 localities; 3662 specimens), *Lepomis macrochirus* (Bluegill Sunfish; from 41 localities; 4301 specimens), and *Gambusia affinis* (Western Mosquitofish; from 38 localities; 3029 specimens). The least common and least abundant fishes included *Amia calva* (Bowfin), *Esox niger* (Chain Pickerel), *Lepisosteus oculatus* (Spotted Gar) and *Luxilus chrysocephalus* (Striped Shiner). These fishes were represented by a single specimen from a single locality (Appendix 1).

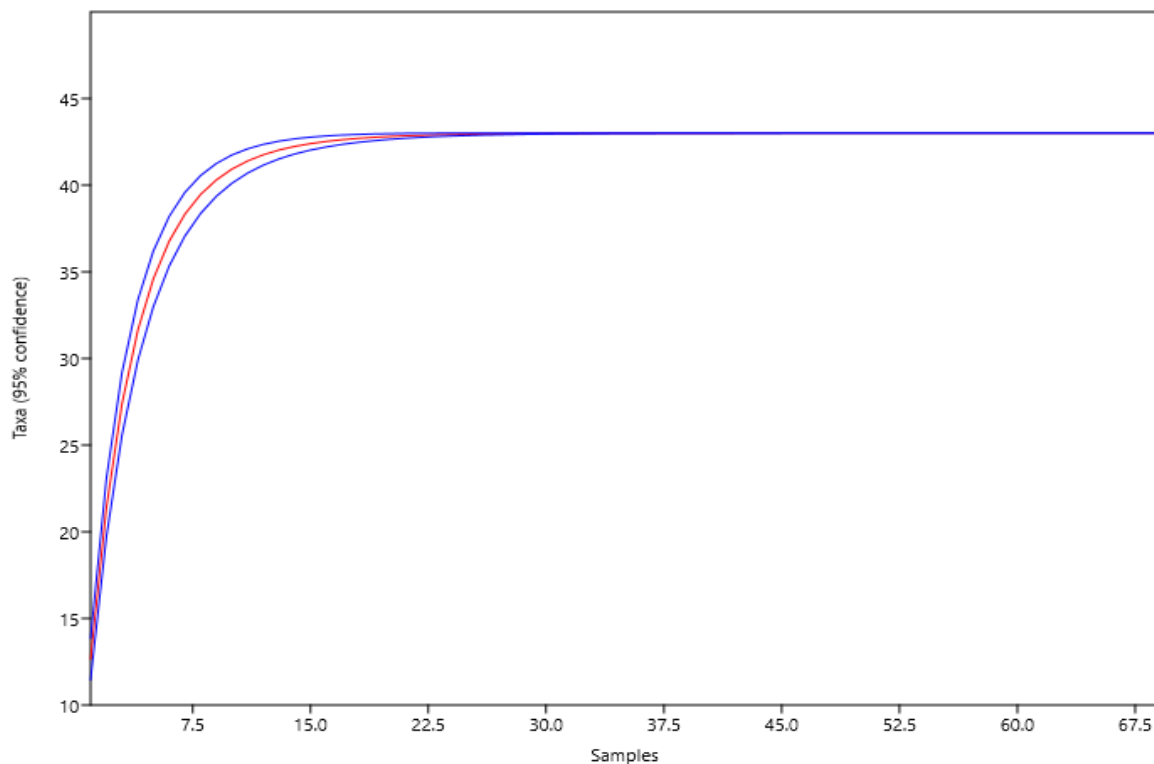


Figure 7. Rarefaction curve (with confidence intervals) based on our rectangular species-locality matrix.

Based on a search of museum records, we identified 76 species that were recorded historically from the Big Cypress/Sulphur Drainage. We collected 69 species. There were 16 species identified in historical records that we did not collect in our study, and we collected 9 species that

were not reported historically. Thus, there are 85 documented species from the Big Cypress/Sulphur Drainage in Texas when you combine historical museum records and species collected in our current study (Table 2).

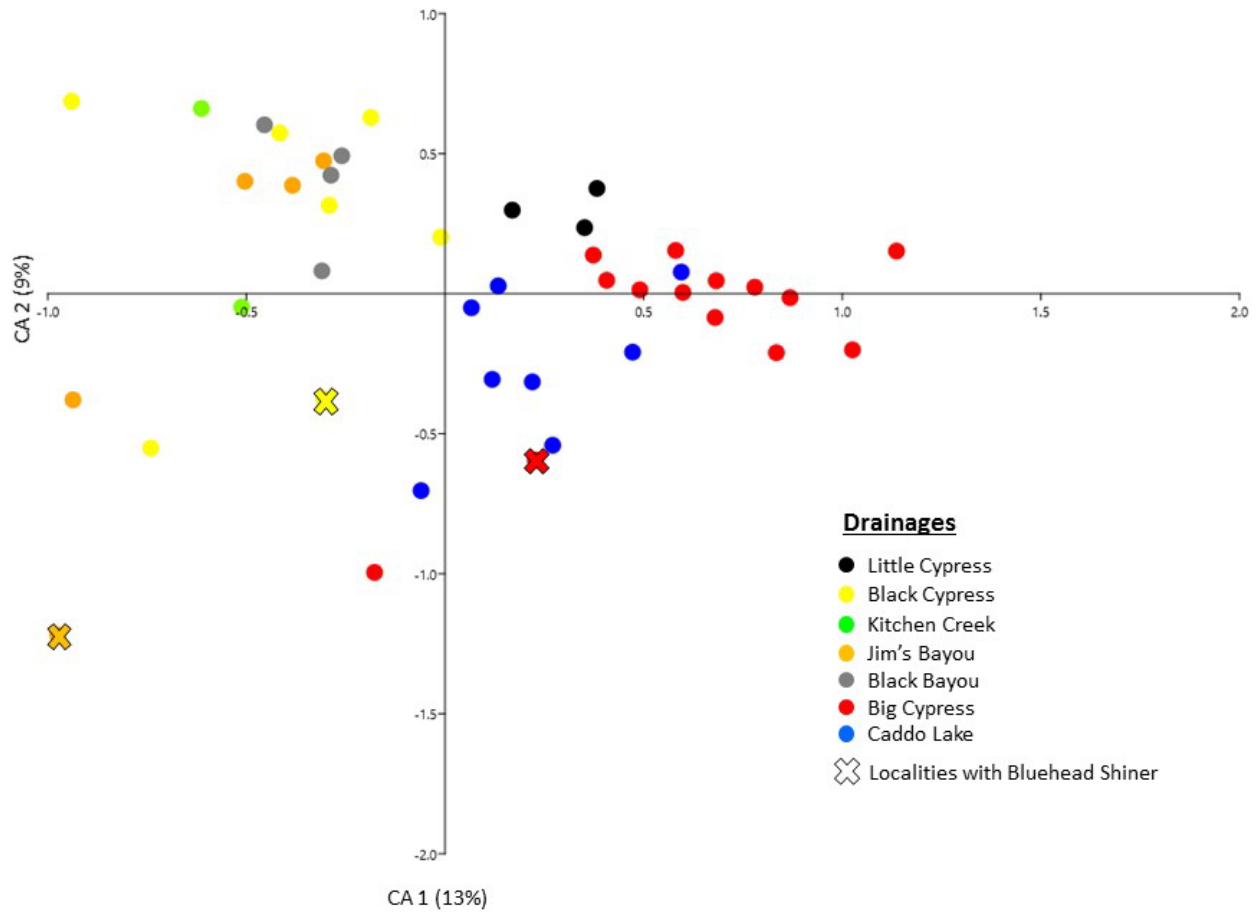


Figure 8. Correspondence analysis showing ordination of sample localities based on species abundances. Colors correspond to major river drainages within the Big Cypress/Sulphur Watershed.

Community assembly varied predictably over the drainage. Sample localities, as defined by their respective assemblages, formed two distinct clusters in CA space (Fig. 8). These clusters correspond to longitudinal position of the sample locality in the watershed. For example, localities clustering on the positive end of CA 1, were the largest, most downstream localities in the watershed (i.e., Caddo Lake, Big Cypress Bayou, and Little Cypress Bayou). The localities clustering on the negative end of CA 1 were smaller, more upstream localities. These localities were located on streams in the Black Cypress, Kitchen Creek, Jim's Bayou, and Black Bayou drainages (Fig. 8). The species that drove the ordination of these groups are illustrated in Fig. 9.



In general, several darter and minnow species ordinated positively on CA 1 and, thus, were characteristic of the largest, most downstream sample localities. Bullhead catfish, sucker, sunfish and topminnow species ordinated on the negative end of CA 1, and, thus, were characteristic of the smaller, upstream localities (Fig. 9).

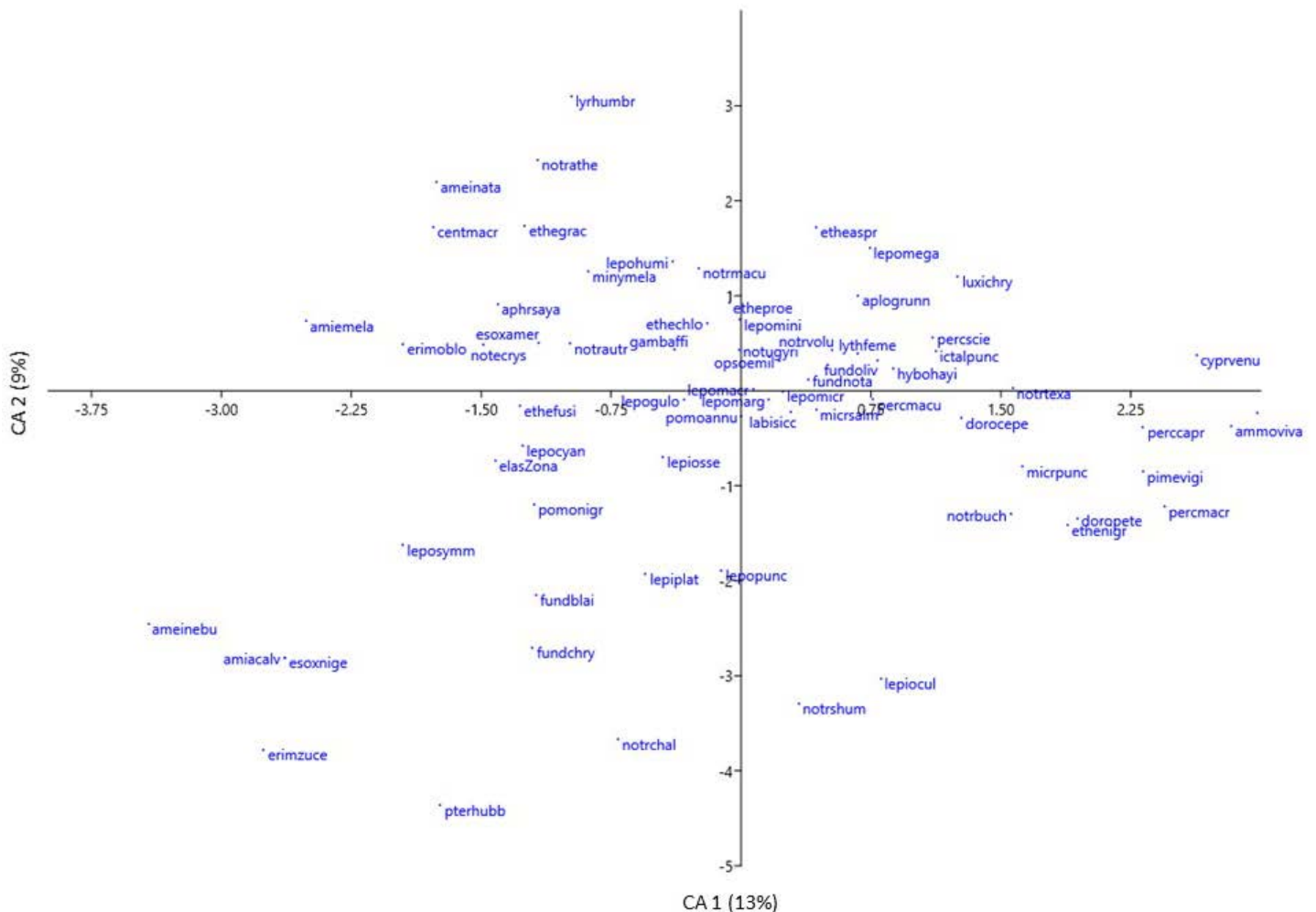


Figure 9. Species ordination scores for correspondence analysis. Four-letter species codes are shown in Table 2.



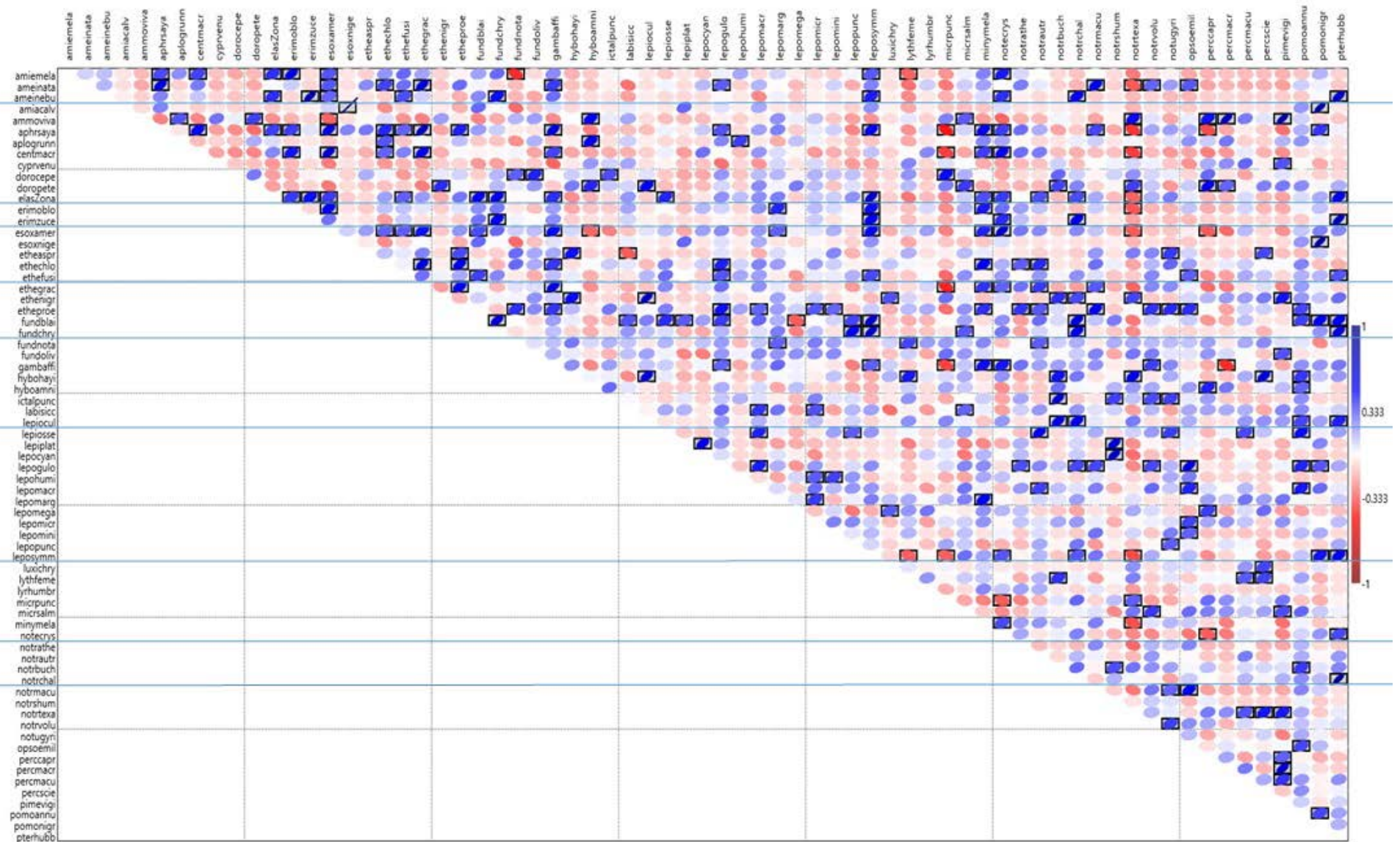


Figure 11. Pairwise correlation coefficients [significant ( $P < 0.05$ ) indicated by boxes] of species abundance.



Table 2. Species names, 4-letter code used in multivariate analyses, and whether the species was documented in historical records and whether the species was collected in the current study.

Fish Species	4-Letter Code	Historical	Current
<i>Ichthyomyzon castaneus</i>	--	yes	no
<i>Lepisosteus oculatus</i>	lepiocul	yes	yes
<i>Lepisosteus osseus</i>	lepiosse	yes	yes
<i>Lepisosteus platostomus</i>	lepiplat	no	yes
<i>Amia calva</i>	amiacalv	yes	yes
<i>Dorosoma cepedianum</i>	dorocepe	yes	yes
<i>Dorosoma petenense</i>	doropete	no	yes
<i>Cyprinella lutrensis</i>	--	yes	no
<i>Cyprinella venusta</i>	cyprvenu	yes	yes
<i>Hybognathus hayi</i>	hybohayi	yes	yes
<i>Hybognathus nuchalis</i>	--	yes	no
<i>Hybopsis amnis</i>	hyboamni	yes	no
<i>Luxilus chrysocephalus</i>	luxichry	yes	yes
<i>Lythrurus fumeus</i>	lythfeme	yes	yes
<i>Lythrurus umbratilis</i>	lyrhumbr	yes	yes
<i>Notemigonus crysoleucas</i>	notecrys	yes	yes
<i>Notropis atherinoides</i>	notrathe	yes	yes
<i>Notropis atrocaudalis</i>	notrautr	yes	yes
<i>Notropis blennius</i>	--	yes	no
<i>Notropis buchanani</i>	notrbuch	no	yes
<i>Notropis chalybaeus</i>	notrchal	yes	yes
<i>Notropis maculatus</i>	notrmacu	yes	yes
<i>Notropis shumardi</i>	notrshum	yes	yes
<i>Notropis texanus</i>	notrtexa	yes	yes

<i>Notropis volucellus</i>	notrvolu	yes	yes
<i>Opsopoeodus emiliae</i>	opsoemil	yes	yes
<i>Pimephales vigilax</i>	pimevigi	yes	yes
<i>Pteronotropis hubbsi</i>	pterhubb	yes	yes
<i>Semotilus atromaculatus</i>	--	yes	no
<i>Erimyzon claviformis</i>	--	yes	no
<i>Erimyzon oblongus</i>	erimoblo	no	yes
<i>Erimyzon sucetta</i>	erimzuce	yes	yes
<i>Minytrema melanops</i>	minymela	yes	yes
<i>Ameiurus melas</i>	amiemela	yes	yes
<i>Ameiurus natalis</i>	ameinata	yes	yes
<i>Ameiurus nebulosus</i>	ameinebu	no	yes
<i>Ictalurus punctatus</i>	ictalpunc	no	yes
<i>Noturus gyrinus</i>	notugyri	yes	yes
<i>Noturus nocturnus</i>	--	yes	no
<i>Pylodictis olivaris</i>	--	yes	no
<i>Esox americanus</i>	esoxamer	yes	yes
<i>Esox niger</i>	esoxnige	yes	yes
<i>Aphredoderus sayanus</i>	aphrsaya	yes	yes
<i>Cyprinodon variegatus</i>	--	yes	no
<i>Fundulus blairae</i>	fundblai	yes	yes
<i>Fundulus chrysotus</i>	fundchry	yes	yes
<i>Fundulus notatus</i>	fundnota	yes	yes
<i>Fundulus olivaceus</i>	fundoliv	yes	yes
<i>Gambusia affinis</i>	gambaffi	yes	yes
<i>Labidesthes sicculus</i>	labisicc	yes	yes
<i>Menidia beryllina</i>	--	yes	no

<i>Morone chrysops</i>	--	yes	no
<i>Morone mississippiensis</i>	--	yes	no
<i>Elassoma zonatum</i>	elaszona	yes	yes
<i>Centrarchus macropterus</i>	centmacr	yes	yes
<i>Lepomis cyanellus</i>	lepocyan	yes	yes
<i>Lepomis gulosus</i>	lepogulo	yes	yes
<i>Lepomis humilis</i>	lepohumi	no	yes
<i>Lepomis macrochirus</i>	lepomacr	yes	yes
<i>Lepomis marginatus</i>	lepomarg	yes	yes
<i>Lepomis megalotis</i>	lepomega	yes	yes
<i>Lepomis microlophus</i>	lepomicr	yes	yes
<i>Lepomis miniatus</i>	lepomini	yes	yes
<i>Lepomis punctatus</i>	lepopunc	no	yes
<i>Lepomis symmetricus</i>	leposymm	yes	yes
<i>Micropterus punctulatus</i>	micrpunc	yes	yes
<i>Micropterus salmoides</i>	micrsalm	yes	yes
<i>Pomoxis annularis</i>	pomoannu	yes	yes
<i>Pomoxis nigromaculatus</i>	pomonigr	yes	yes
<i>Ammocrypta vivax</i>	ammoviva	yes	yes
<i>Etheostoma artesiae</i>	--	yes	no
<i>Etheostoma asprigene</i>	etheaspr	yes	yes
<i>Etheostoma chlorosoma</i>	ethechlo	yes	yes
<i>Etheostoma fusiforme</i>	ethefusi	yes	yes
<i>Etheostoma gracile</i>	ethegrac	yes	yes
<i>Etheostoma histrio</i>	--	yes	no
<i>Etheostoma nigrum</i>	ethenigr	no	yes
<i>Etheostoma parvipinne</i>	--	yes	no

<i>Etheostoma proeliare</i>	etheproe	yes	yes
<i>Etheostoma pulchellum</i>	--	yes	no
<i>Percina caprodes</i>	perccapr	yes	yes
<i>Percina macrolepida</i>	percmacr	no	yes
<i>Percina maculata</i>	percmacu	yes	yes
<i>Percina sciera</i>	percscie	yes	yes
<i>Aplodinotus grunniens</i>	aplogrunn	no	yes

### **TASK 3: Estimate Bluehead Shiner Population Size and Structure in Texas**

*Objective:* Determine population-level information for BHS in select populations.

*Question 1:* What is the population size and dispersion patterns of extant BHS populations?

#### **Methods**

With approval of the Technical Advisory Panel (TAP), this task was modified from that originally proposed. Originally, we proposed to conduct seasonal population estimates of BHS in localities identified in 2015 and 2016 fish surveys. Season sampling would have allowed us to estimate population dynamics (e.g., size/age structure, annual mortality and recruitment) and build population growth models of BHS in Texas. However, following the field surveys of 2015 and 2016, we determined that the densities of currently documented populations were too small to adequately conduct a robust population-level analysis. Thus, the TAP and I determined that any benefits of a population-level analysis were outweighed by the negative impacts of repeated sampling of the current BHS populations. Thus, we scaled back the objective of this task to simply evaluate the local densities and dispersion patterns of BHS in the localities presently supporting BHS in Texas.

We estimated density of BHS in Iron Ore Lake and Pruitt Lake on October 2016 & July 2017. To estimate population size of BHS, we first established 8 sampling plots in Iron Ore Lake and 9 sampling plots in Pruitt Lake. The plots were sampled exhaustively for all fishes by repeated seining through the plot. All fishes were removed, preserved and identified in the laboratory. Following sampling, we measured the area of each sample plot and delineated local habitat parameters within each sample plot. The density of BHS was then plotted across each habitat to evaluate the dispersion of the individuals within each locality and the density was estimated per sample plot.

We estimated local microhabitat parameters within each sample plot to evaluate potential relationship between BHS density and habitat. To estimate local microhabitat, we established a transect that bisected the sample plot. Sample points were established every 0.5m along the transect and the following microhabitat-specific parameters were estimated/measured at each transect point: microhabitat size (area), average water depth, average water velocity, substrate composition, percent cover of emergent, submergent and floating vegetation, percent detrital cover, abundance of submerged structure. We calculated the average of each microhabitat parameter for the entire sample plot and used these values to analyze any covariance between BHS density and habit.

We calculated a Principal Components Analysis (PCA) based on local environmental parameters measured from each sample plot. These variables were square-root transformed, standardize and centered prior to analysis. Fish densities collected from each sample plot were then overlayed on top of locality scores from the PCA. The environmental loadings were plotted in conjunction with PCA scores and used to identify environmental variables that covaried with BHS density.

## Results and Discussion

We sampled 8 plots in Iron Ore Lake in October 2016 and July 2017. A total of 39 & 65 BHS were collected in Iron Ore Lake from 3 of these sample plots in October and July, respectively. The average density of BHS between sample periods from these plots was 0.1 fish/m<sup>2</sup>, 1.2 fish/m<sup>2</sup>, and 2.1 fish/m<sup>2</sup> (Fig. 12).

In Pruitt Lake, we collected fishes from 9 sample plots in October 2016 and July 2017. A total of 30 & 22 BHS were collected from 2 of the 9 plots in October and July, respectively. The average density of BHS from these sample plots was 0.4 fish/m<sup>2</sup> and 0.98 fish/m<sup>2</sup> (Fig. 13).

PCA axes 1 and 2 explained a large proportion of the environmental variation across sample plots in both Iron Ore Lake and Pruitt Lake. Sample plots supporting BHS in both Iron Ore Lake and Pruitt Lake had local abiotic parameters that differed from sample plots that did not support BHS. For example, plots in Iron Ore Lake that supported BHS had substrates composed of a high proportion of clay and had high percent cover of emergent vegetation (Fig. 14). Plots in Pruitt Lake that supported BHS had substrates composed of a high proportion of clay and silt, and had high percent cover of floating vegetation (Fig. 15).



Figure 12. Heat map showing dispersion pattern and relative densities of Bluehead Shiner in Iron Ore Lake.



Figure 13. Heat map showing dispersion patterns and relative densities of Buehead Shiner in Pruitt Lake.

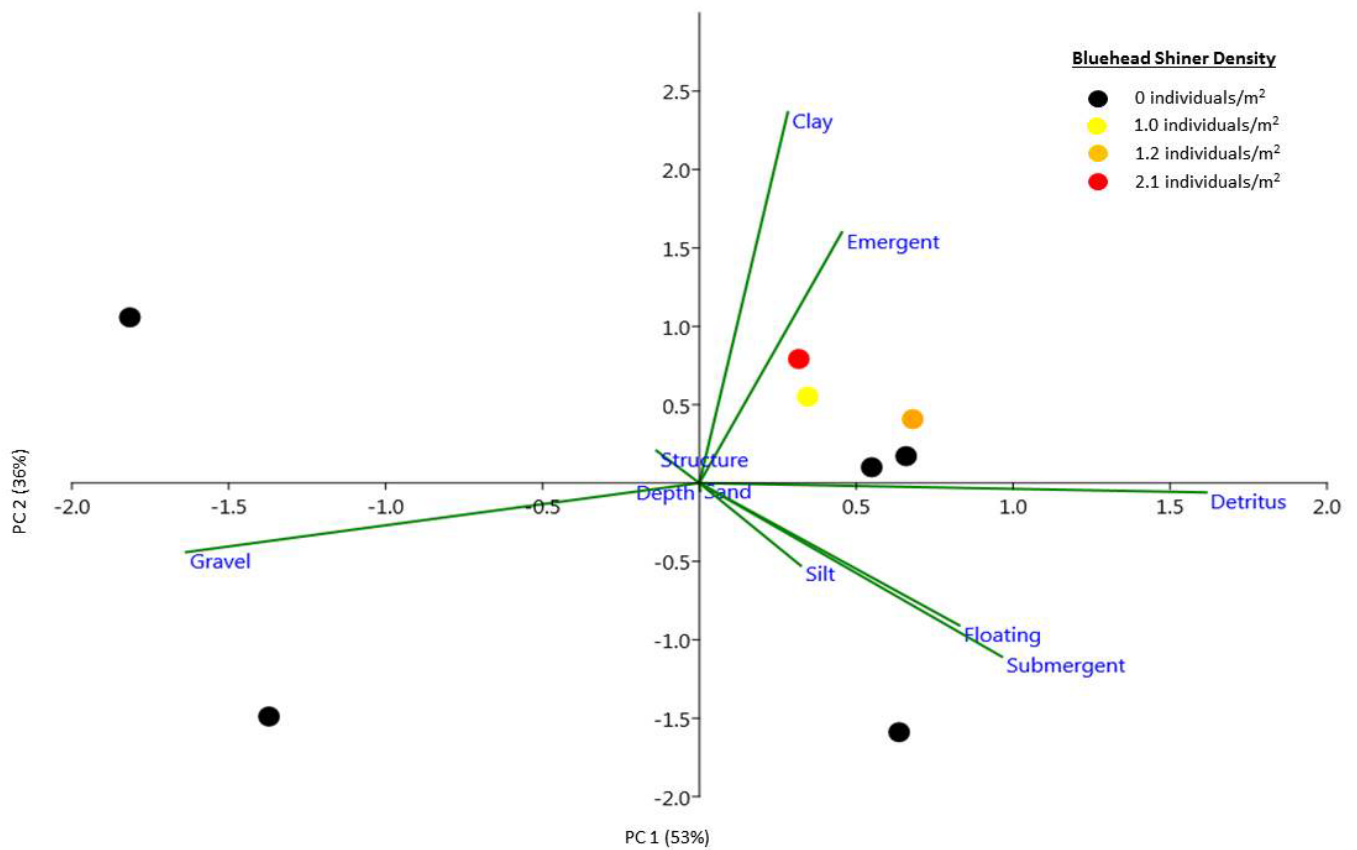


Figure 14. PCA ordination of sample plots in Iron Ore Lake showing environmental similarities.

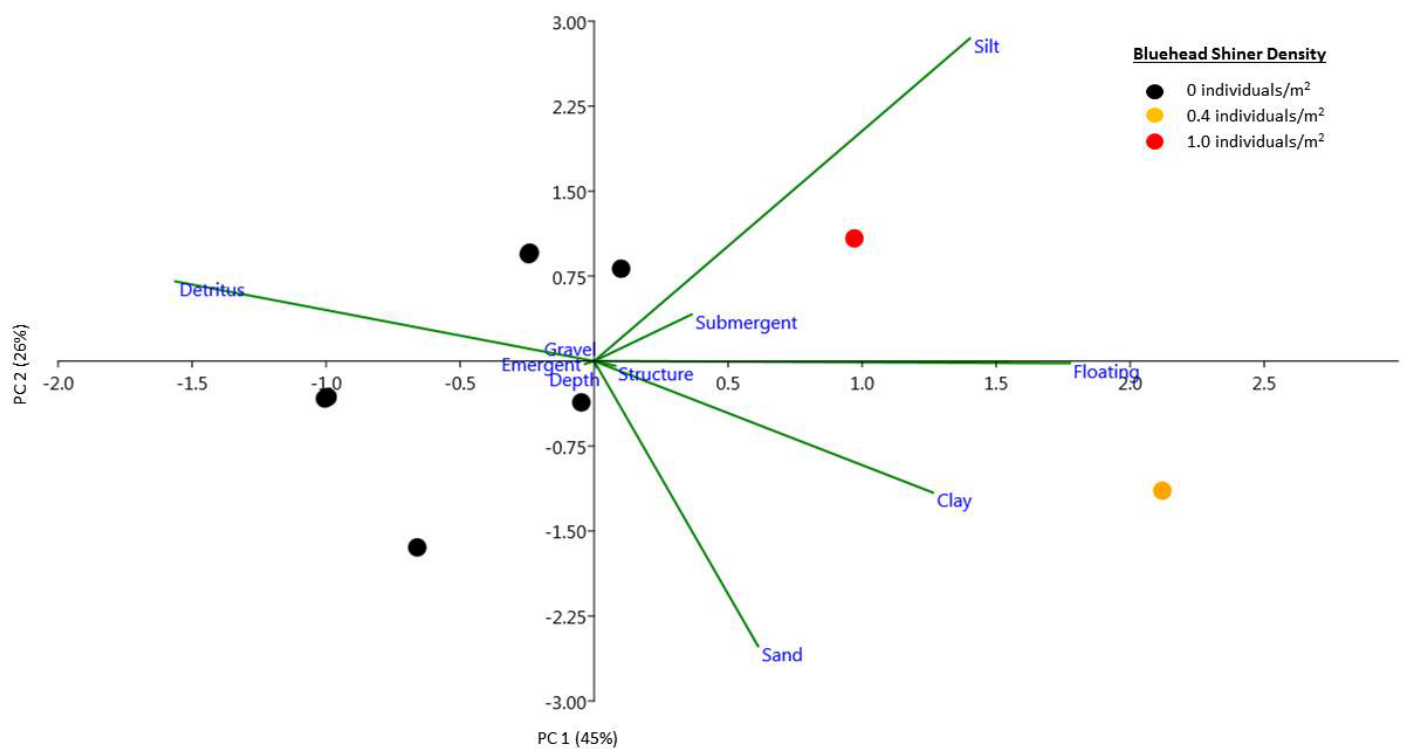


Figure 15. PCA ordination of sample plots in Iron Ore Lake showing environmental similarities.



#### **TASK 4: Compare Habitat Characteristics for Localities Supporting and not Supporting Bluehead Shiner in Texas**

*Objective:* Quantify the abiotic characteristics of habitats supporting BHS in Texas.

*Question 1:* Do abiotic parameters present in localities supporting BHS differ from abiotic parameter in all localities not supporting BHS in Texas?

*Question 2:* Do abiotic parameters present in localities supporting BHS differ from abiotic parameters in historical localities and not historical localities not supporting BHS in Texas?

#### **Methods**

We measured regional-scale abiotic parameters (i.e., ecosystem type and soil type) using GIS layers from the Ecological Mapping Systems of Texas (EMST). To quantify these parameters, we identified the corresponding ecosystem polygon at each sample locality using the GIS from EMST. A categorical score for the respective ecosystem and soil type was then assigned to each locality and used in subsequent analyses.

In addition to the regional-scale abiotic parameters, we estimated mesohabitat and microhabitat-level parameters at each sample locality. Mesohabitat parameters were measured once for each locality, including temperature (C), conductivity ( $\mu$ S), dissolved oxygen (mg/L), pH, turbidity (NTU), and chlorophyll concentration ( $\mu$ g/L). These parameters were measured with a YSI-EXO2 meter. We estimated microhabitat-scale parameters within each sample locality, including microhabitat size (area), average water depth, average water velocity, substrate composition, percent cover of emergent, submergent and floating vegetation, percent detrital cover, abundance of submerged structure. The microhabitat-scale parameters were estimated by point sampling along a transect that bisected the sample area. Sample points were established every 3 m along the transect and all microhabitat-scale parameters were measured at each transect point. We calculated the average of each microhabitat parameter for the entire sample locality and used these values to analyze any covariance between BHS presence/absence and abiotic habitat structure.

We performed two Discriminant Function Analyses (DFA) to compare statistically the regional, mesohabitat, and microhabitat parameters between historical and non-historical localities supporting and not supporting BHS. The first DFA compared abiotic parameters between two groups, i.e., localities supporting and not supporting BHS in the current study. The second DFA compared abiotic parameters between three groups, i.e., localities supporting BHS, historical localities not supporting BHS, and non-historical localities not supporting BHS in this study. In addition to generating ordination scores for each locality based on DFA, we calculated abiotic loadings to determine the abiotic drivers of the separation in DFA space, and we calculated a confusion matrix to determine accuracy of the DFA model in assigning sample localities to the predetermined grouping based on the discriminant function.

## Results and Discussion

We first used DFA to analyze environmental differences between only two groups – current localities supporting BHS and current localities not supporting BHS. This analysis helped us evaluate whether a subset of unique abiotic variables differed between localities with and without BHS. Localities supporting BHS had highly negative scores in the DFA and 95% of the predicted classifications were correctly assigned (Fig 16). We identified several local abiotic variables measured in each sample plot that differed between localities with and without BHS. The abiotic conditions in localities supporting BHS were best characterized by high percent coverage of floating and emergent vegetation, and high composition of detrital substrate. Localities not supporting BHS had high percent coverage of submergent vegetation, and greater percent composition of sand and gravel substrates.

In a second DFA, we analyzed three groups – current localities supporting BHS, non-historical localities not supporting, and historical localities not supporting BHS. In this analysis, localities supporting BHS had highly negative scores and 74% of the predicted classifications were correctly assigned (Fig 17). The DFA correctly classified 100% of the localities supporting BHS, 85% of the historical localities not supporting BHS, and 65% of the non-historical localities not supporting BHS. There were several abiotic variables that were characteristic of localities supporting BHS. They included deeper localities with a high percent cover of emergent and floating vegetation and high composition of detritus in the substrate (Fig. 17). There was substantial overlap between historical and non-historical localities not supporting BHS. These localities generally had more positive DFA scores and were characterized by high percent cover of submergent vegetation, substrates with percent composition dominated by gravel, gravel, sand, and clay substrates (Fig 17). Non-historical localities not supporting BHS were more similar, environmentally, to localities with BHS. However, these had less percent coverage of emergent and floating vegetation, and they had less percent detritus and were shallower than the localities supporting BHS.

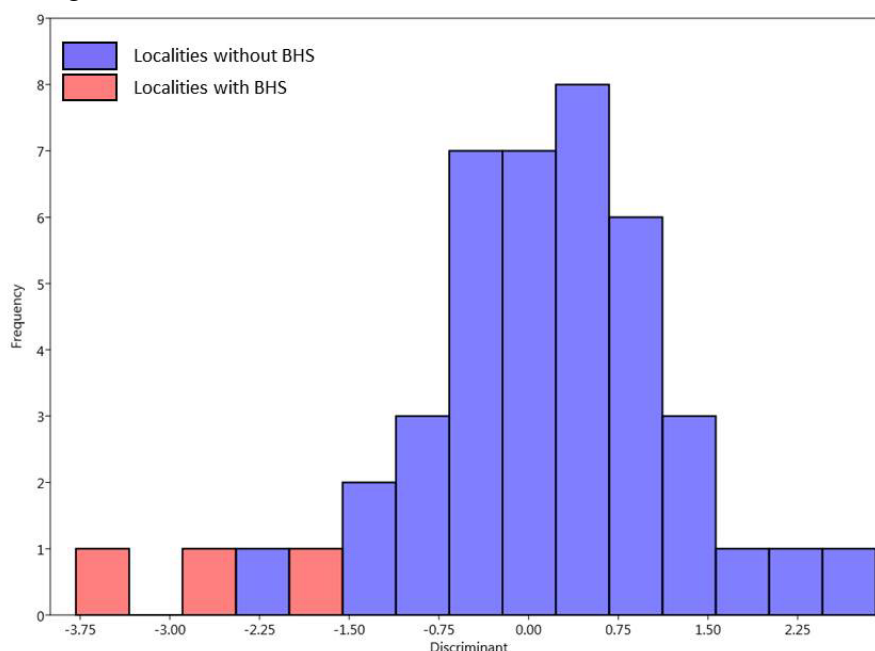


Figure 16. DFA for two groups (localities with and without BHS) calculated using environmental variables measured at each sample locality.

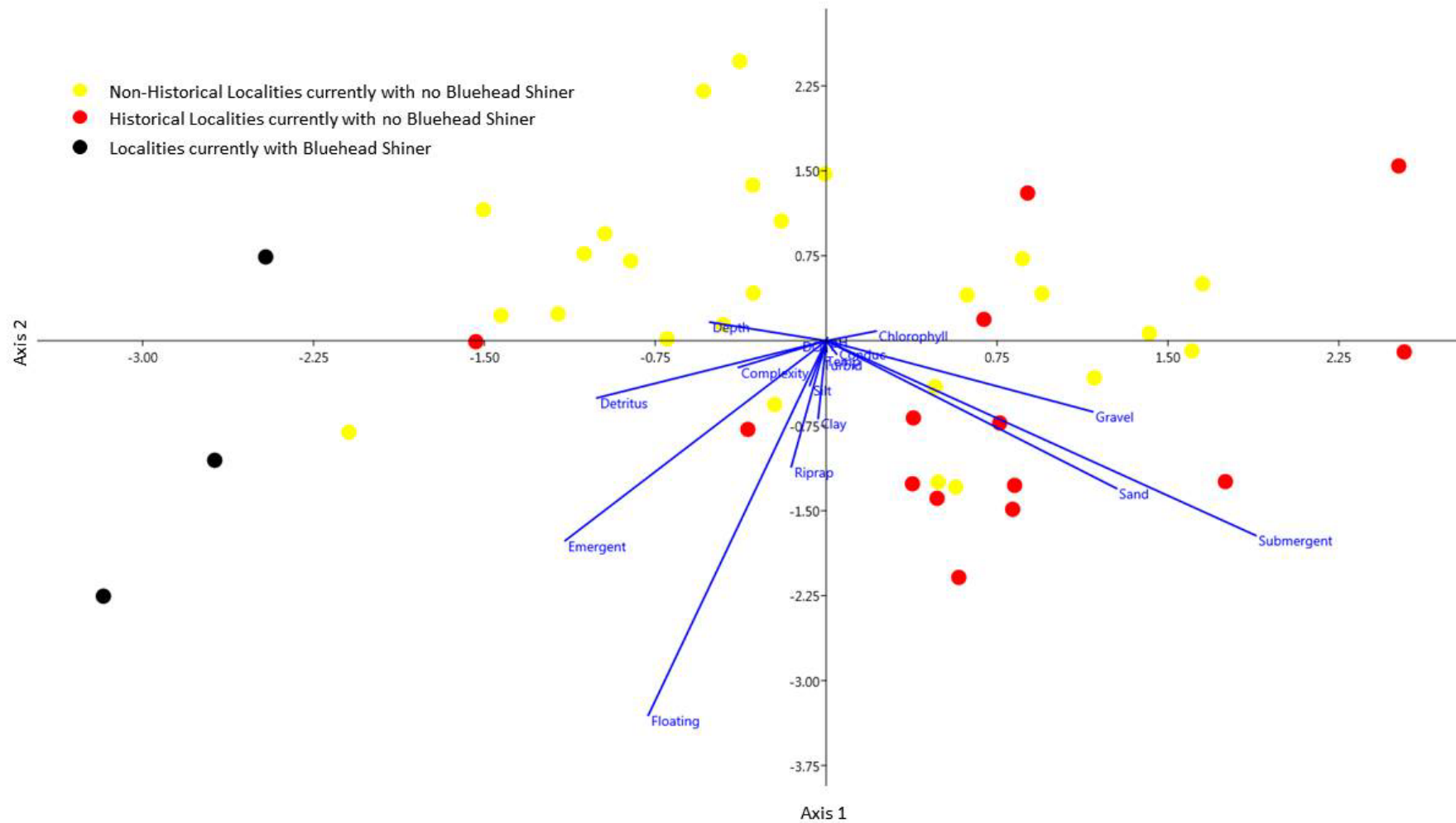


Figure 17. DFA for two groups (localities with and without BHS) calculated using environmental variables measured at each sample locality.

## **TASK 5: Document Feeding Ecology and Potential for Captive Breeding of Bluehead Shiner**

*Objective:* Quantify the food items consumed by BHS in natural populations and determine spawning requirements for BHS for potential captive rearing.

*Question 1:* What are the gut contents of wild caught BHS?

*Question 2:* Will BHS spawn in a captive rearing facility and does successful rearing in this facility depend on the presence of emergent vegetation?

### **Methods**

We preserved 53 BHS out of the 572 total number of individuals captured throughout the course of this experiment. These individuals were preserved in 10% formalin and 20 individuals were dissected for gut contents. The viscera for these fish was removed, separated on a Petri dish with distilled water, and the gut contents examined for major food items. Percent occurrence of each food item was calculated for each fish stomach.



Figure. 19. Bluehead Shiners collected from Iron Ore Lake by seine (left) and in a cooler for transport to CBFS for the rearing experiment (right).



On 10 March 2017, we collected 230 adult BHS (in breeding colors) from Iron Ore Lake by seine and transferred these live fish to a large cooler with stream water (Fig. 19). These fish were returned the SHSU Center for Biological Field Studies (CBFS) for an outdoor, captive rearing experiment. Nineteen mesocosms were prepared for this experiment in Spring 2016, by cleaning, adding a ~15cm layer of sand for substrate, and filling with well water. This allowed natural assemblages of algae and aquatic invertebrates (i.e., potential food sources) to become established prior to the addition of fish in the spring. Emergent vegetation (water iris – a common emergent vegetation in localities supporting BHS) was added to ten randomly selected mesocosms (Fig. 20). The 9 mesocosms without vegetation had only sand substrate.

Prior to stocking, fishes were separated into males and females based on breeding coloration, photographed, and then added to the mesocosms. Each mesocosm received as close to a 50:50 ratio of males to females as possible. Reproductive signals (coloration) and presence of larval fishes was monitored weekly by visual observation. Although we had successful reproduction in this trial, we decided to allow this experiment to run for an additional year for a more robust dataset. Therefore, this experiment is on-going and the second year of data is not be presented in this report.



Figure 20. Mesocosms at CBFS with emergent vegetation (left) and no vegetation (right) 2 weeks following initial BHS stocking.

## Results and Discussion

Wild caught BHS consumed an array of food items, including food stuffs from terrestrial and aquatic sources (Fig. 18). The most abundant items in BHS guts included terrestrial Dipterans (mainly adult midges), aquatic Dipteran larvae (i.e., Chironomidae), and aquatic algae/vegetation. Other items that occurred in much lesser abundance included terrestrial spiders (1 individual), aquatic beetle larvae (Coleoptera), aquatic mites (Acari), aquatic Odonate larvae, and rotifers.

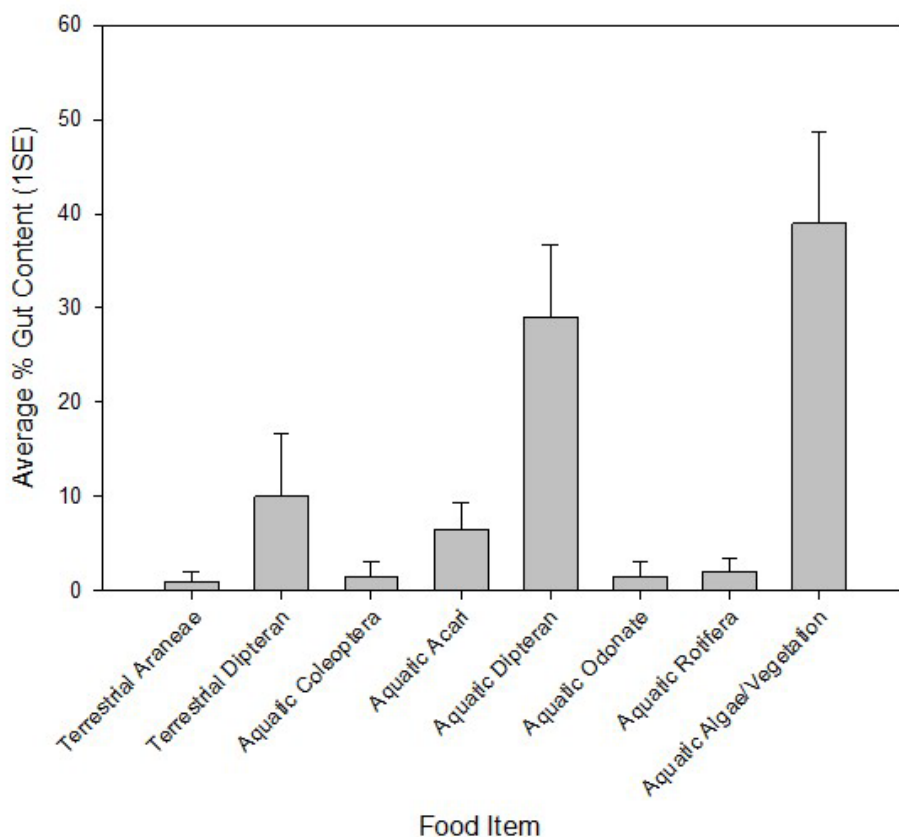


Figure 21. Average percent occurrence of major food items identified from 20 BHS guts.

Bluehead Shiner were captured for the artificial rearing experiment on 10 March 2017. At this time, males were in full breeding color but females did not appear fecund at this time. Fishes were in a large congregation around floating and emergent vegetation in Iron Ore Lake (Fig. 21). Males lost breeding coloration after being returned to the CBFS and stocked into mesocosms. However, this loss of coloration was short lived. By 20 March 2017, males again were in full coloration and appeared to be schooling (Fig. 22). We observed young of year fishes (~2.5cm) on 6 June 2017 in 6 of the mesocosms. All of the mesocosm with larval fishes had emergent vegetation; thus, 60% of vegetated mesocosm supported successful reproduction. No young of year were observed in mesocosms lacking emergent vegetation.





Figure 21. Localities in Iron Ore Lake where large congregation (~500) of BHS in breeding colors were collected for captive rearing study.





Figure 22. Bluehead Shiners displaying breeding coloration (males) photographed using an underwater camera in artificial mesocosms at CBFS.



## **TASK 6: Produce Model to Predict Potential Distribution of Bluehead Shiner in Texas.**

*Objective:* Identify the abiotic parameters that best explain the current distribution BHS in Texas, and using the parameters create a predictive model to estimate potential distribution in Texas.

*Question 1:* What abiotic parameters best predict the distribution of BHS in Texas?

*Question 2:* What is the predicted distribution of BHS in Texas based on abiotic and biotic parameters?

### **Methods**

Regional scale environmental parameters derived from GIS did not provide enough resolution to predict accurately the presence-absence of BHS throughout the drainage. Rather, finer meso- and local-scale parameters provided the best resolution for potential model building (Appendix 2). Therefore, we elected to use a hierarchical model building approach using field data rather than an ecological niche modeling approach using GIS layer data.

We used high performance logistical regression in a hierarchical linear modeling approach to develop predictive models from our field measurements. To build these models, we first evaluate which environmental parameters were best predictors of the presence-absence of BHS across sample localities. We used forward model building approach and evaluated change in AIC and *P*-value of the Chi-square statistic bases using maximum likelihood. Because we only had 3 localities with BHS, we used a liberal *P*-value cutoff of 0.25 as the determinant for parameter inclusion into the model.

Once the most predictive environmental parameters were identified, we built multi-parameter models and determined the single best model (based on AIC and *P*-Value) for explaining the observed presence-absence of BHS. To evaluate potential distribution of BHS based on this best-case linear model, we fit the model to a several potential BHS distributions. These distributions were derived based on similarity among sites from UPGMA cluster analyses. We regressed our best-case model against these different distribution scenarios and evaluated AIC and *P*-value across these distribution scenarios. Here, we constrained model significance to  $P \leq 0.05$ , as we were using this technique to determine localities that should theoretically support BHS based solely on environmental parameters.

### **Results and Discussion**

Logistical regression indicated that depth ( $P=0.01$ ), habitat complexity ( $P=0.1$ ), percent emergent and submergent vegetation cover ( $P=0.13$  &  $0.19$ , respectively), and percent detritus and sand comprising the substrate ( $P=0.23$  &  $0.25$ , respectively) were important variables for predicting the presence-absence of BHS across sample localities. Based on this model, 10 sample localities (including 3 localities supporting BHS) fall within the 90% confidence interval for the predicted distribution of BHS (Fig. 23). Sixteen sample localities (including 3 localities supporting BHS) fall within the 80% confidence interval for the predicted distribution (Fig. 23). Twenty-nine localities (including the 3 localities supporting BHS) fall within the 70% confidence interval for the predicted distribution (Fig. 23). Fourteen localities showed no support for BHS.

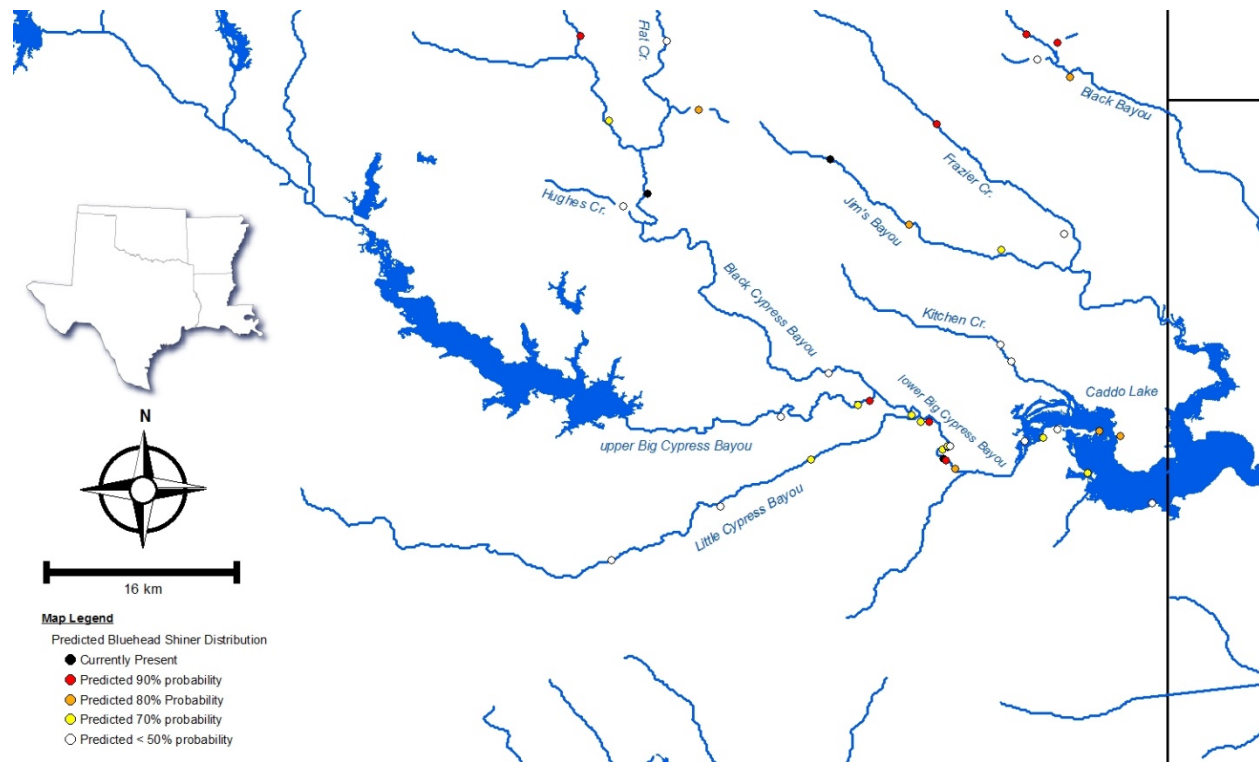


Figure 23. Predicted distribution of BHS based on logistic regression model of local environmental parameters measured at each locality. Color of each point indicates level of confidence in the predicted presence of BHS.

Most localities predicted to support BHS from by this model were non-historical localities and were located in the upper reaches of the Black Cypress Bayou, Jim's Bayou and Black Bayou drainages (Fig. 24). Moreover, many of the non-historical localities were predicted with high confidence (90%) to support BHS, indicating a high degree of similarity in environmental conditions between these localities and localities actually supporting BHS in this study. The historical localities predicted to support BHS occurred primarily in lower Big Cypress Bayou (Fig. 24) but the confidence of these predictions was much lower (70%) than that for the non-historical localities.

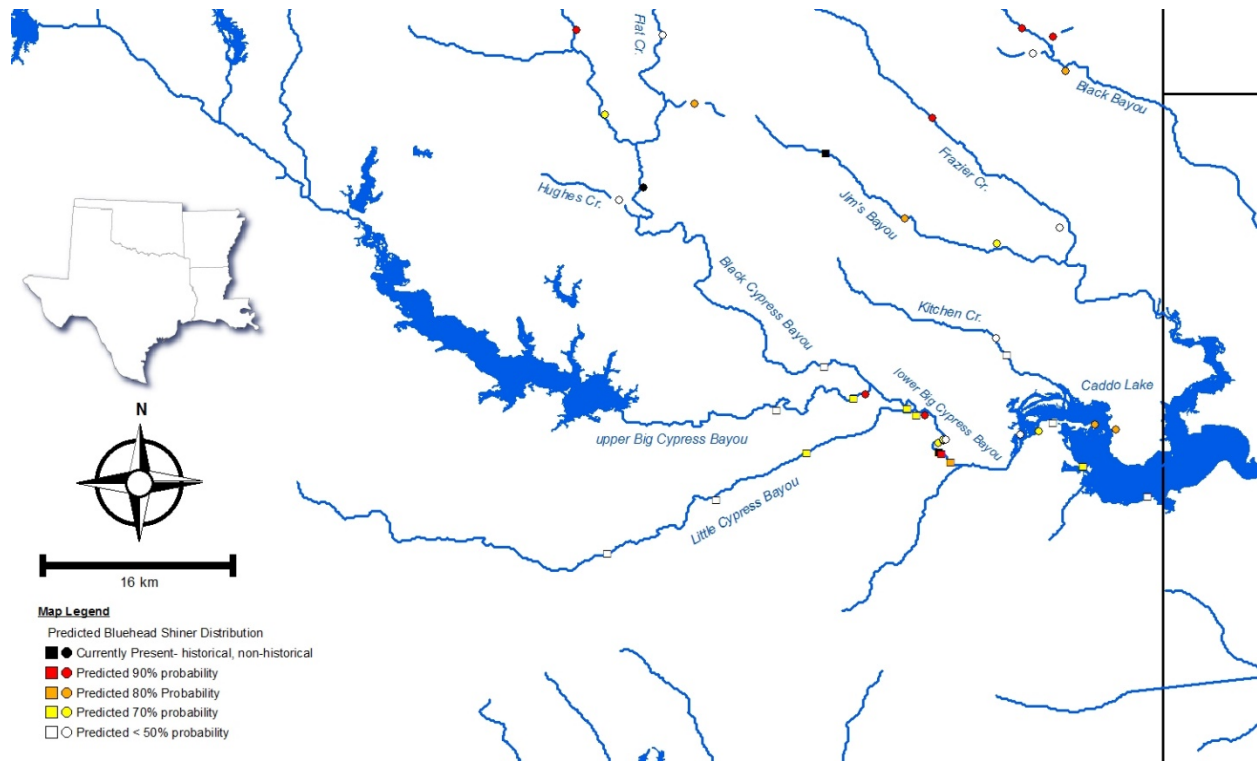


Figure 23. Predicted distribution of BHS based on logistic regression model of local environmental parameters measured at each locality. Color of each point indicates level of confidence in the predicted presence of BHS and shape of the point indicates whether the locality supported BHS historically.

## GENERAL CONCLUSIONS

In this study, we documented the historical and current distributions of BHS in Texas. Our results show that the distribution of BHS in Texas was smaller than the historical distribution reported in museum records. Although failure to identify the presence of a species within a locality does not necessarily indicate absence from that locality, we felt that our sample efficiency (3 time periods) and effectiveness (high fish richness and density) was robust enough to accurately document the presence of BHS in all localities. Therefore, we are relatively confident that the current distribution of BHS reported in this study was representative of the presence/absence of BHS across the drainage during our sampling events.

Although we collected BHS from only 3 localities, these localities were located in different watersheds within the basin. This suggests that BHS could be relatively widespread in the drainage and the distribution potentially dynamic and constantly changing, i.e., a metapopulation. For example, we collected BHS from Iron Ore Lake during all 3 sample years, we collected BHS from Pruitt Lake during 2 of the 3 sample years, and we collected BHS from Big Cypress Bayou near Marshal Pump Station during 1 of the 3 sample years. However, the Big Cypress collection of BHS was the largest collection of individuals from all localities, so it is highly unlikely that BHS was missed the first two years of sampling. Rather, evidence suggests that BHS moved into that habitat sometime between 2016 and 2017 sample periods. Movement into and out of habitats is indicative of a metapopulation.

Localities supporting BHS had unique habitat characteristics that correspond to reports in the literature. For example, BHS occurred in still waters, with aquatic vegetation and soft substrates. Likewise, the gut contents identified from BHS in this study also correspond to reports in the literature. This species is likely dependent on aquatic macroinvertebrates, but will consume some terrestrial foods when available. Aquatic macroinvertebrate densities and production are greatest in vegetated habitats, so aquatic vegetation may play an important role in BHS foraging.

In addition to providing a potential food source, we examined the importance of vegetation for reproduction. We determined experimentally that BHS require vegetation for successful reproduction, and we determined that BHS does not require a sunfish nest associate to successfully spawn. This suggests that BHS eggs may be adhesive and the species uses aquatic vegetation as attachment sites for eggs. This mode of reproduction would be advantageous in habitats with soft, silty, detrital substrates like those supporting BHS. Thus, aquatic vegetation may be a critical environmental factor required for BHS reproduction, which would explain the documented concordance between this fish and aquatic

The environmental analysis and predictive model suggest that there were a number of localities within the watershed that did not support BHS but have environmental parameters suitable for this species. These localities had similar vegetation, substrates and depths as the localities supporting BHS. Therefore, if the BHS fits a metapopulation model in Texas, it is possible that the BHS distribution expands and contracts from these habitats over time. Thus, long-term, repeated sampling at sentinel sites throughout the drainage may provide a robust method for testing the metapopulation hypotheses proposed here. Regardless, any management efforts to protect or restore habitat for BHS should begin with promoting growth of emergent vegetation.

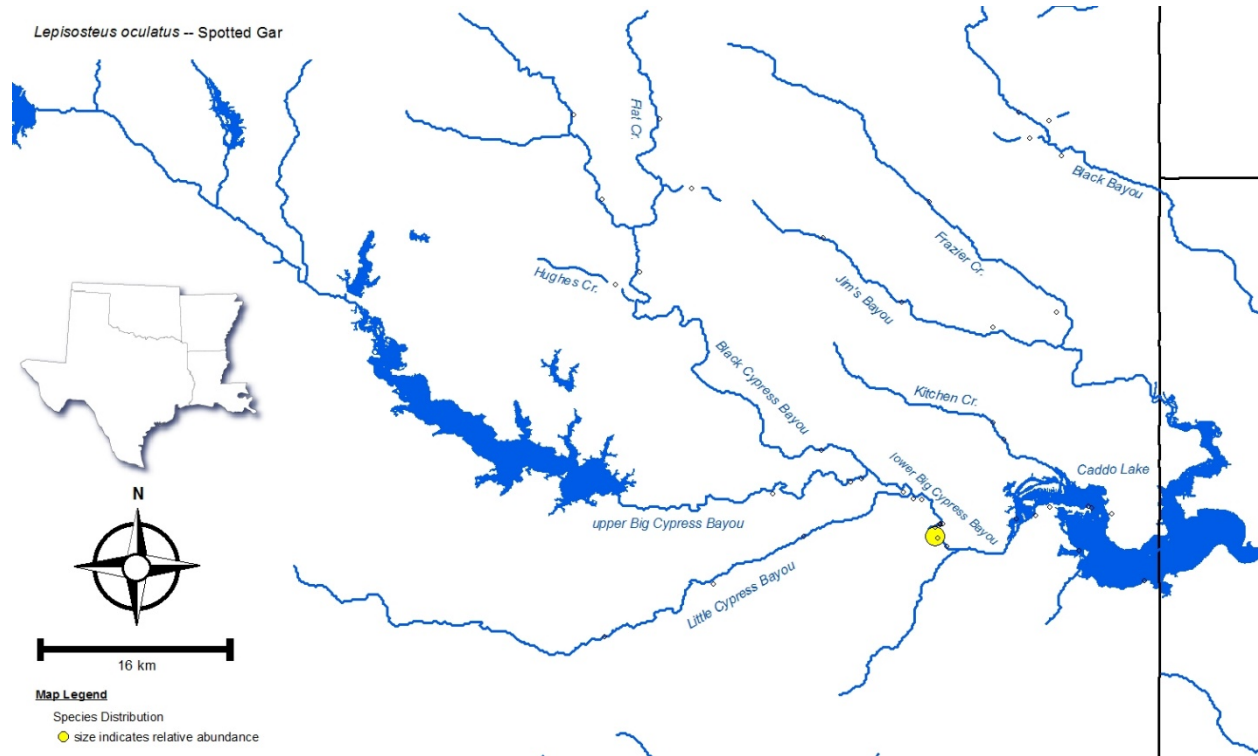
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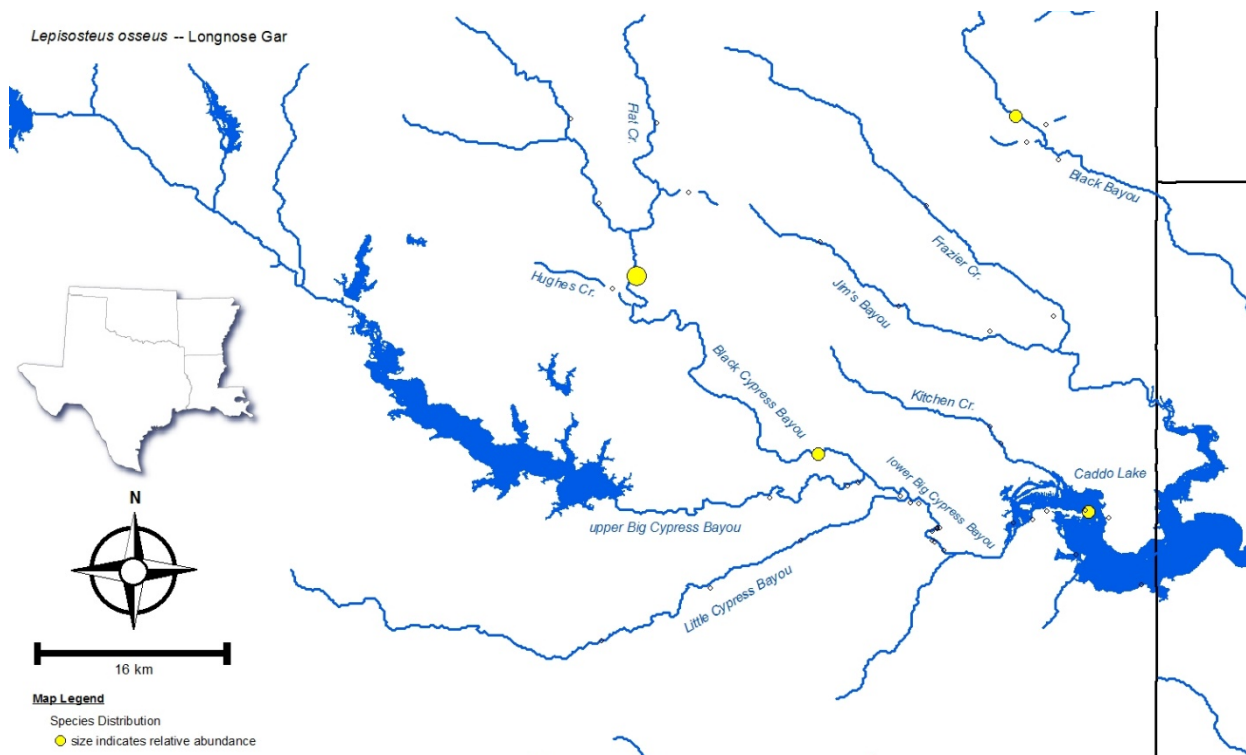
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Appendix 1. Distribution maps of all fish species collected from 2015-2017 in the Big Cypress-Sulphur River Basin, Texas. Distribution maps are grouped by Family and order corresponds to Table 2 in text of the report.

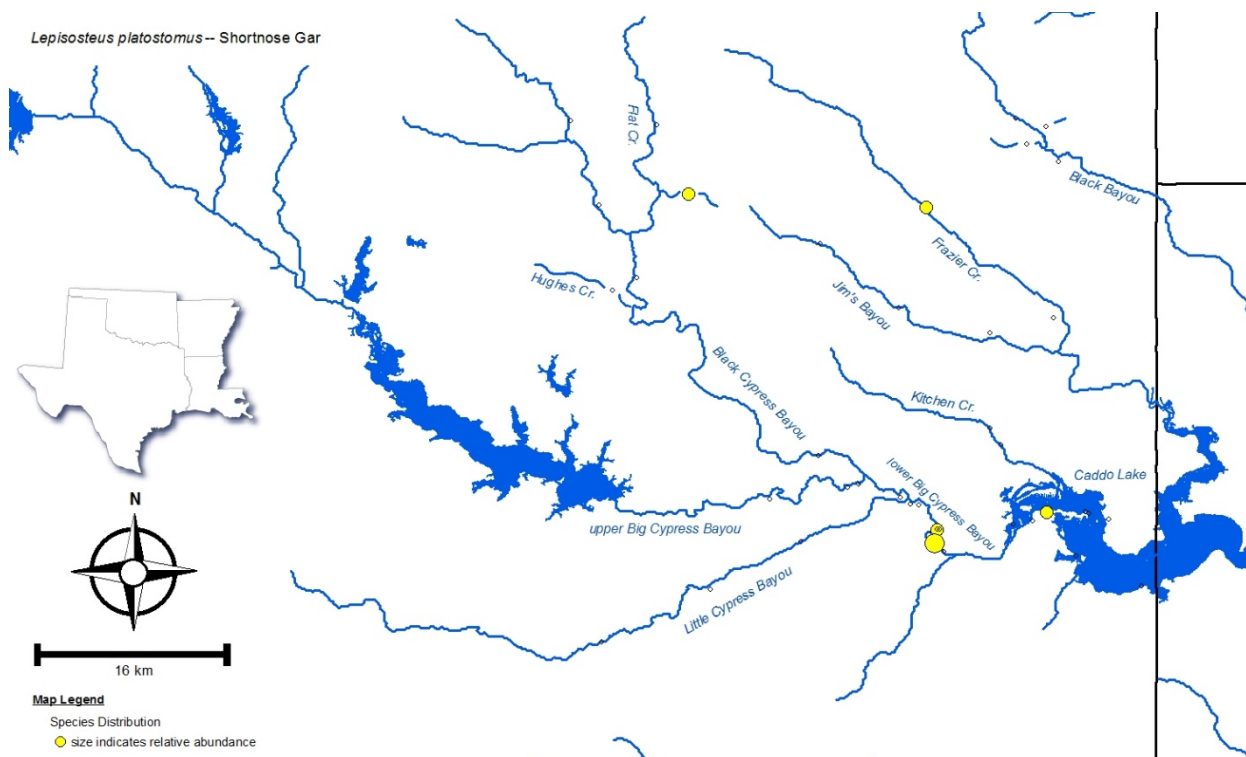
### LEPISOSTEIDAE - GARS



*Lepisosteus osseus* -- Longnose Gar

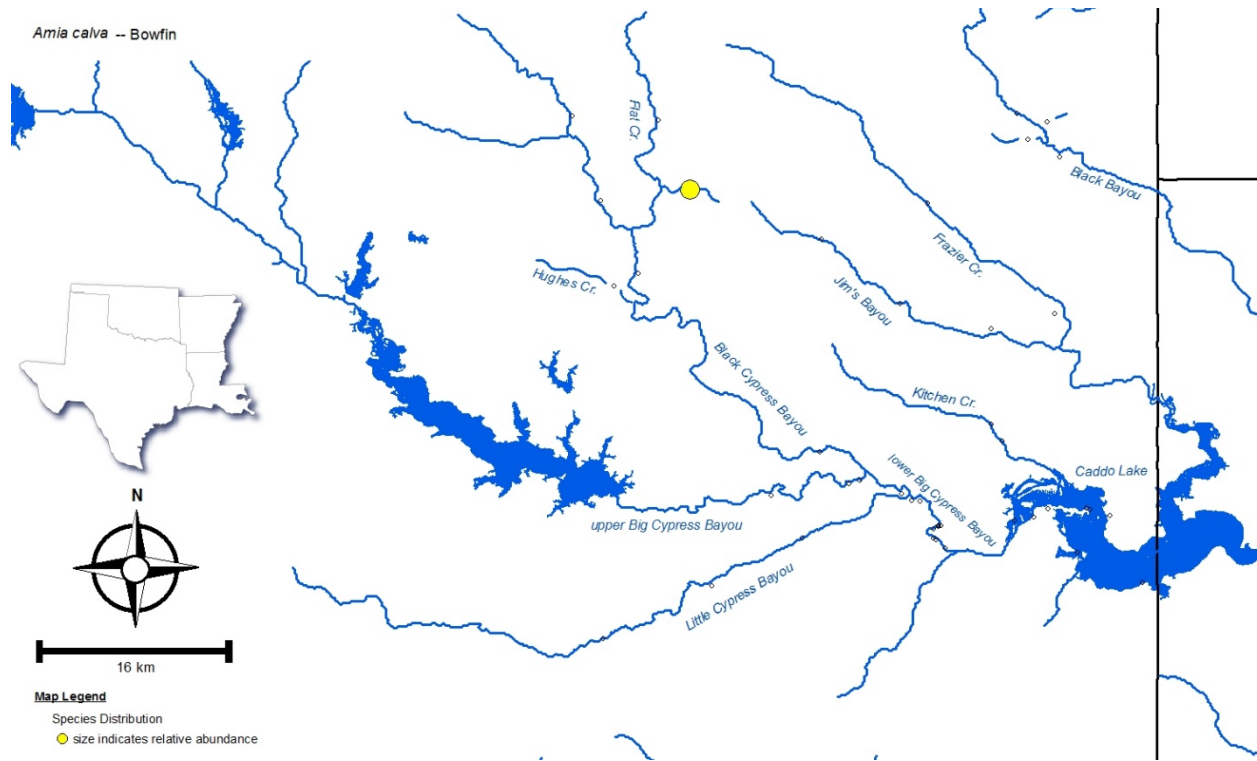


*Lepisosteus platostomus* -- Shortnose Gar

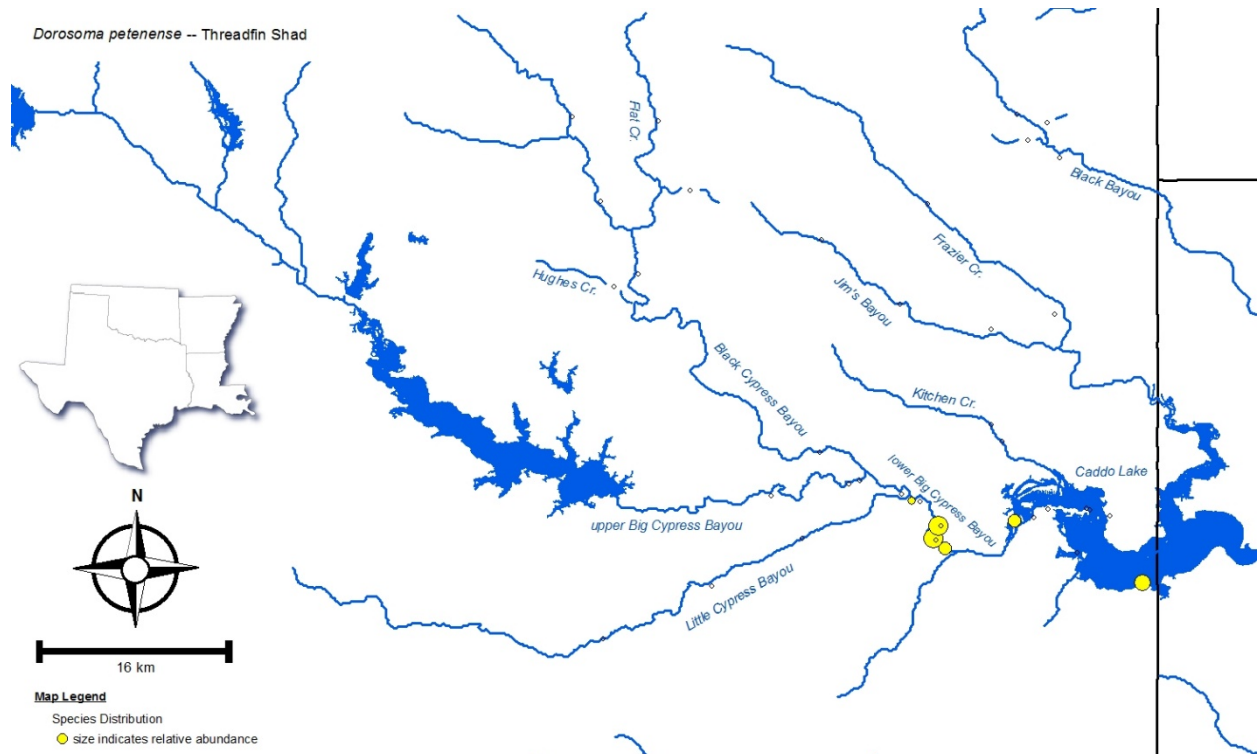
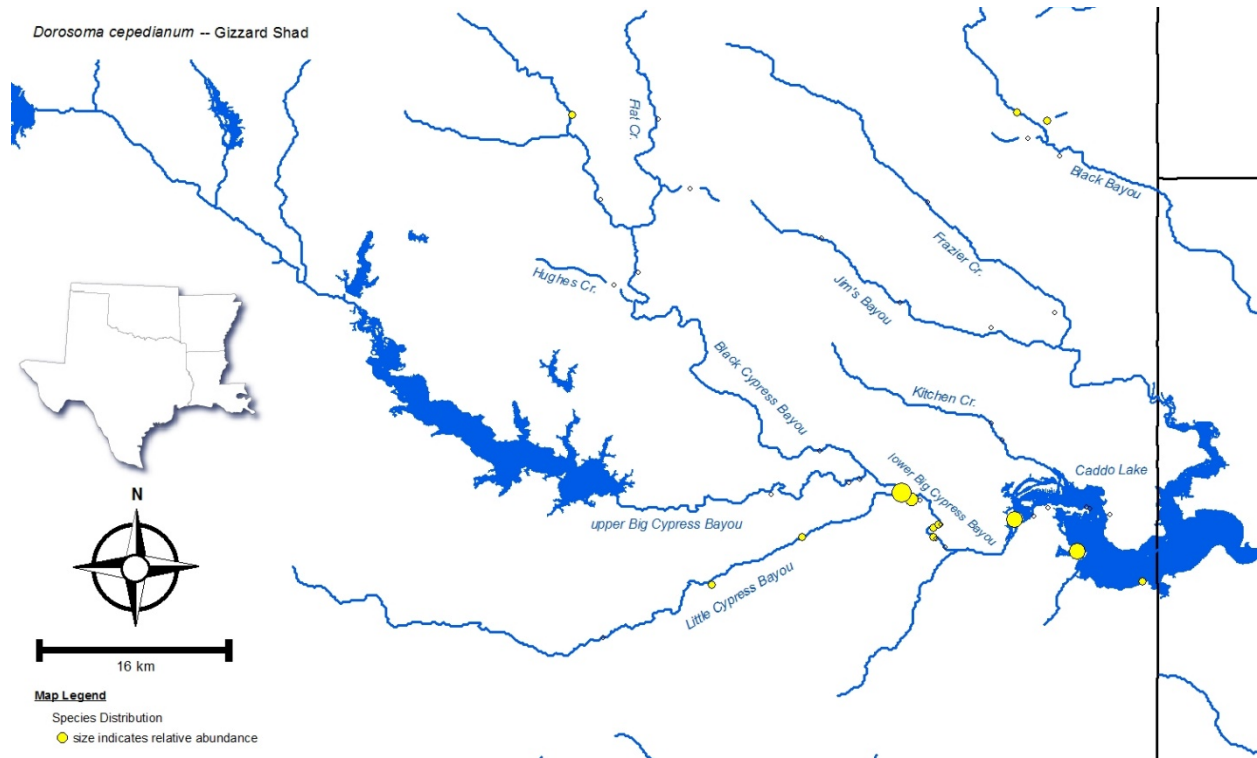




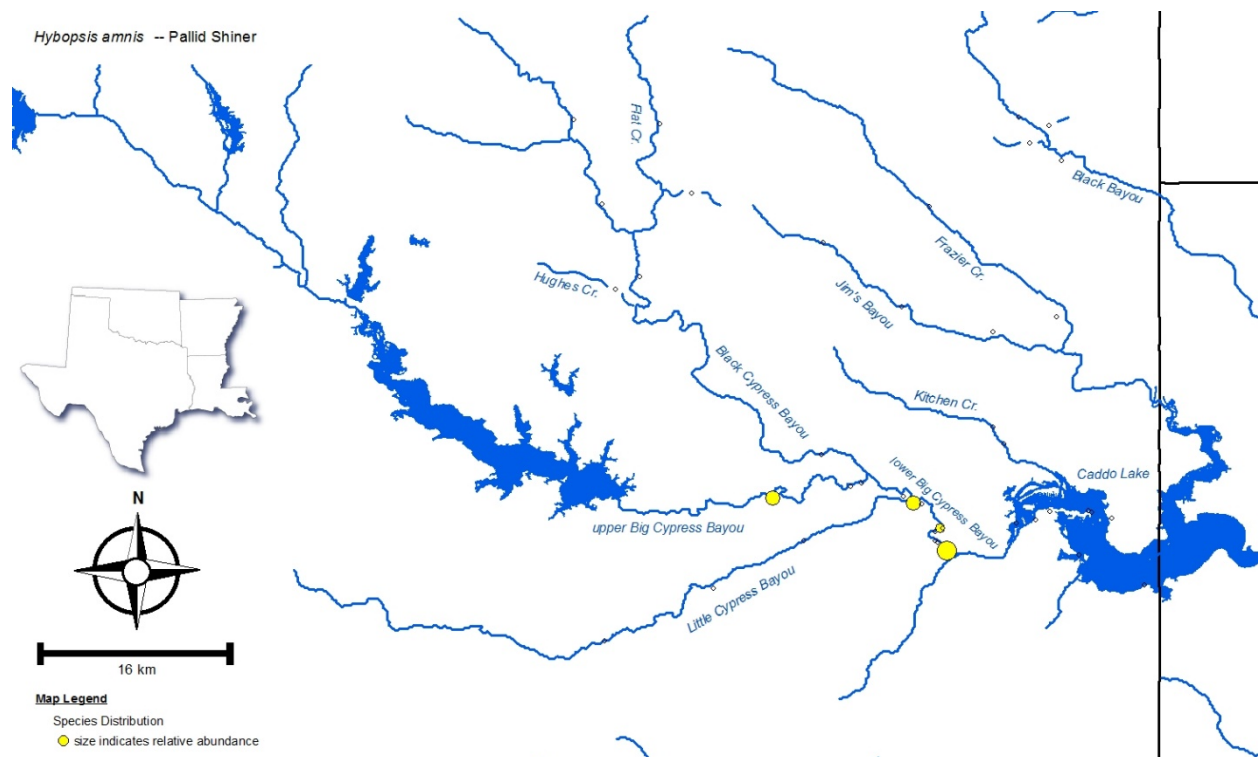
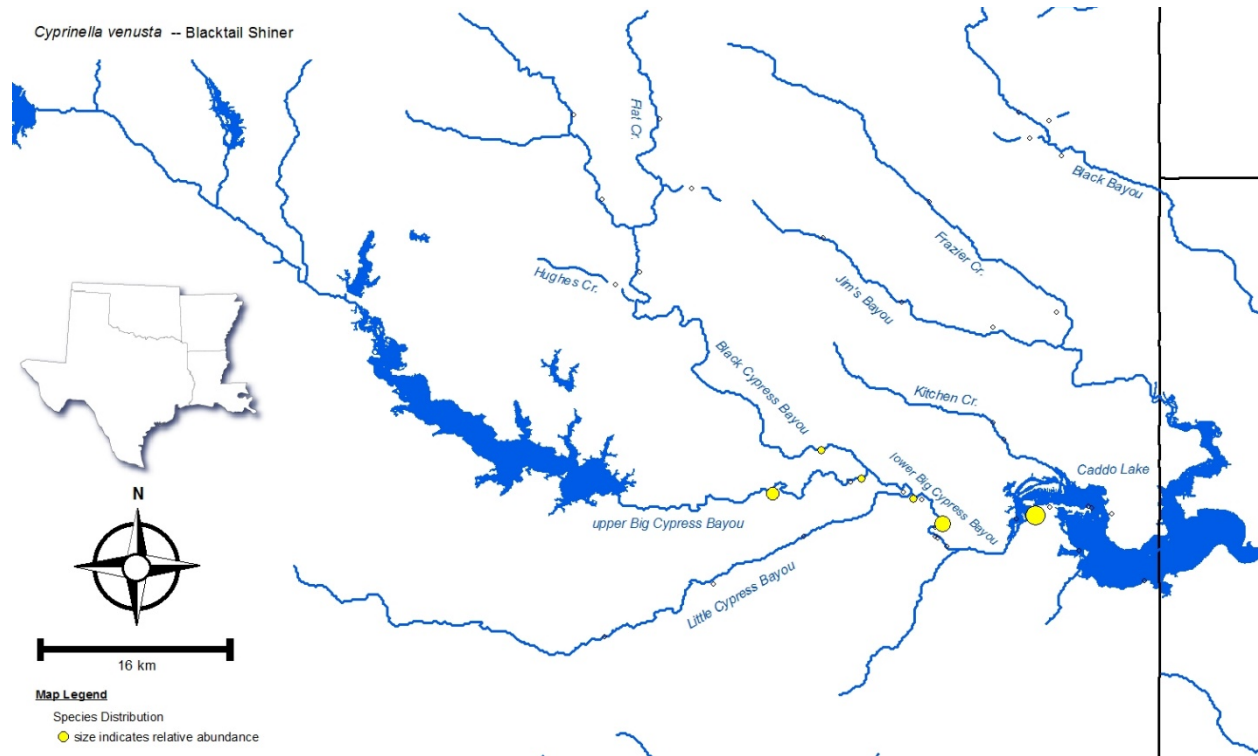
## AMIIDAE - BOWFINS

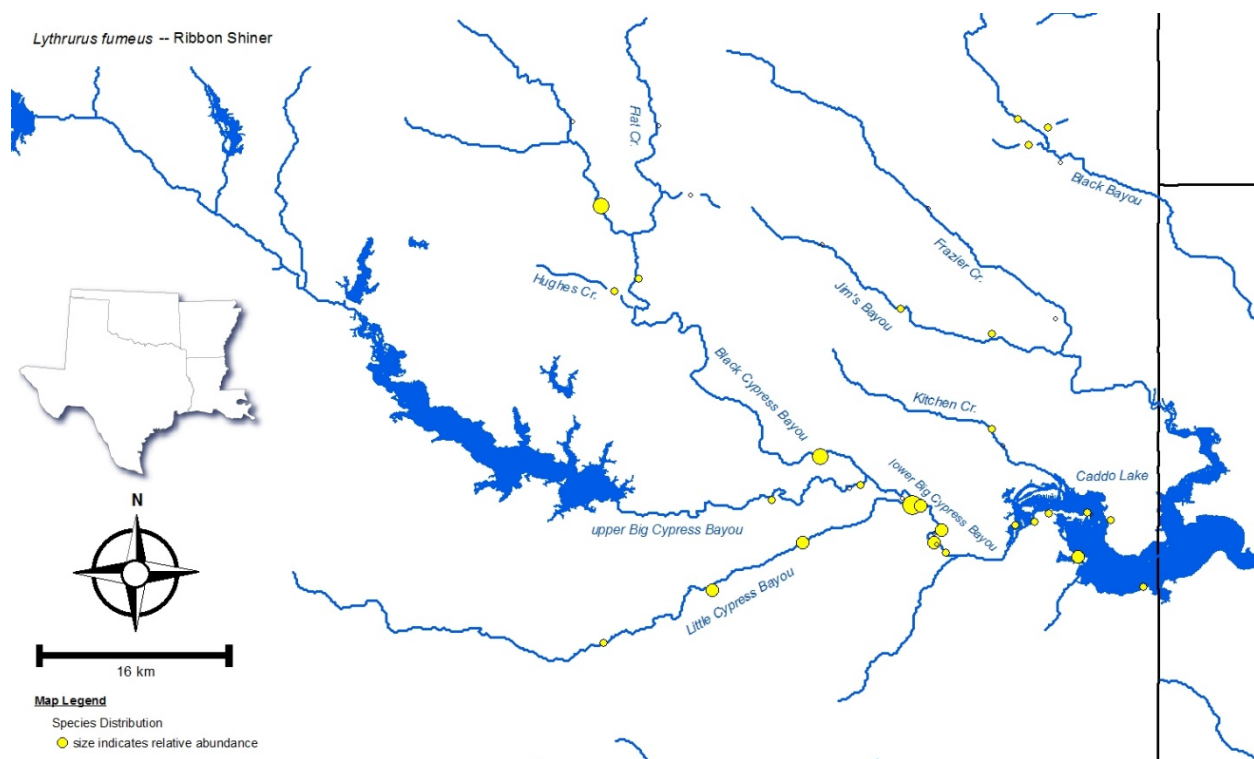
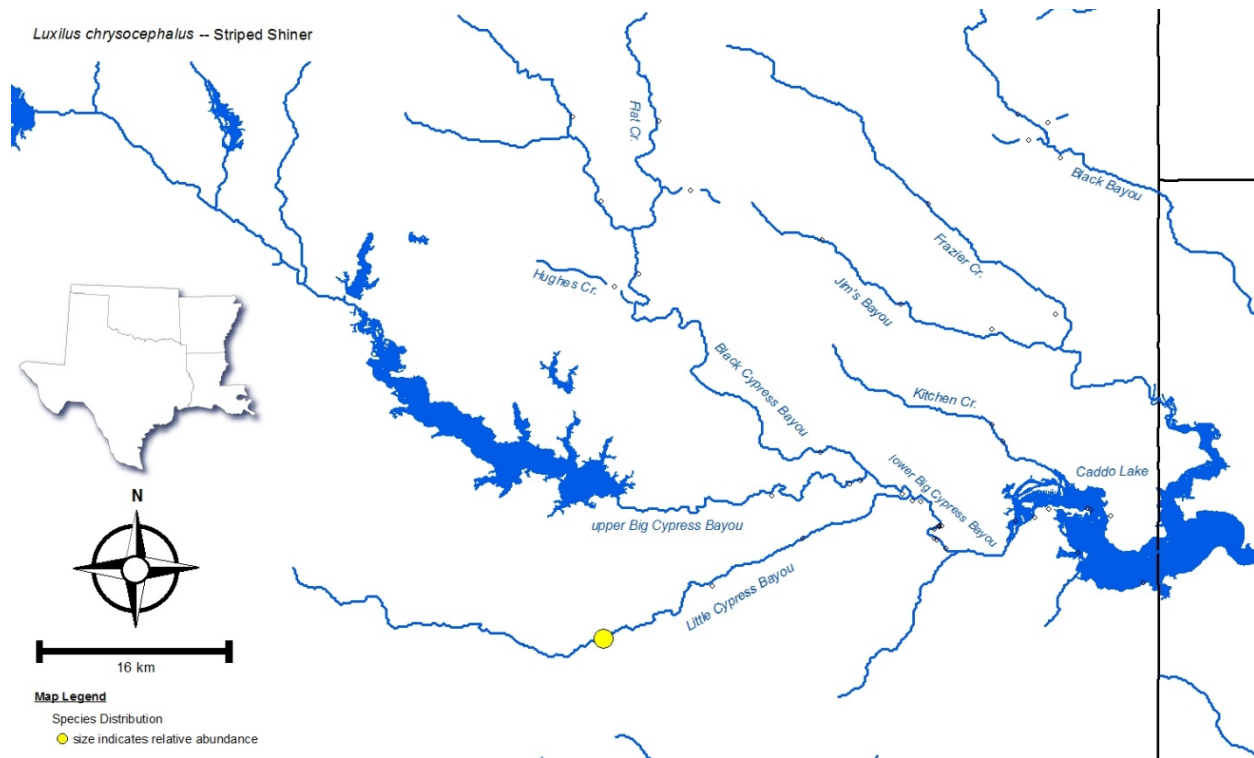


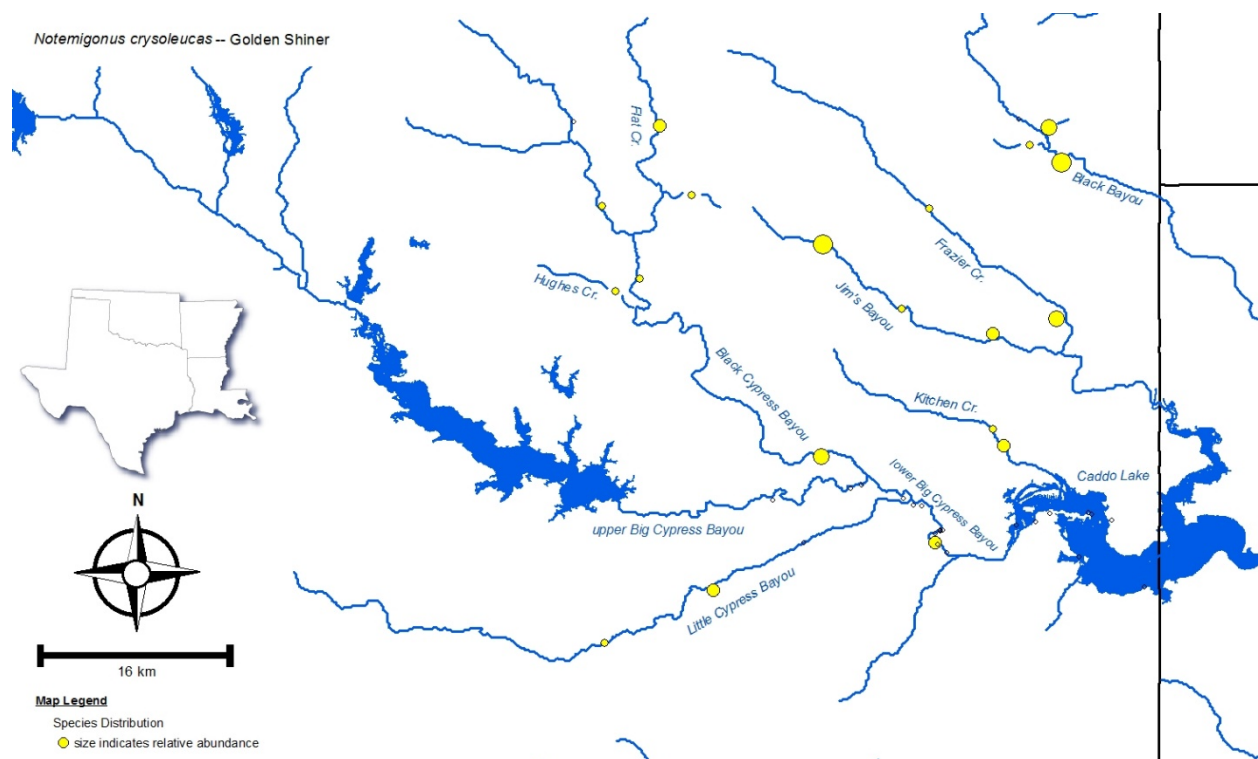
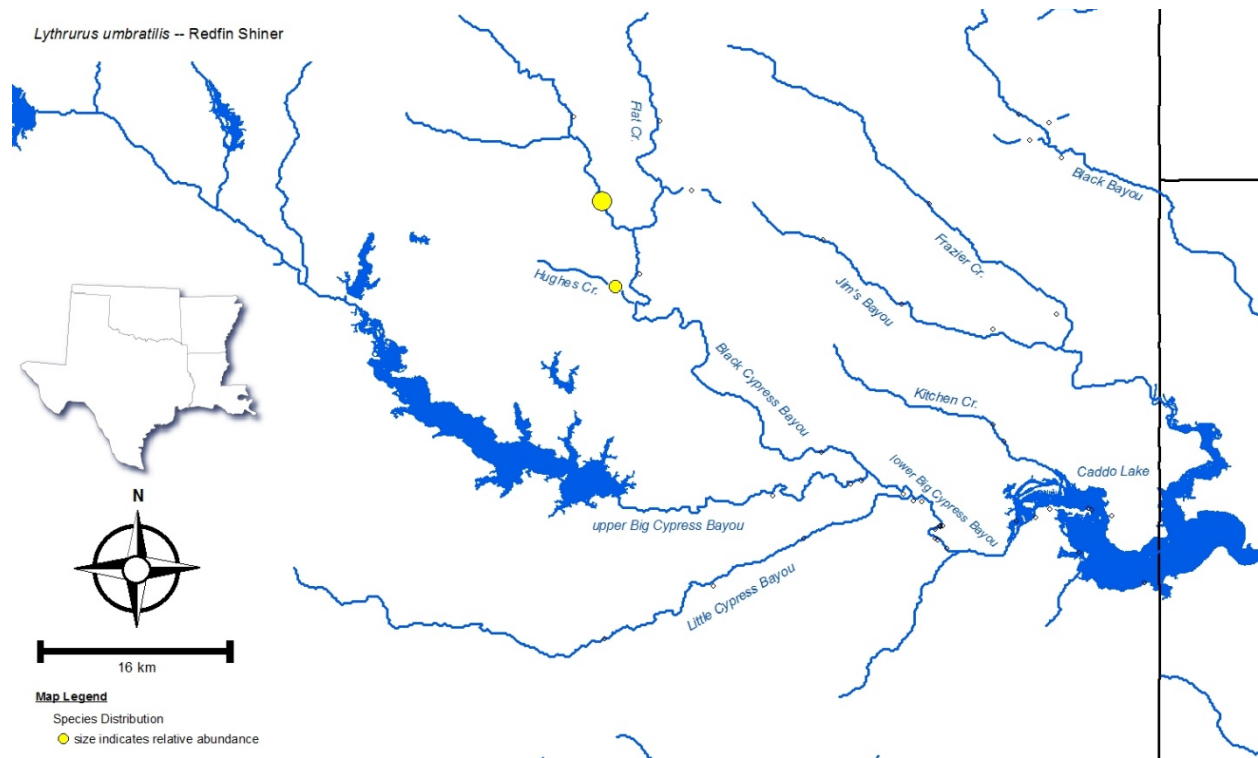
## CLUPEIDAE – SHADS



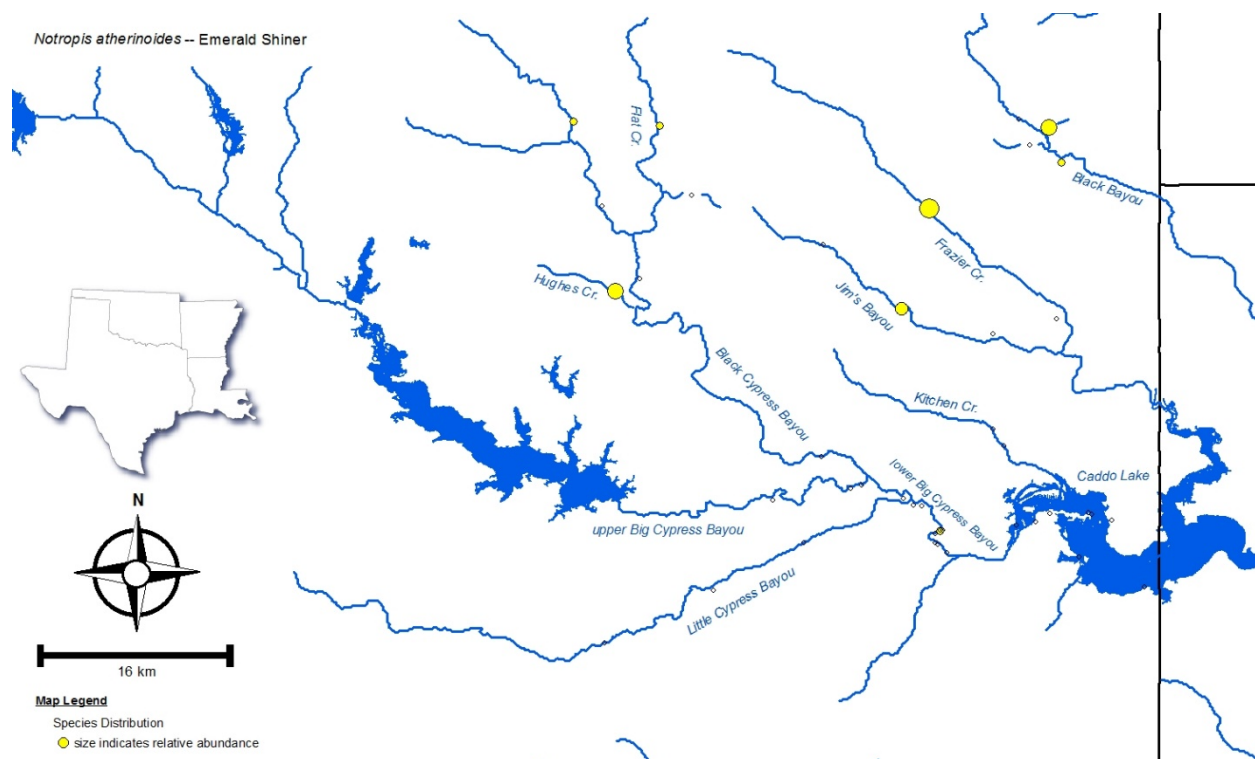
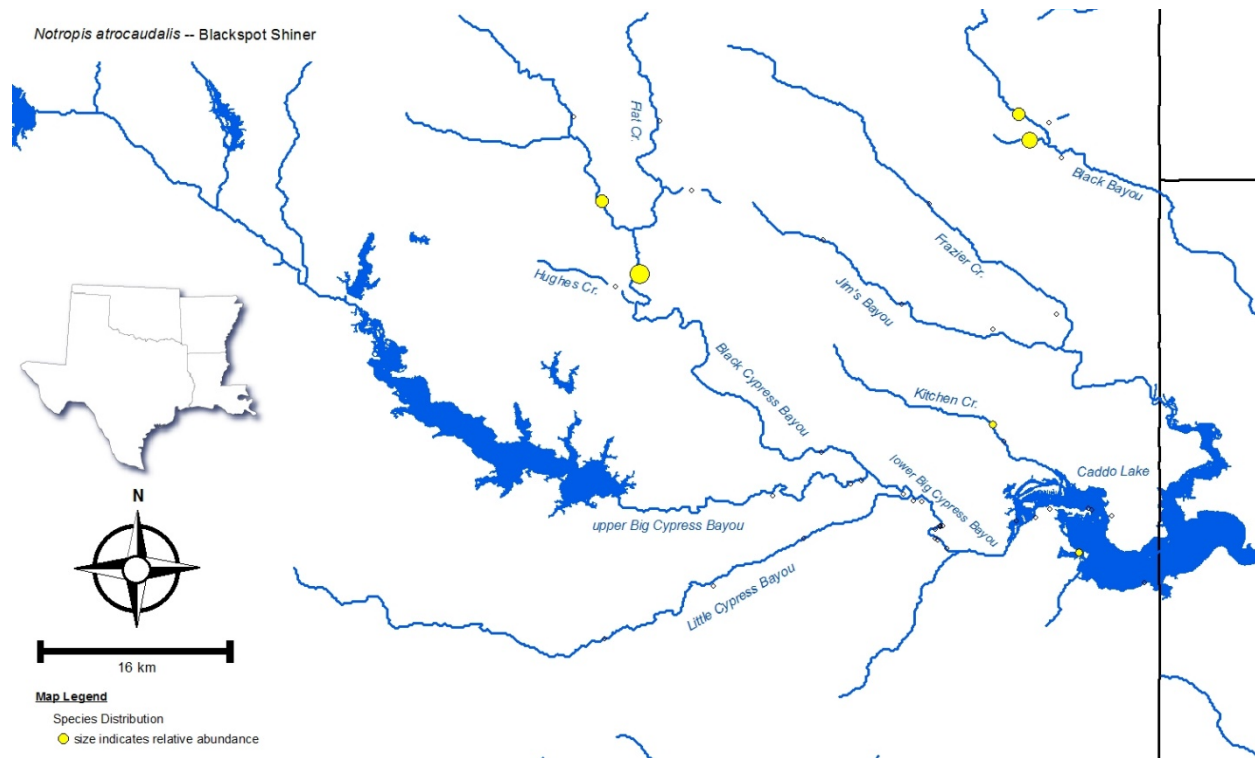
## CYPRINIDAE – MINNOWS

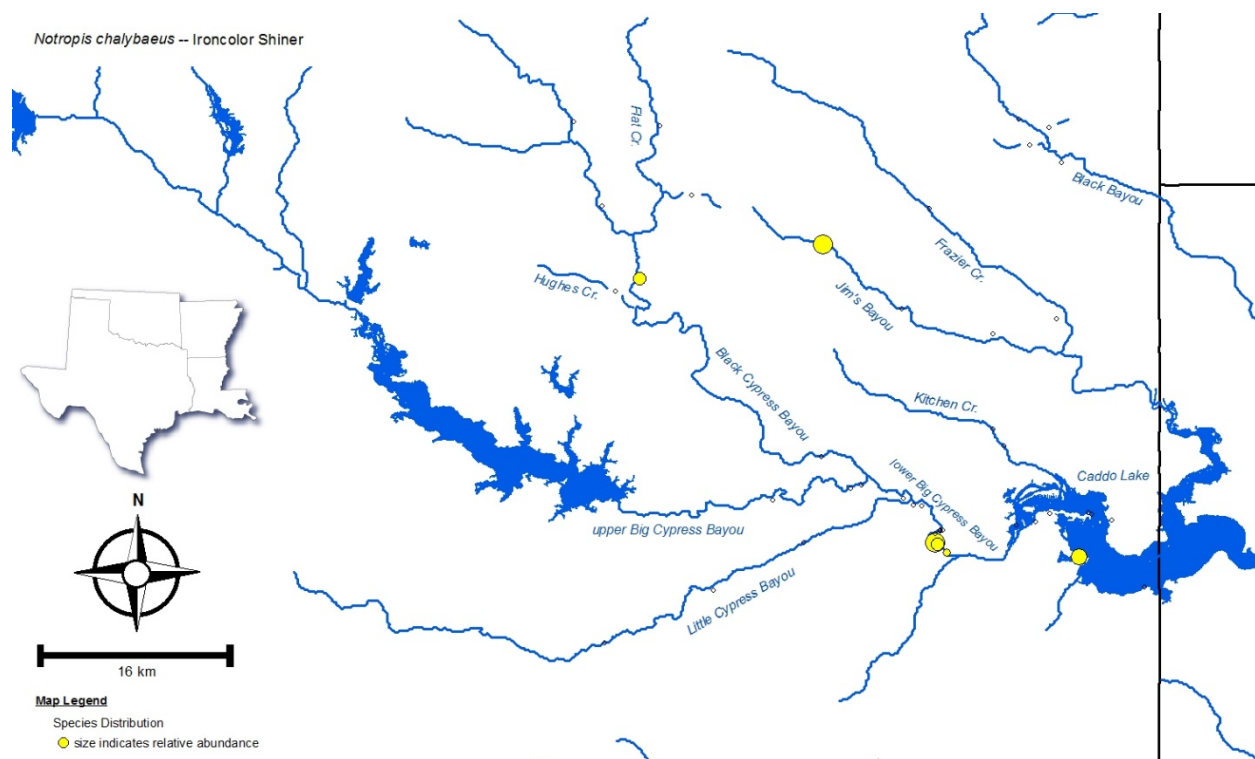
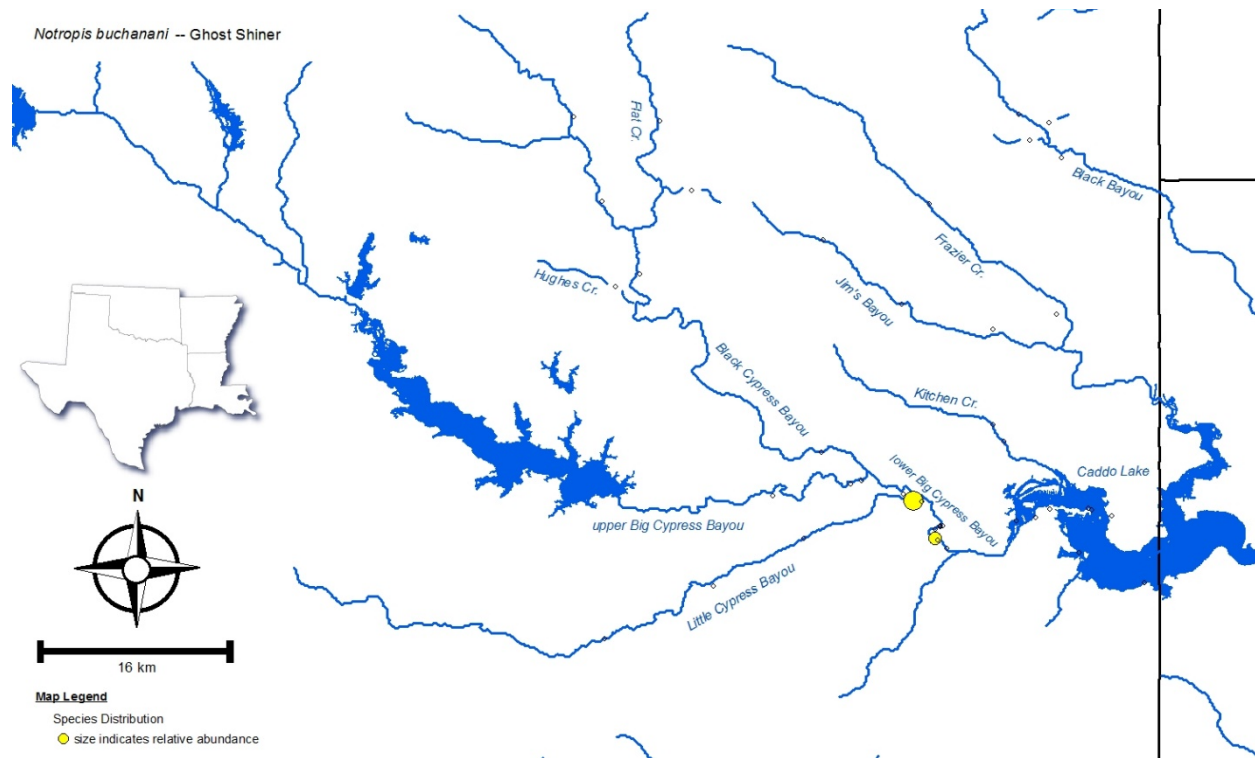




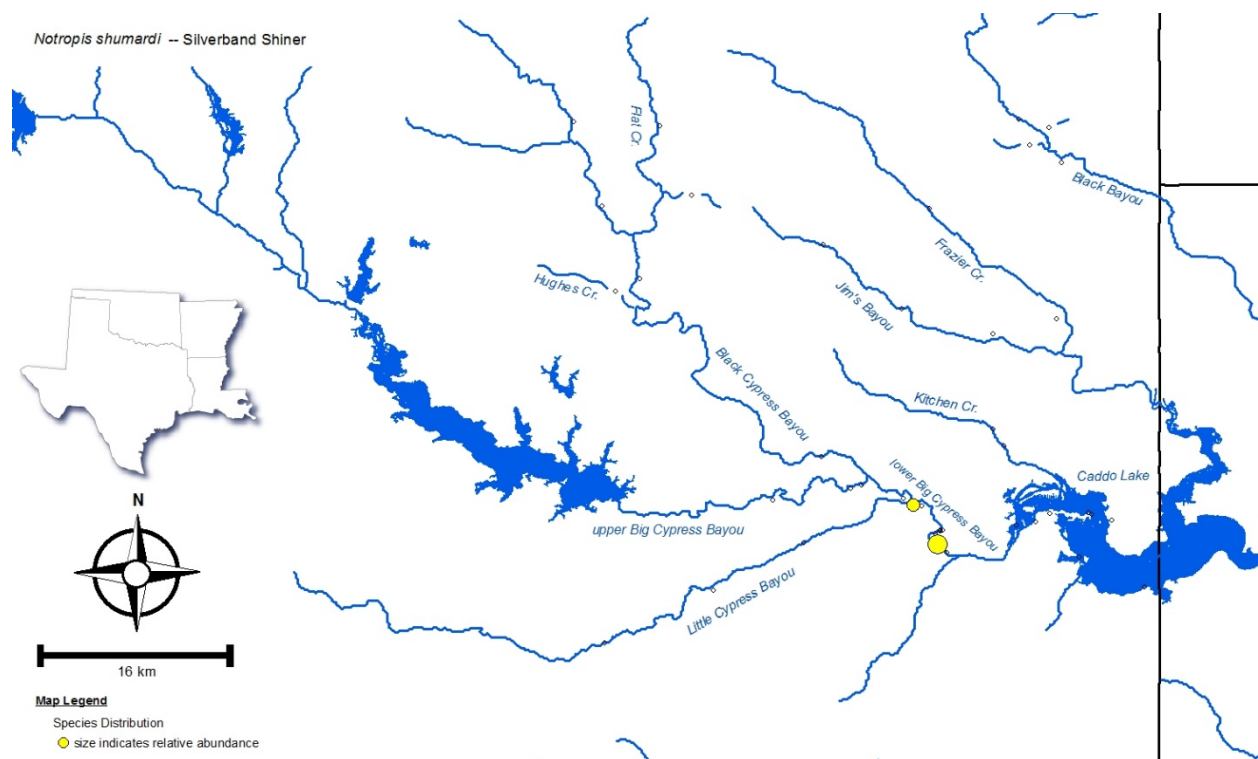
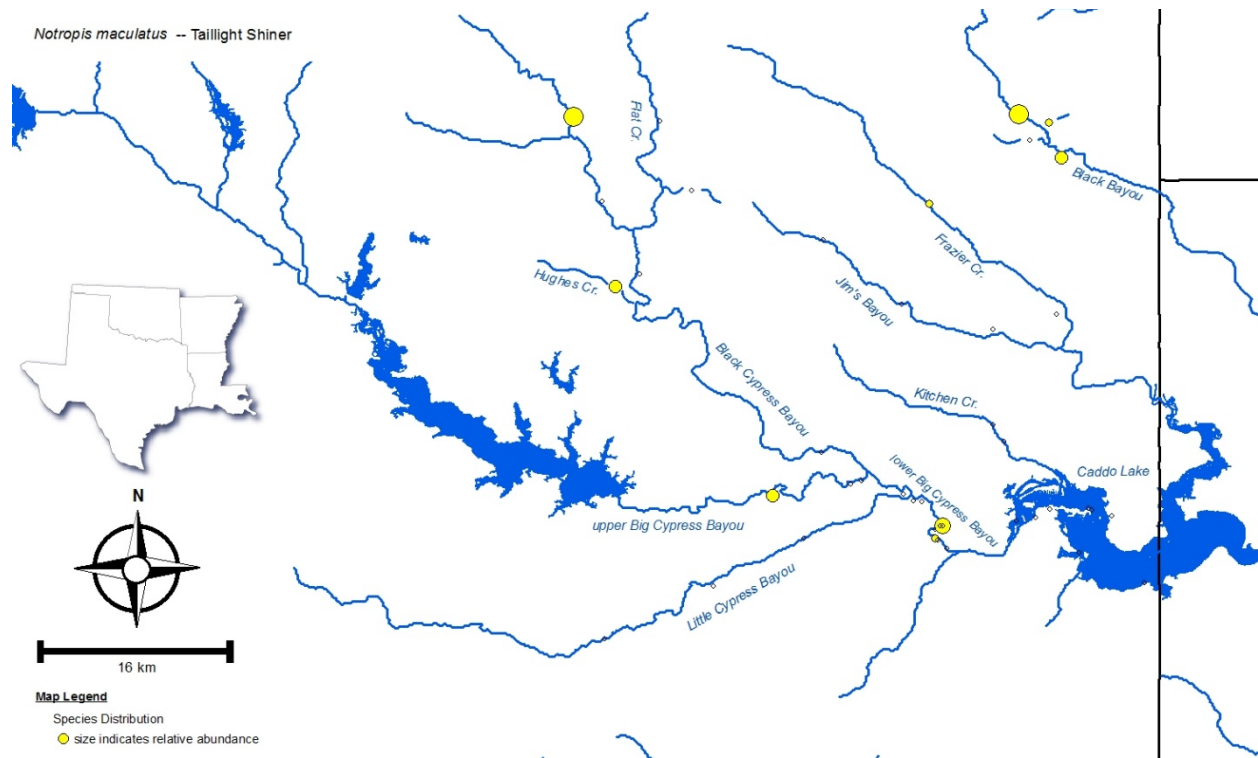


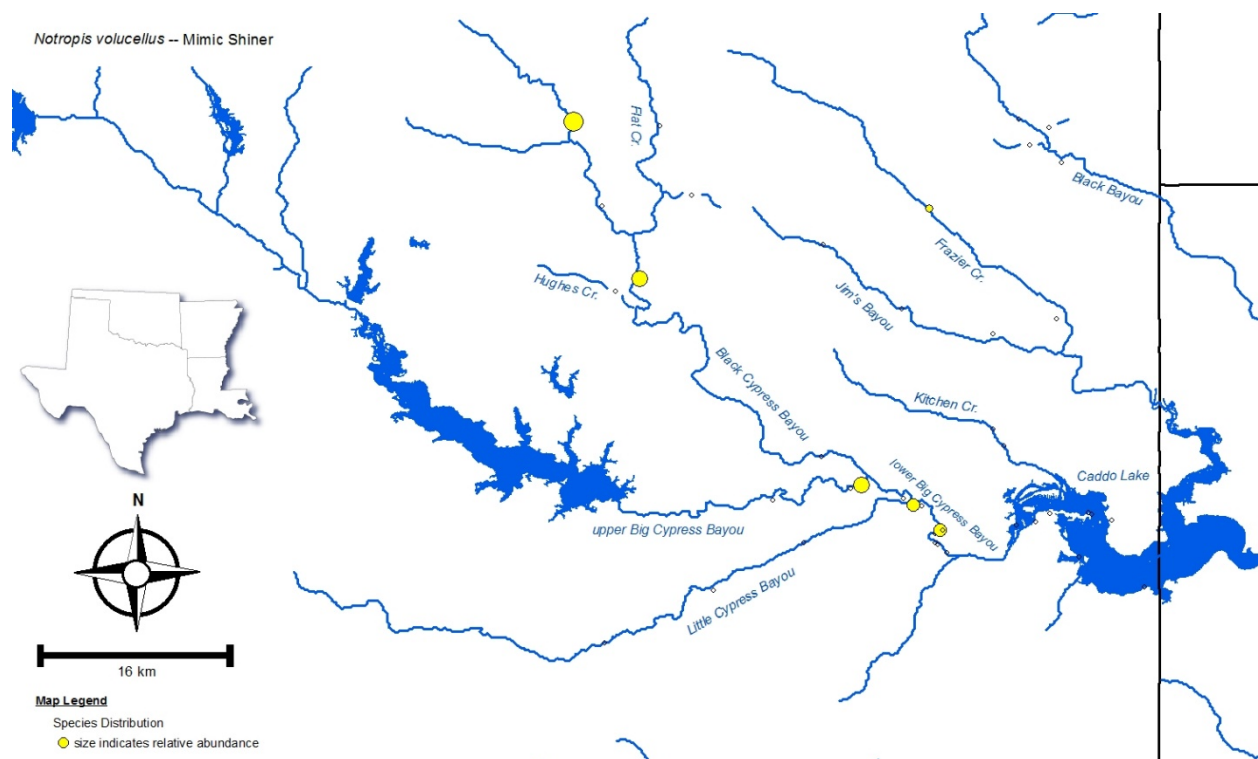
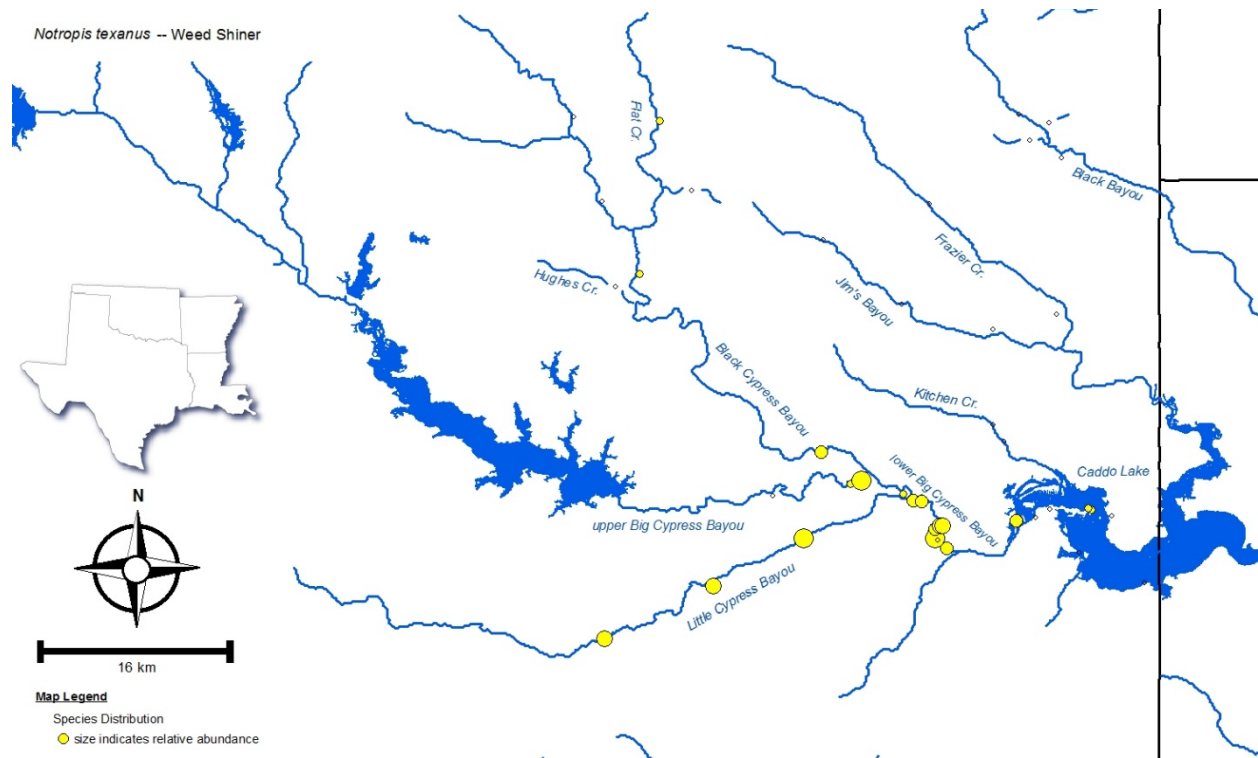


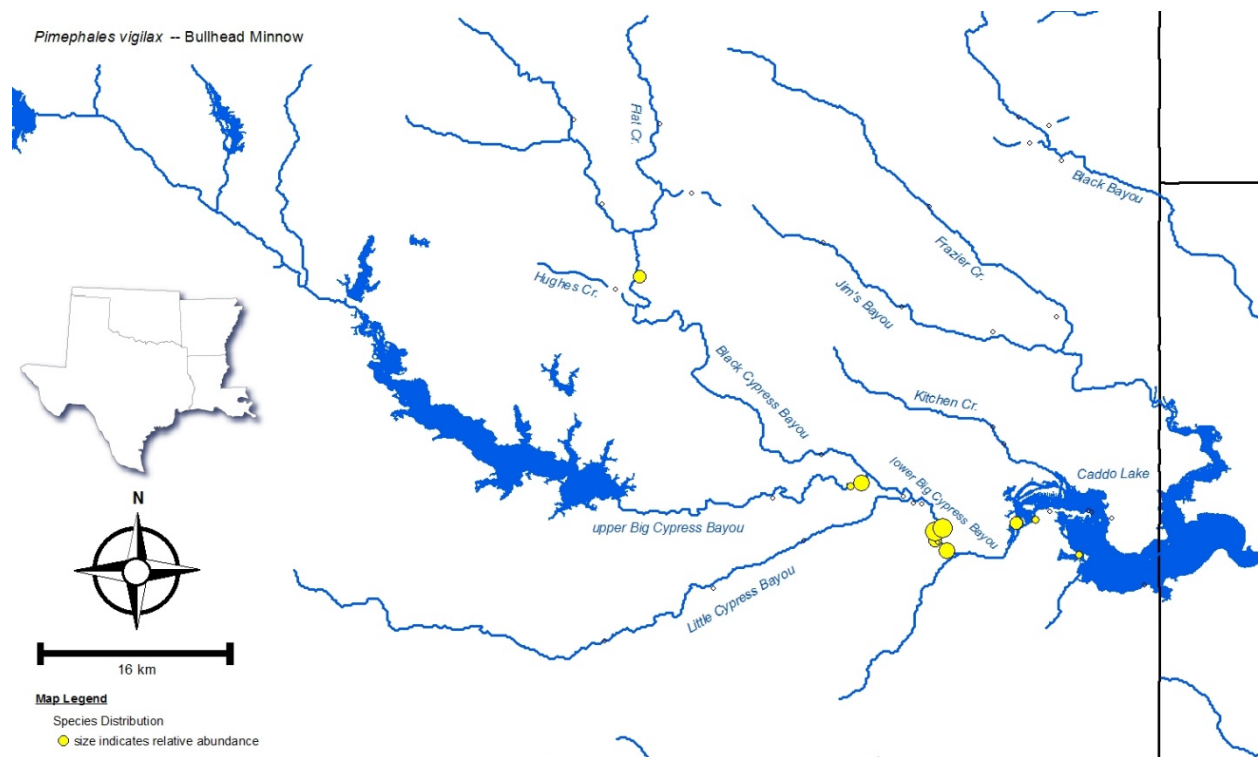
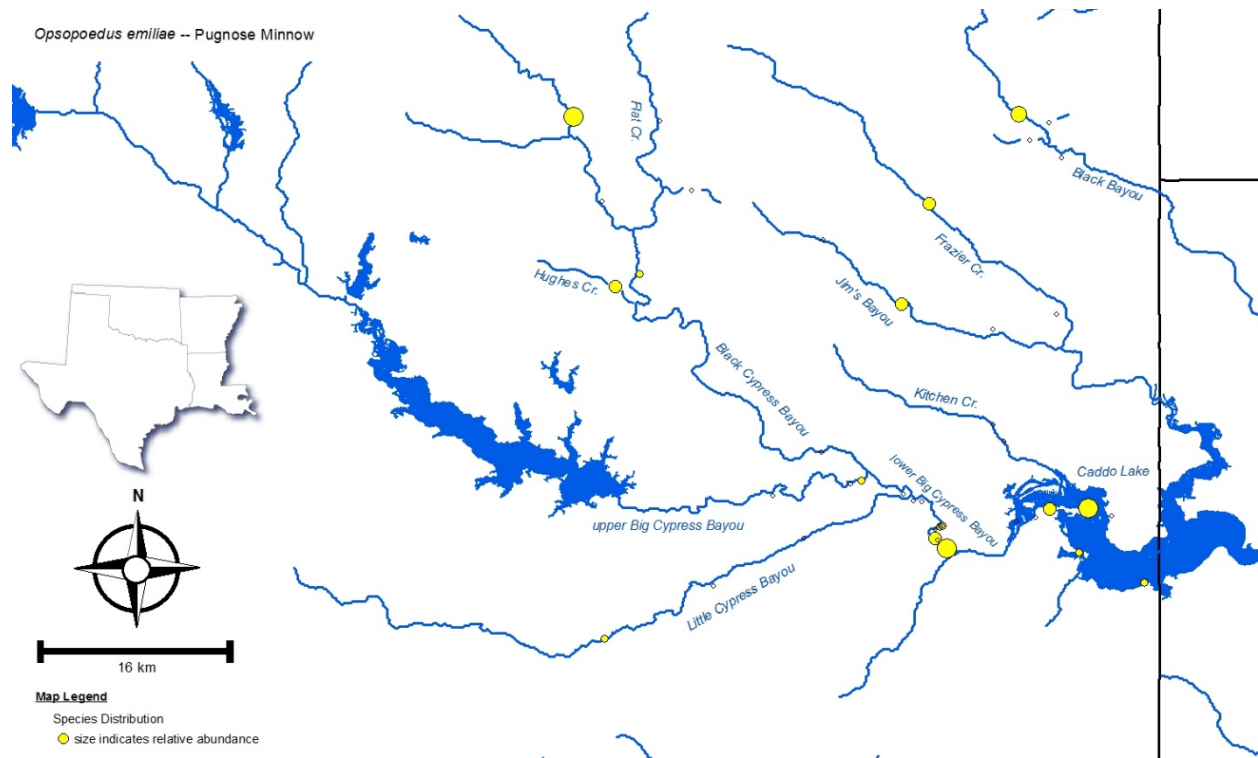




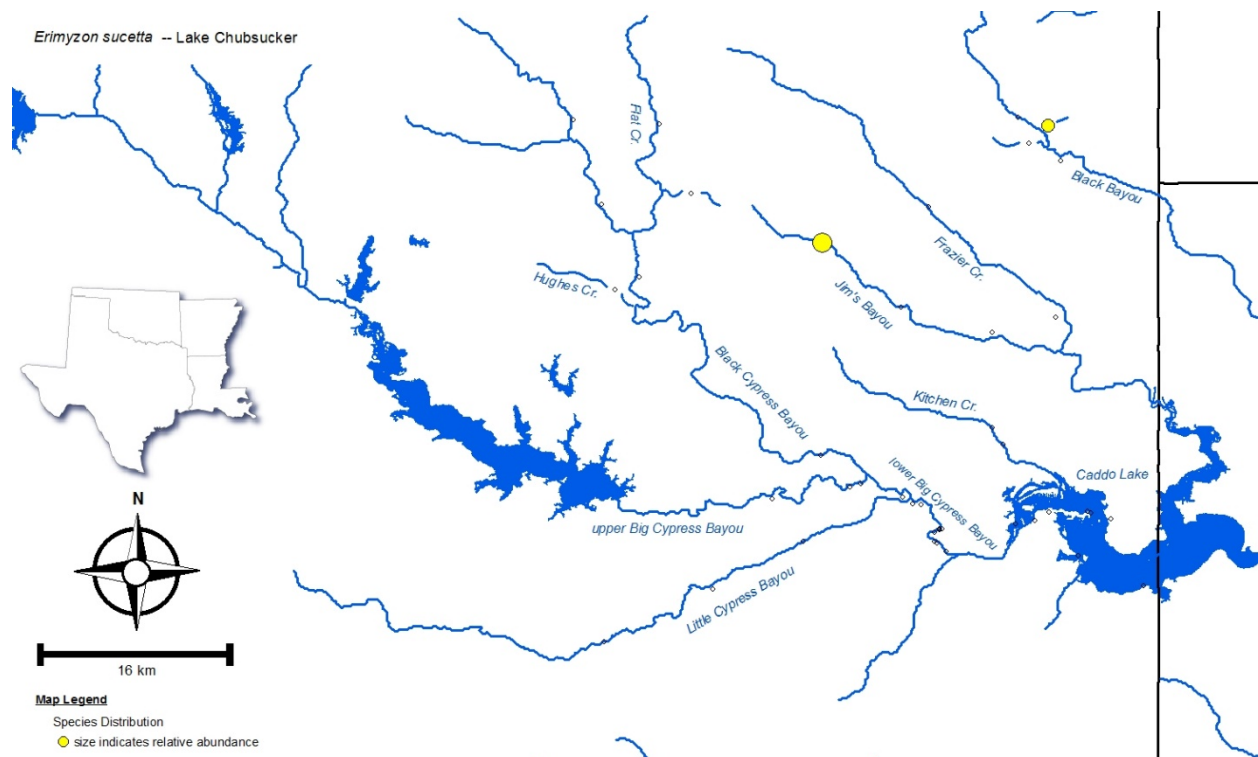
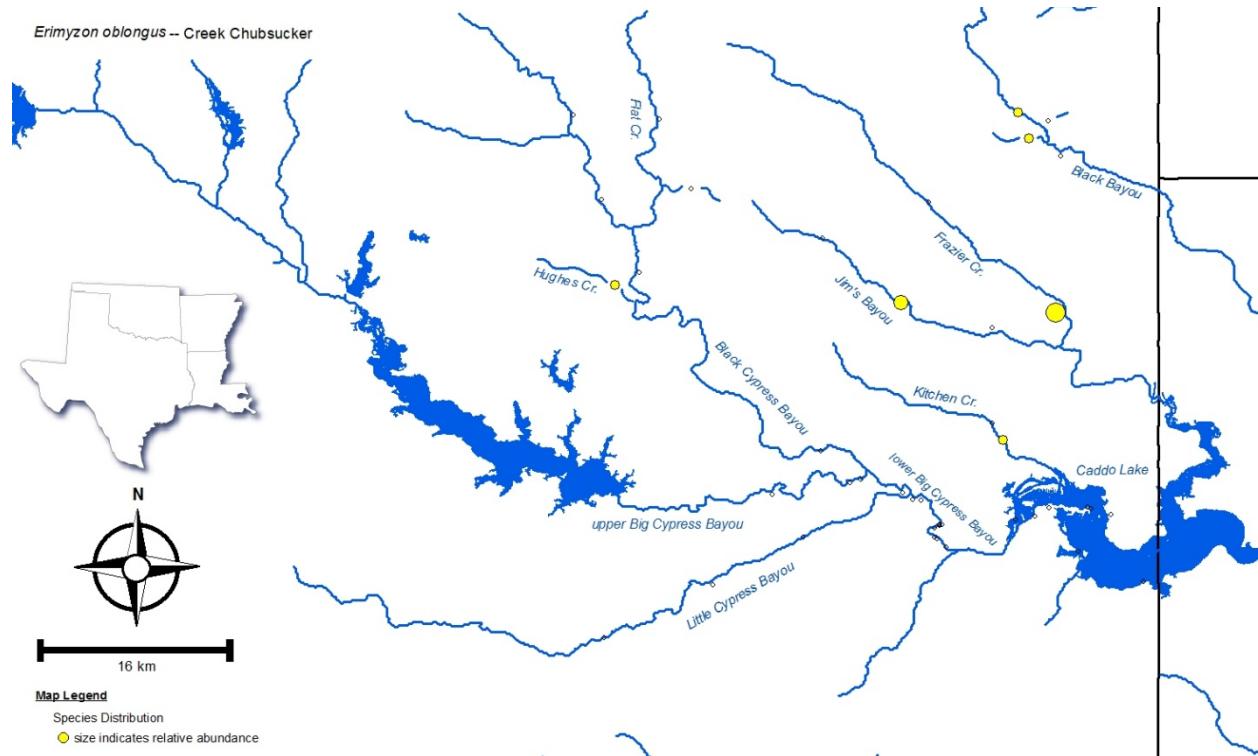


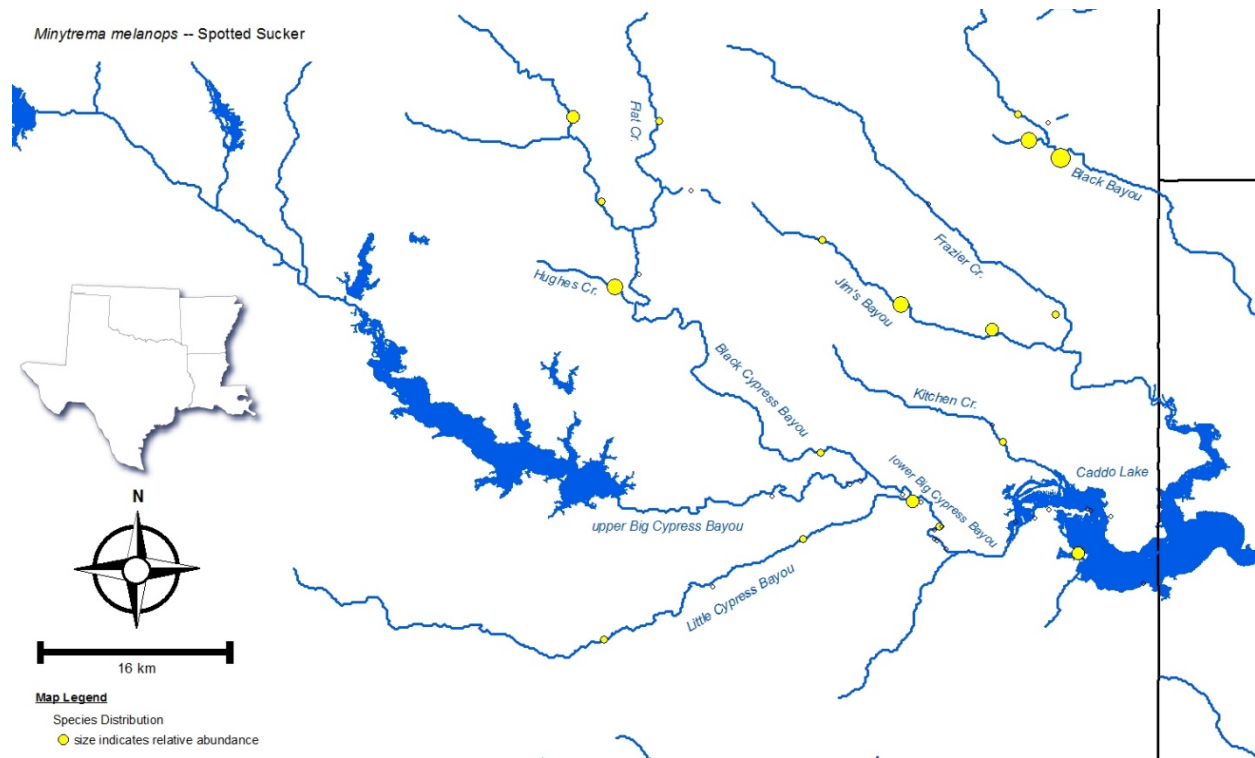






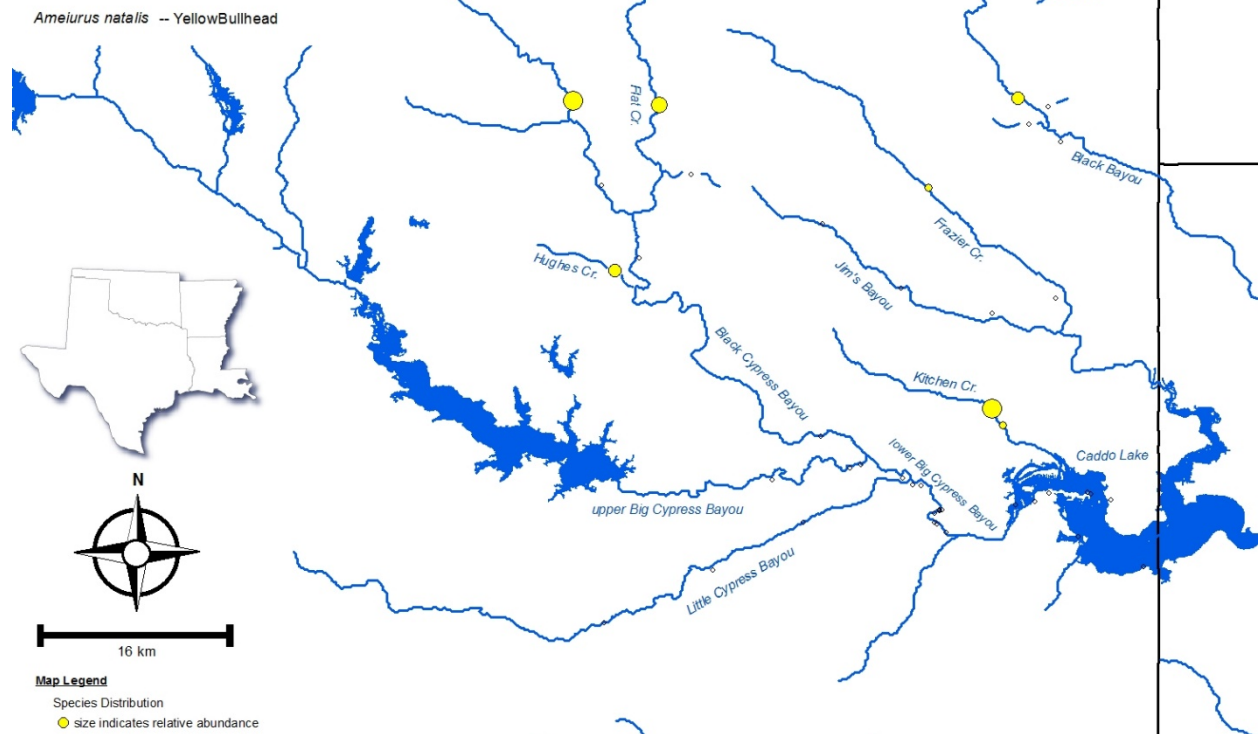
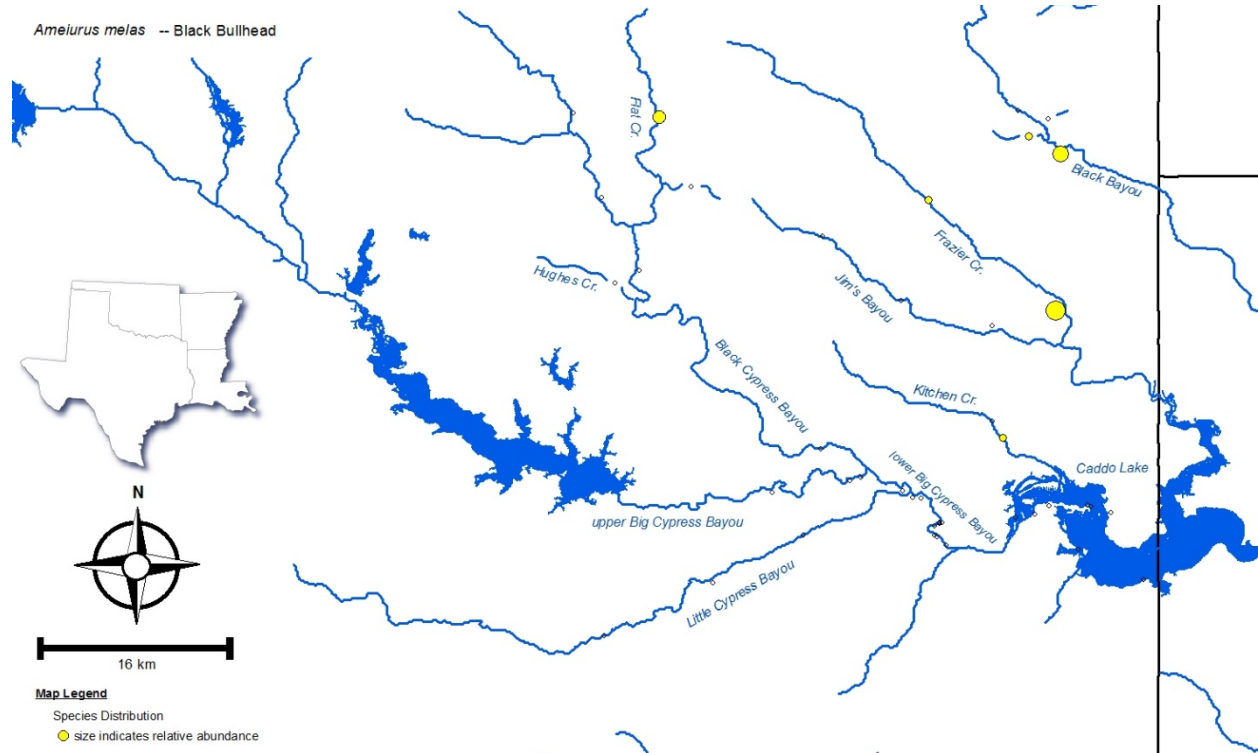
## CATOSTOMIDAE - SUCKERS





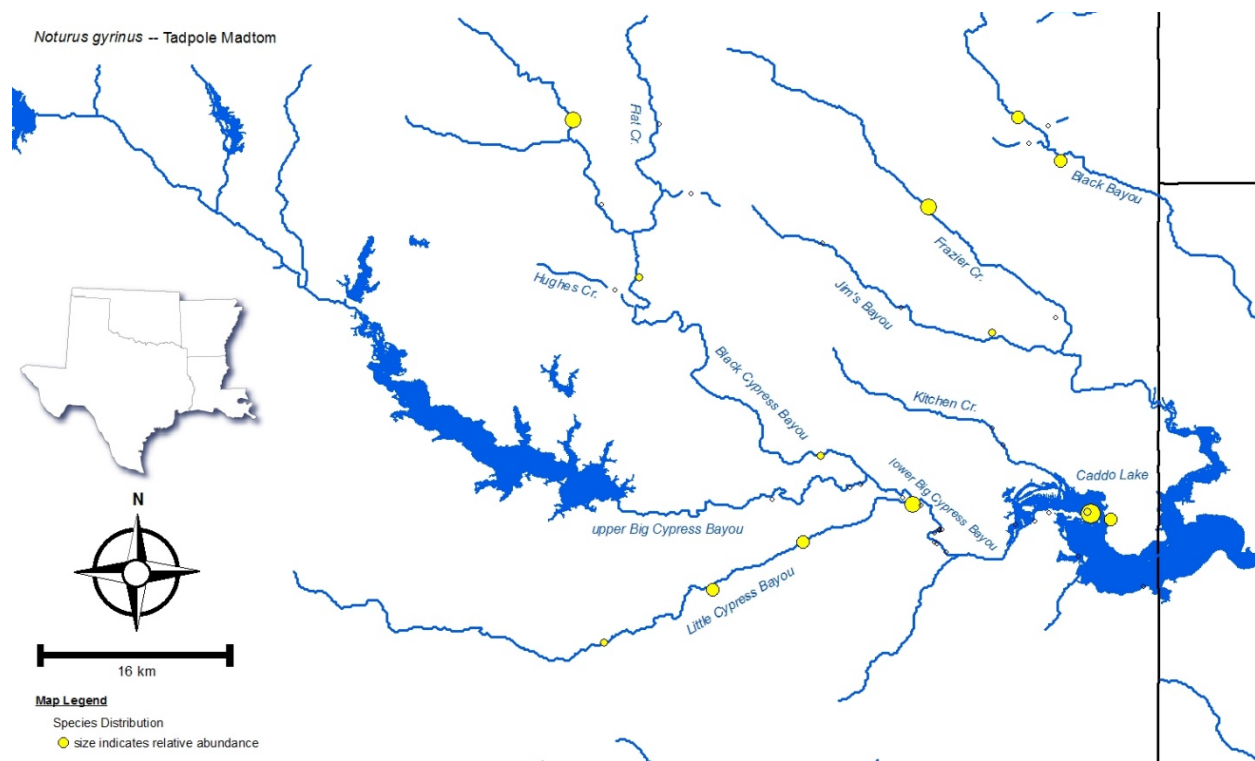


## ICTALURIDAE – CATFISHES

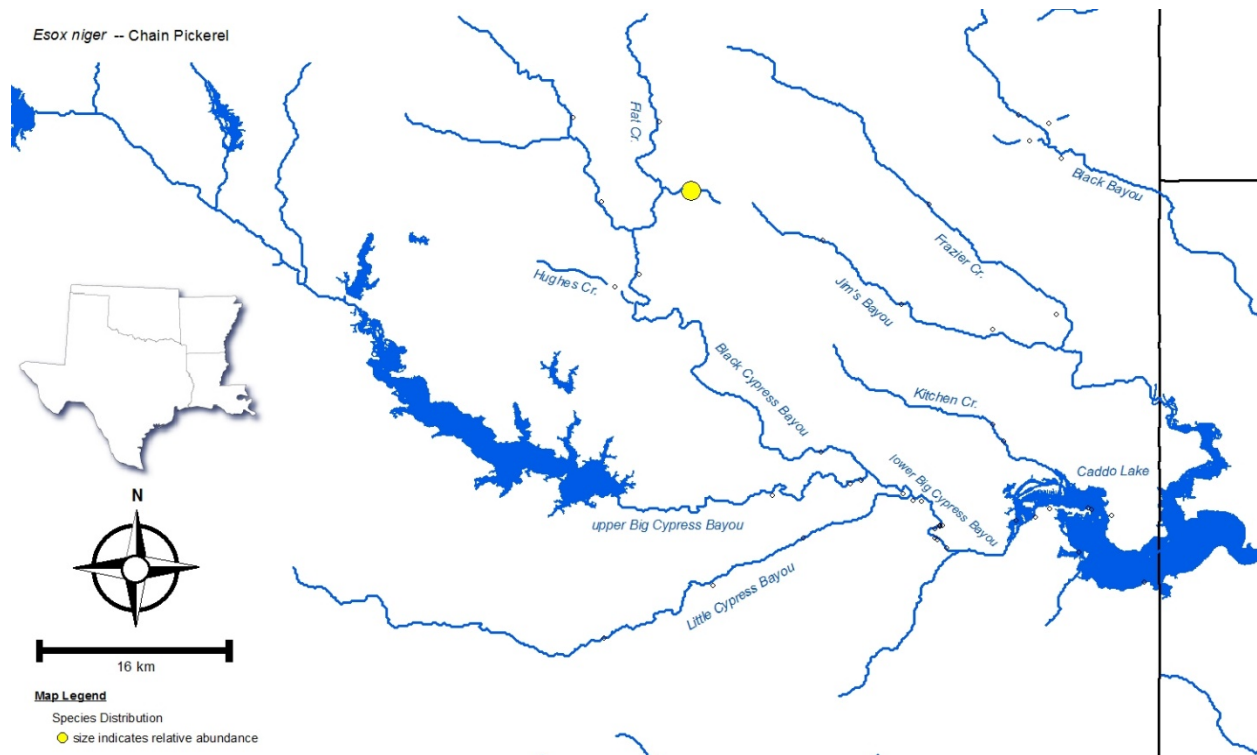
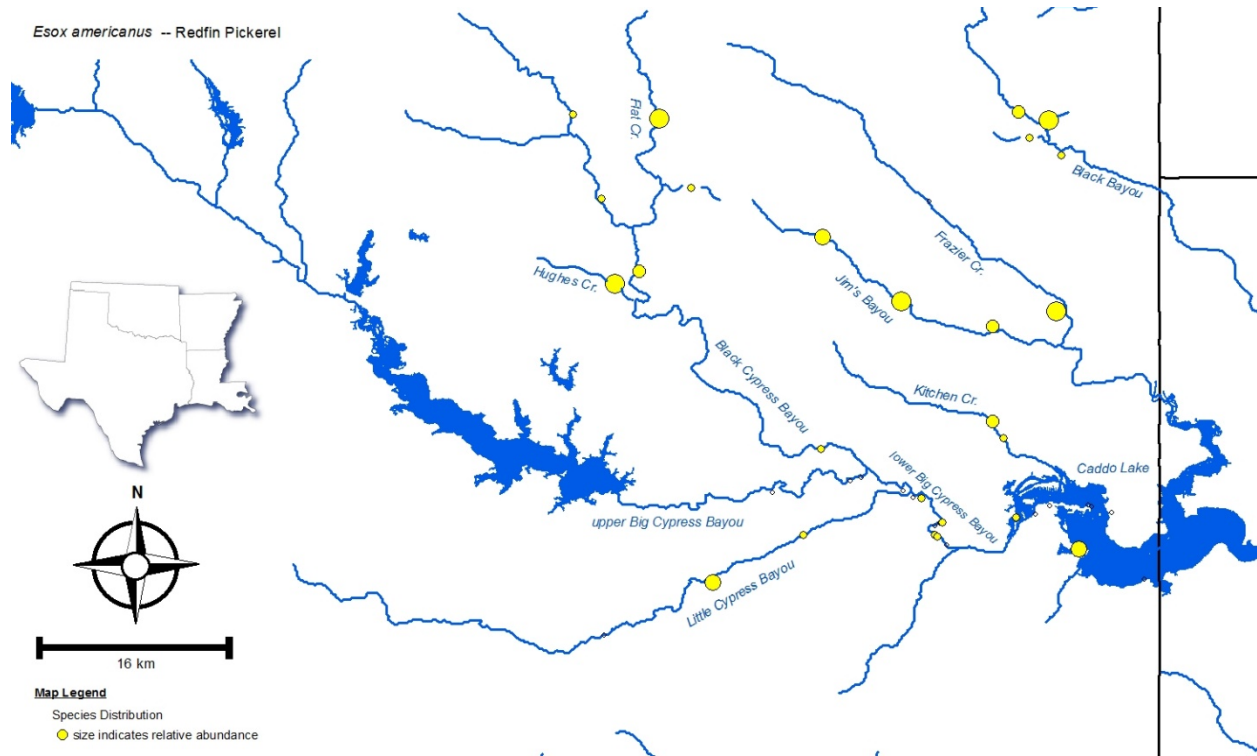




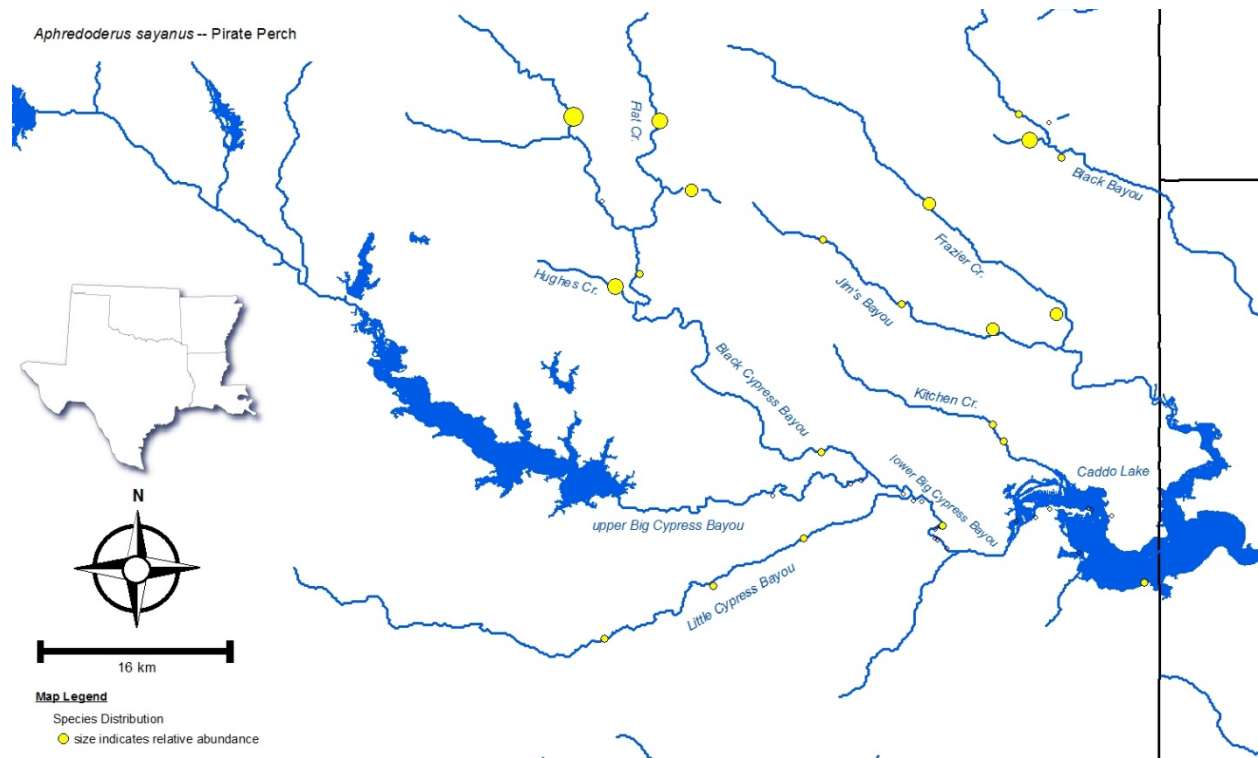




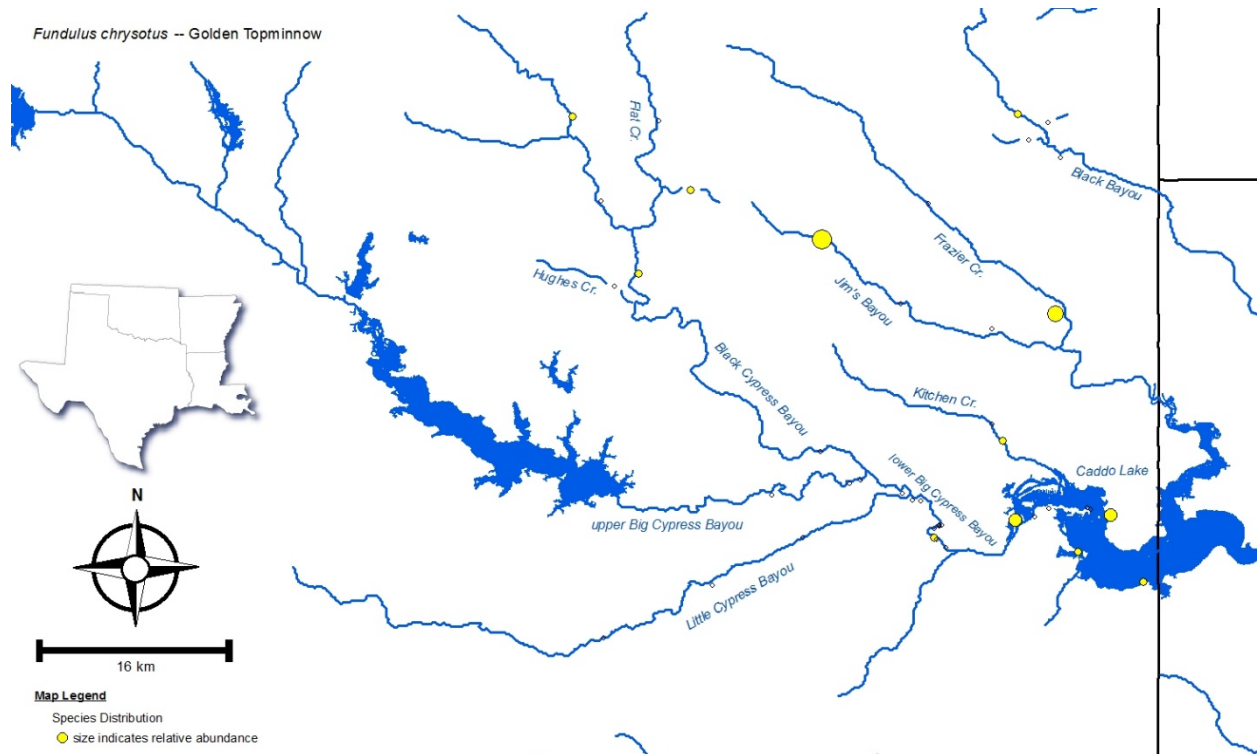
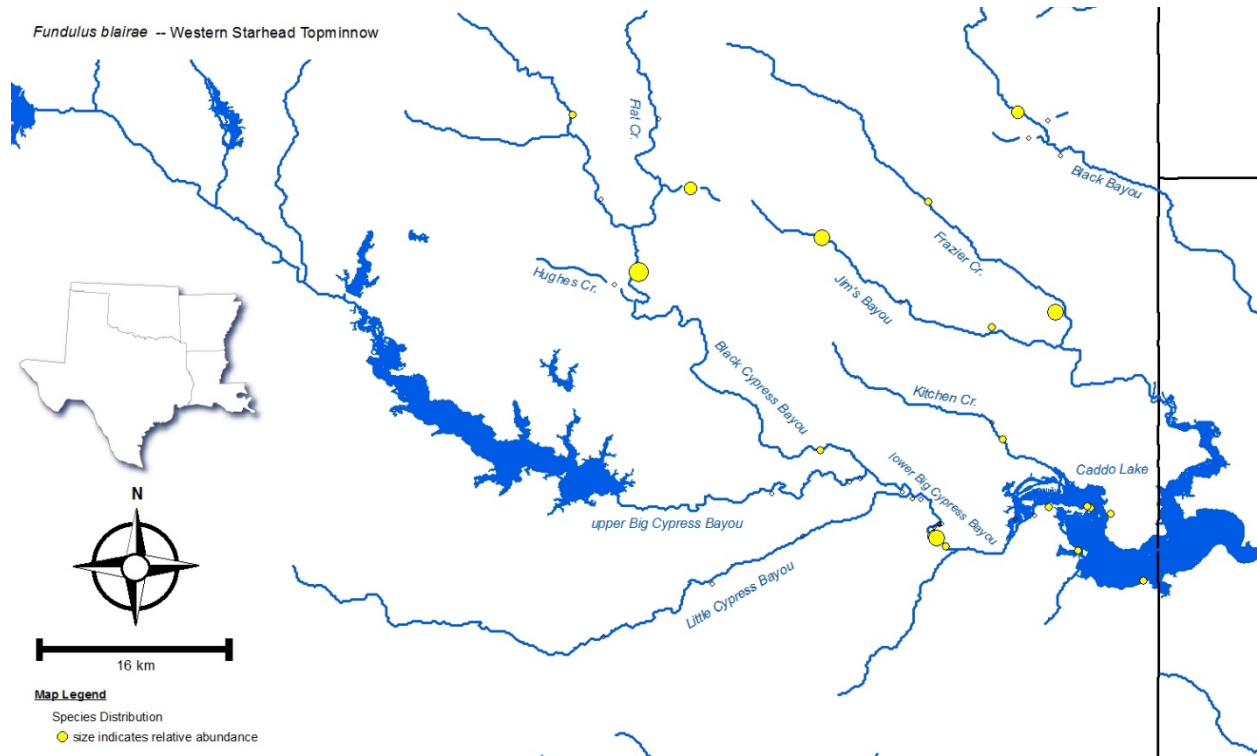
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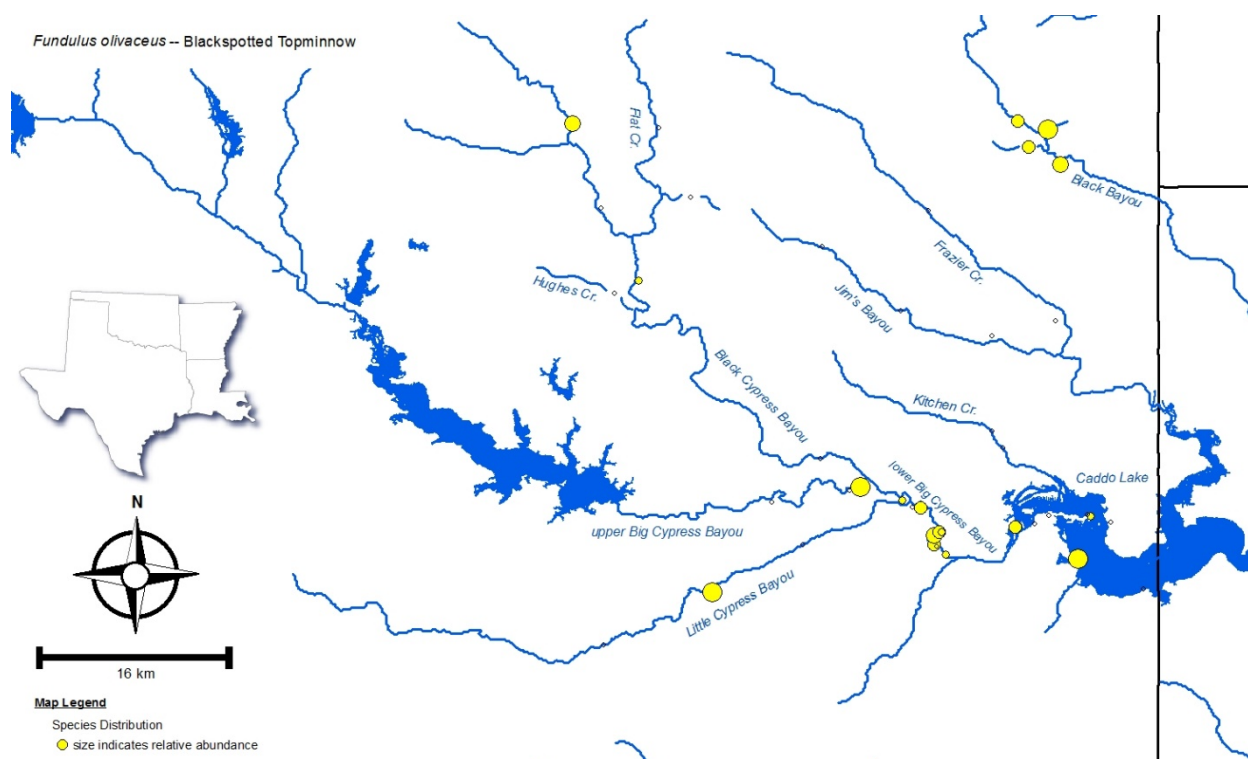
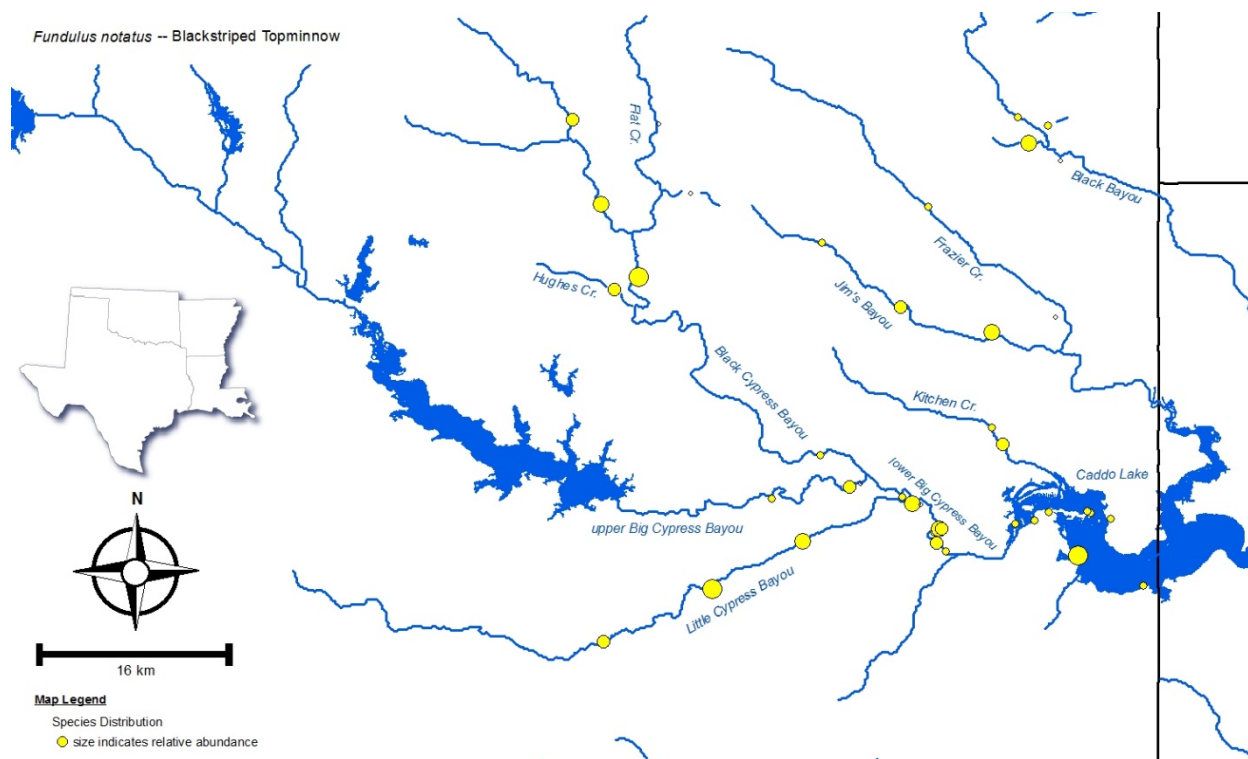


## APHREDODERIDAE – PIRATE PERCHES

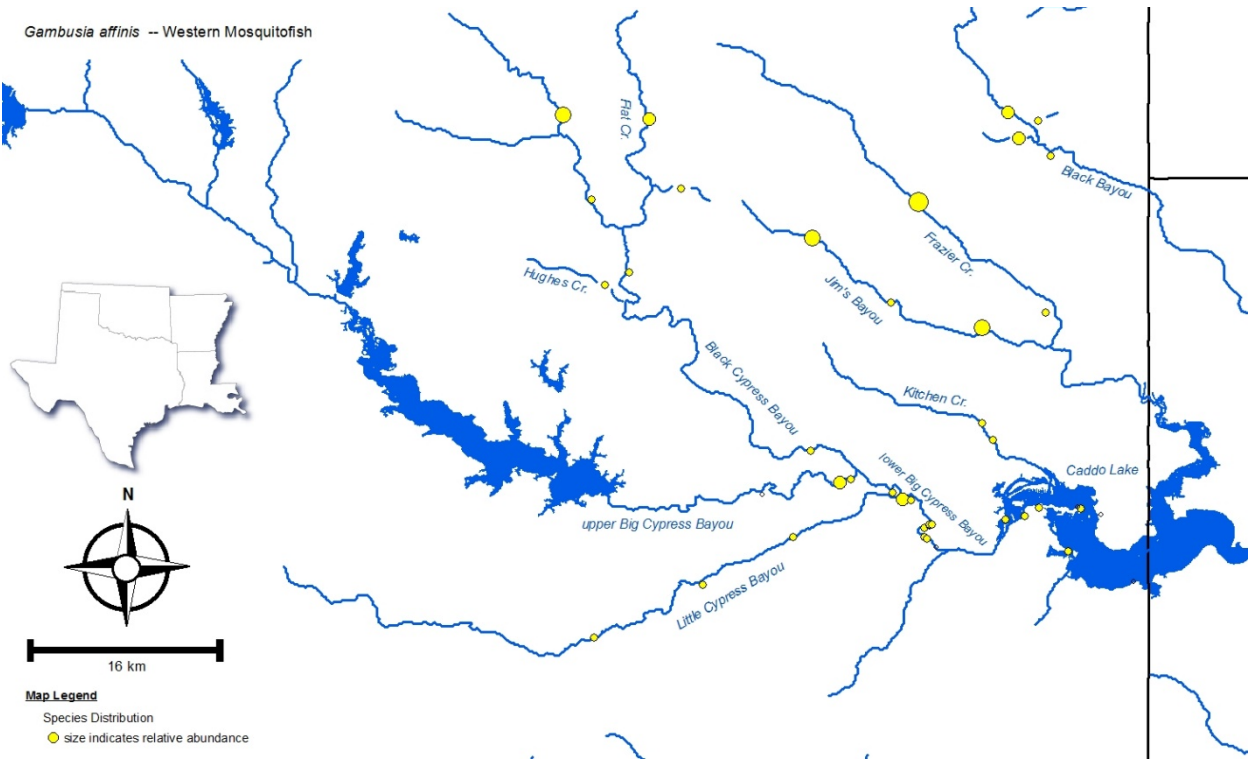


## FUNDULIDAE – TOPMINNOWS & KILLIFISHES



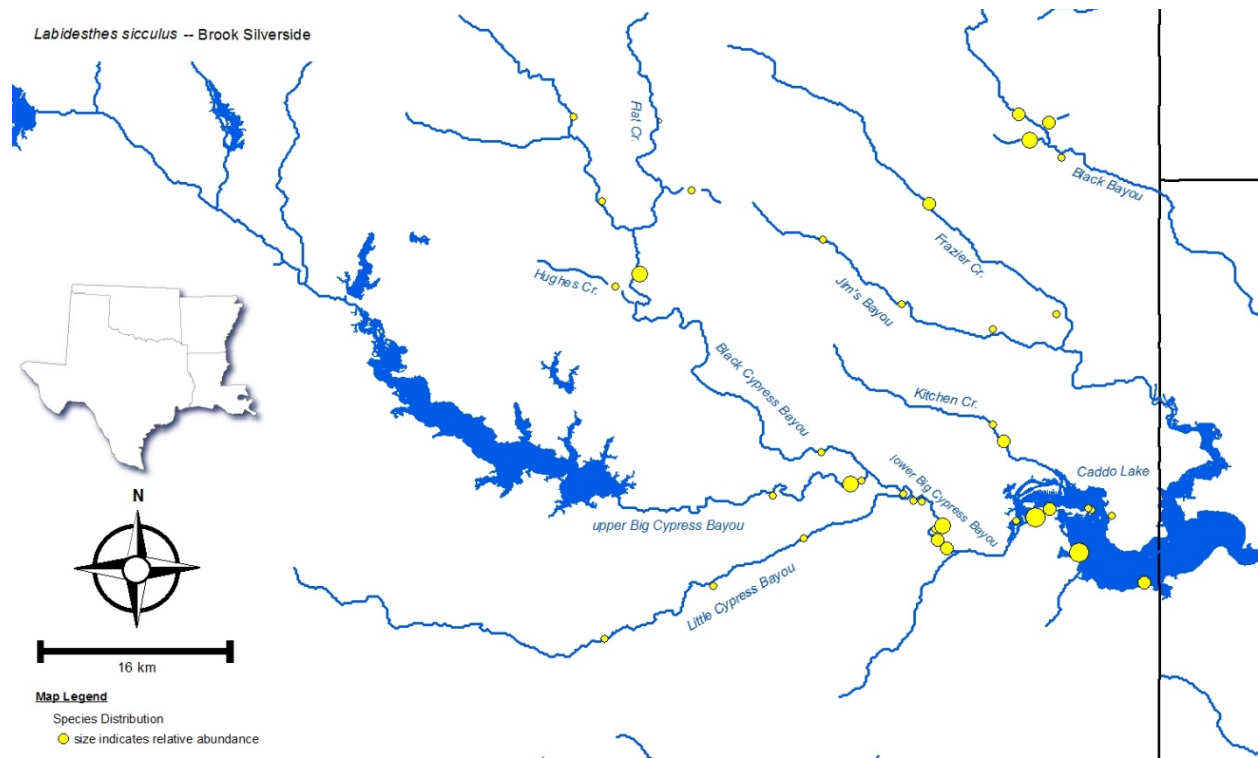


**POECILIIDAE – LIVEBEARERS**

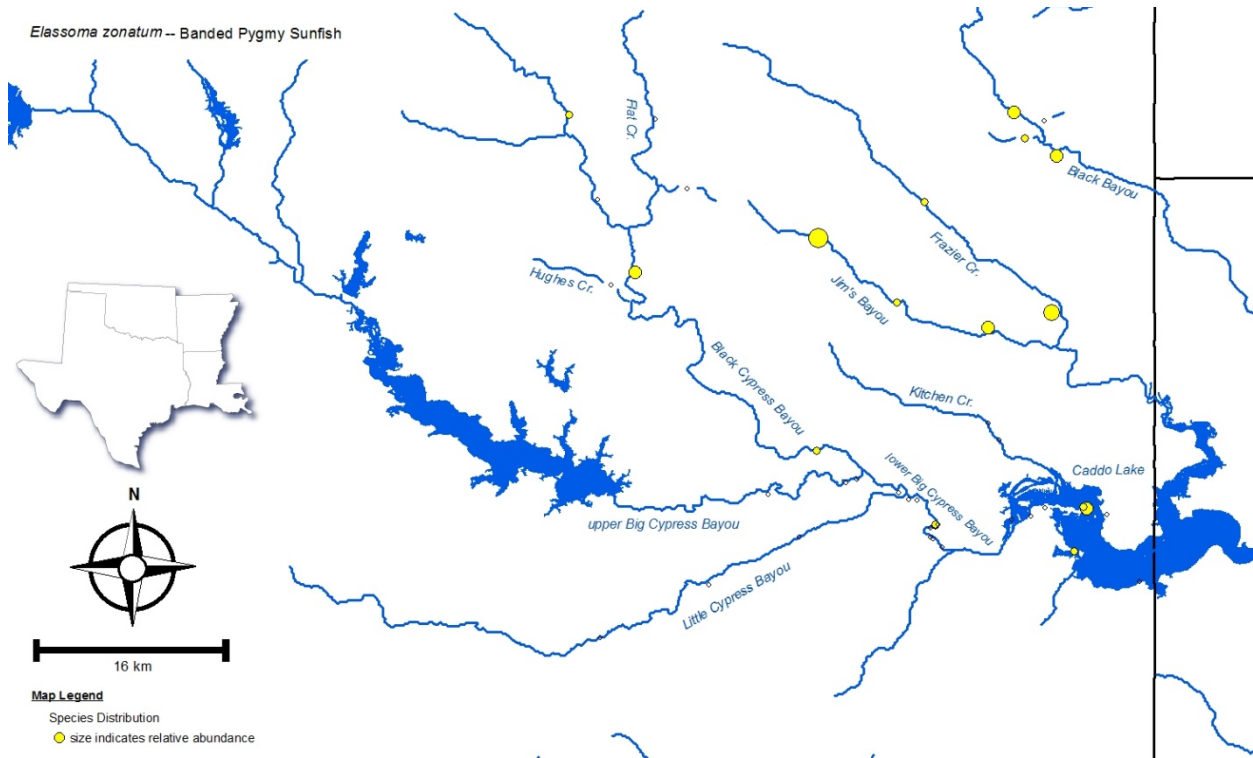




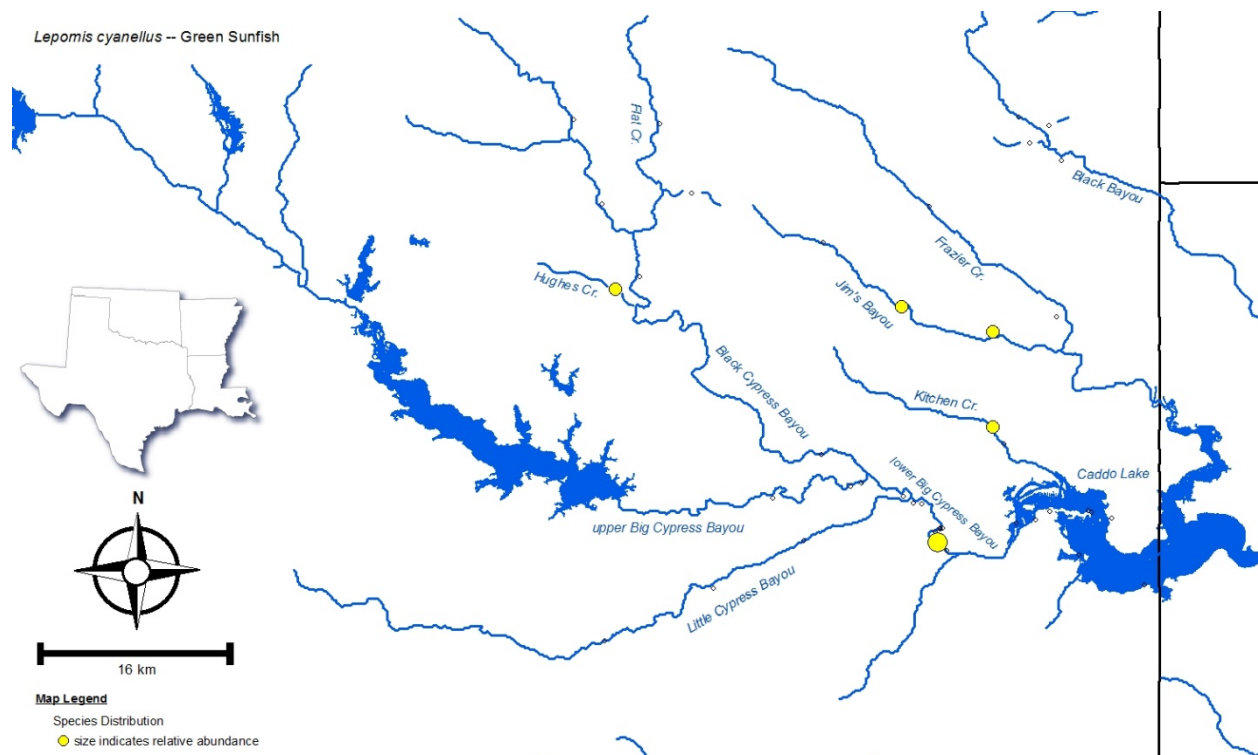
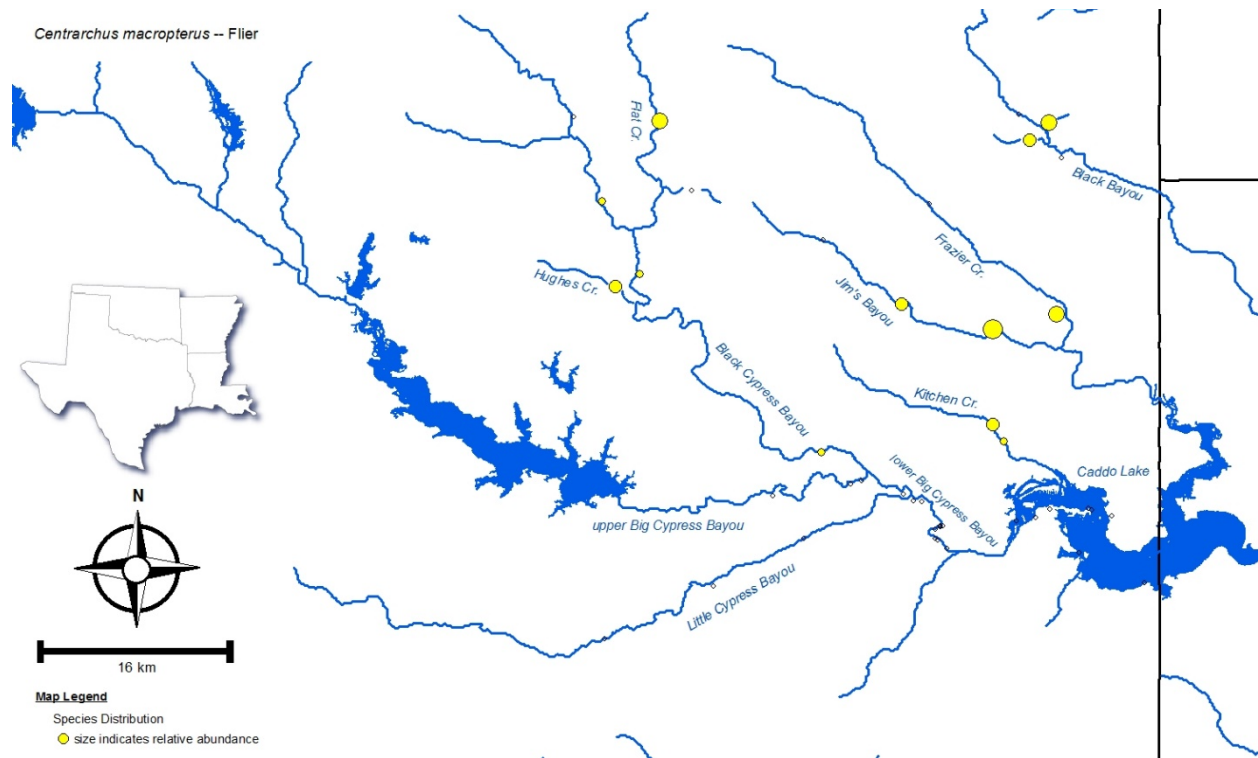
## ATHERINOPSIDAE – SILVERSIDES



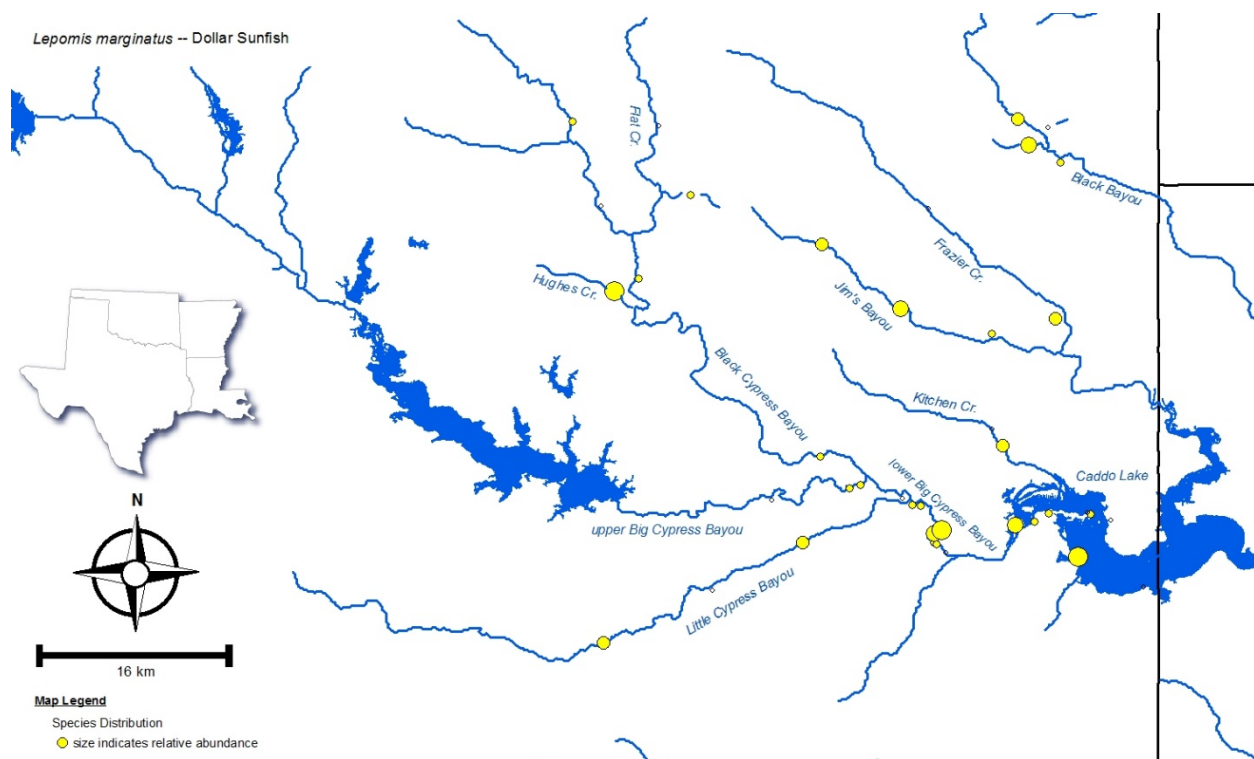
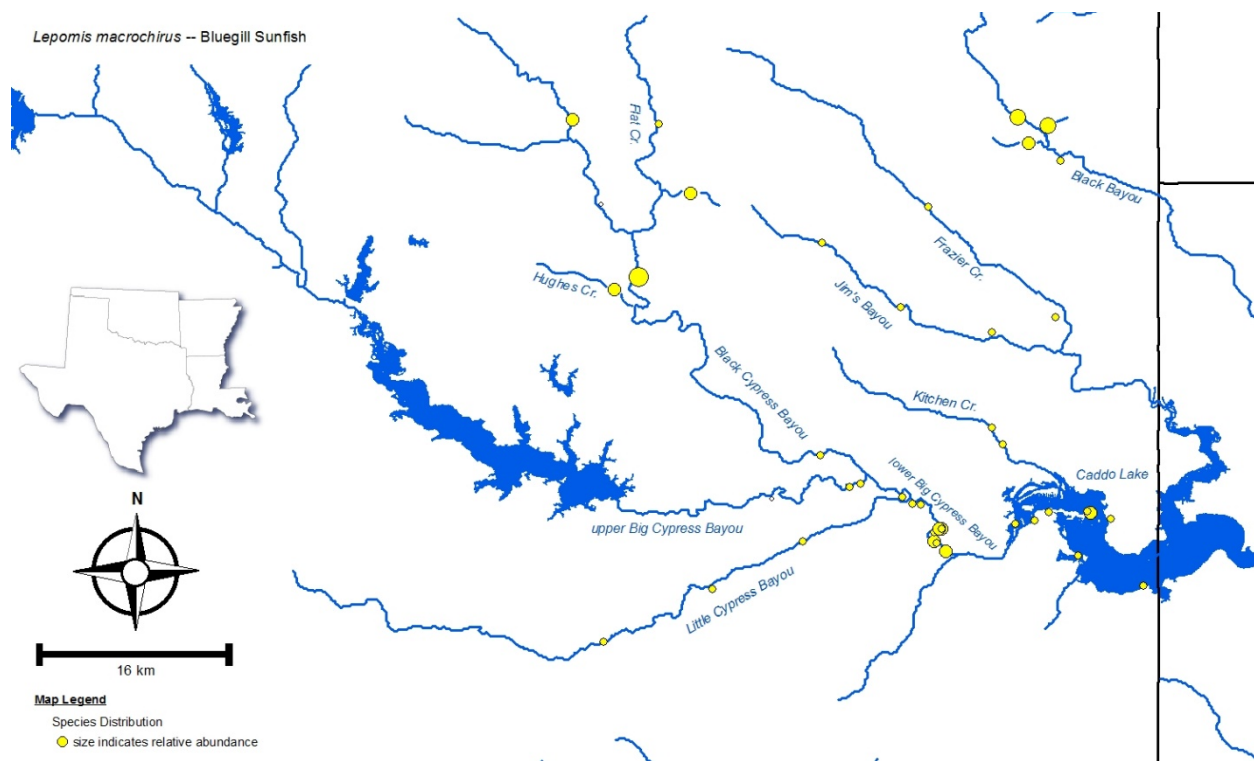
## ELASSOMATIDAE – PYGMY SUNFISHES



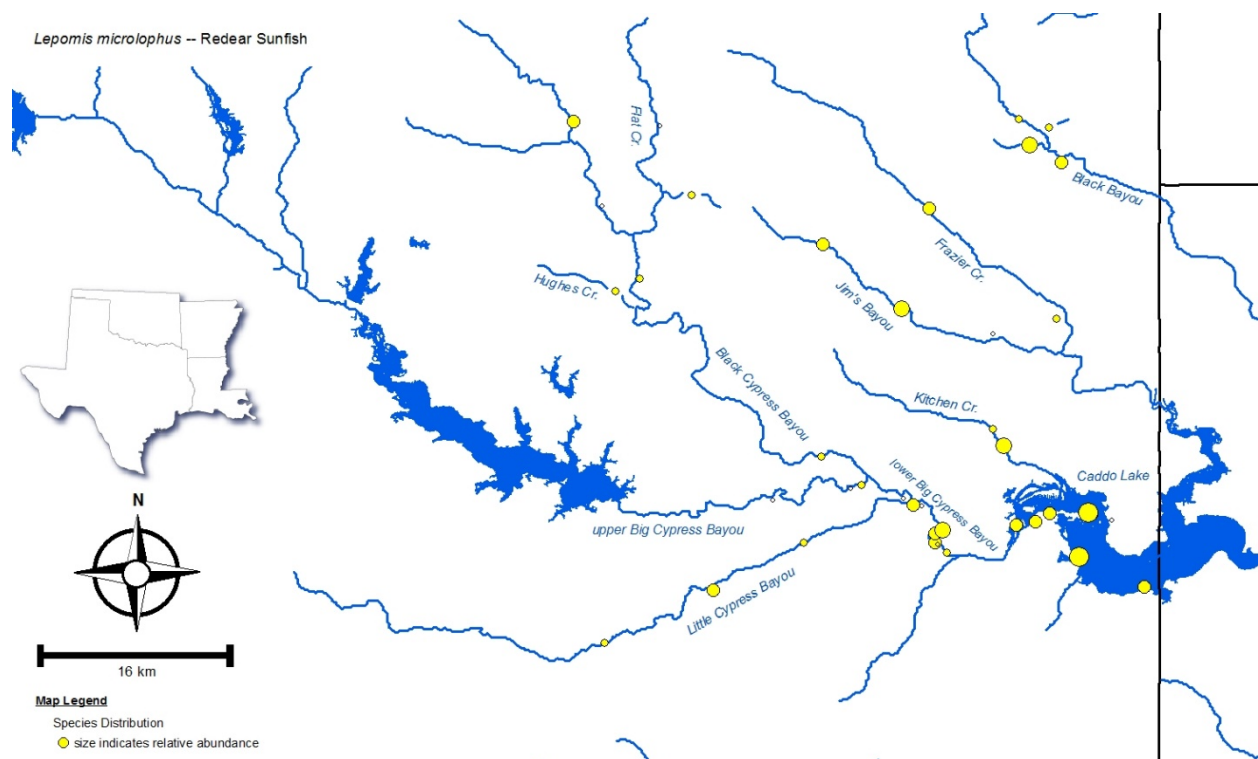
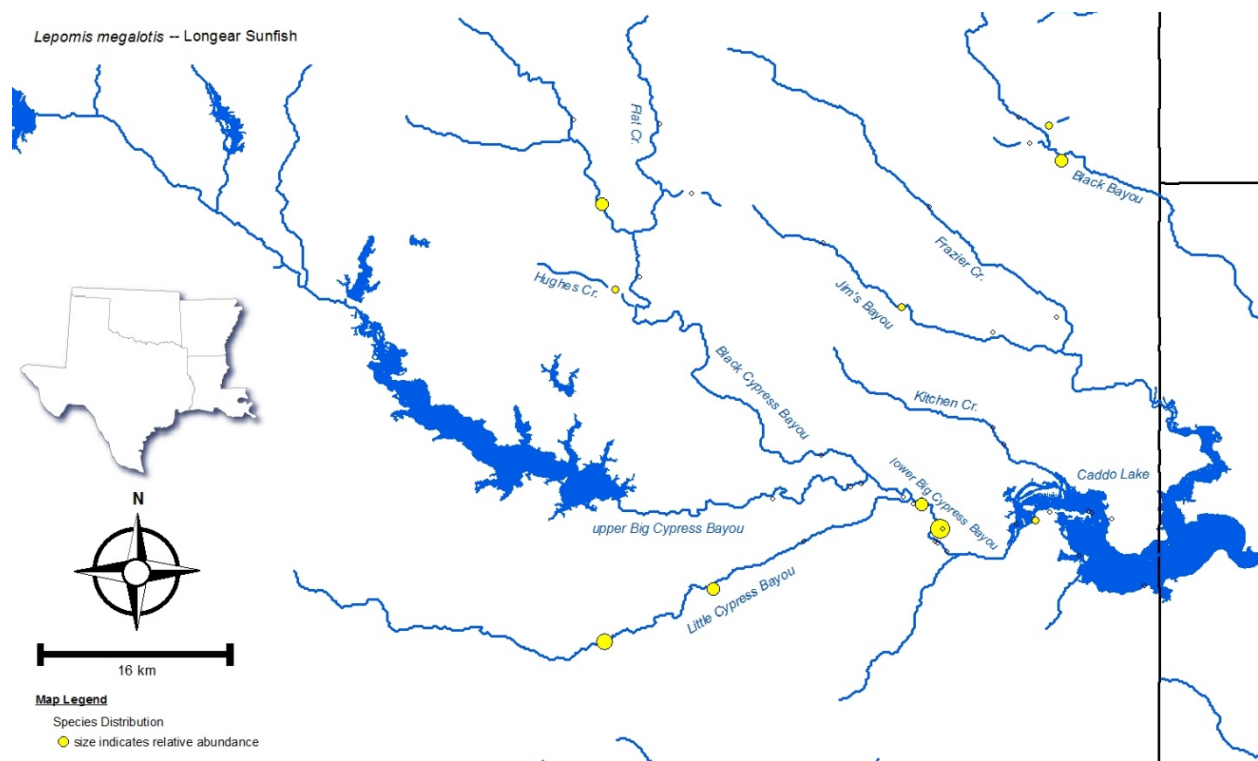
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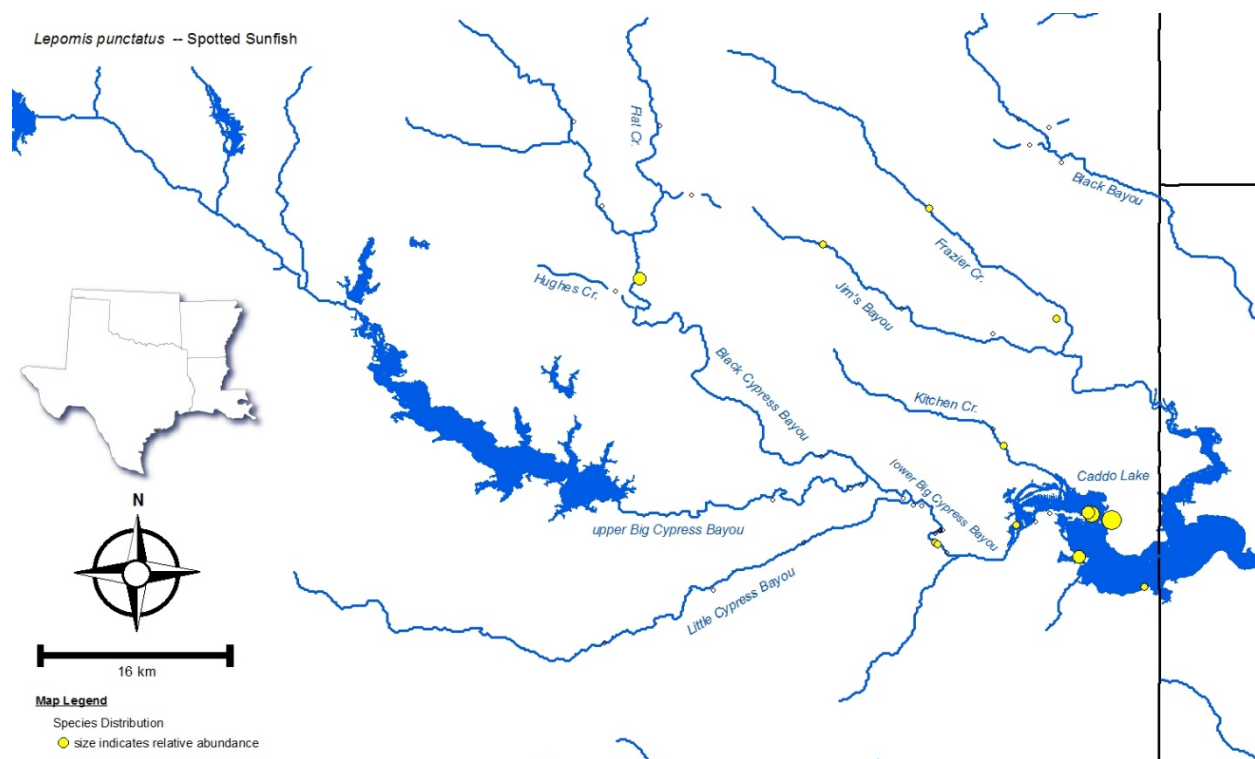
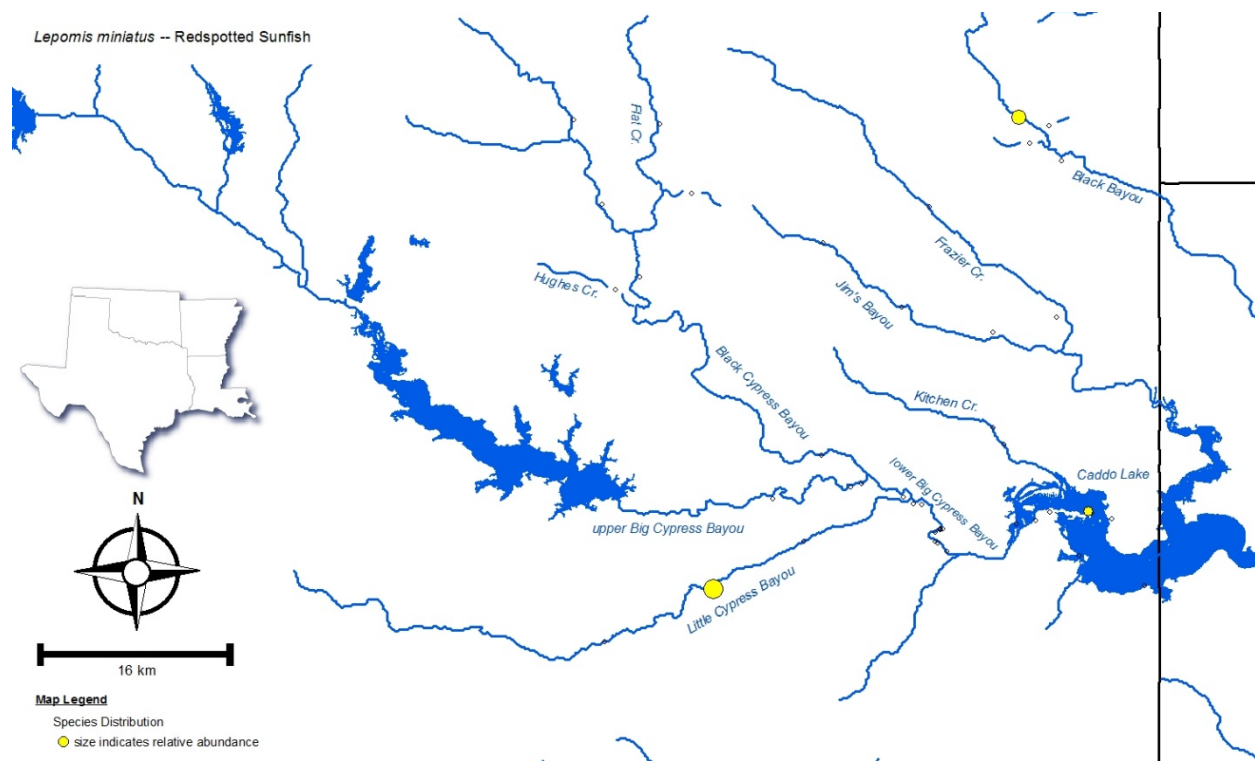


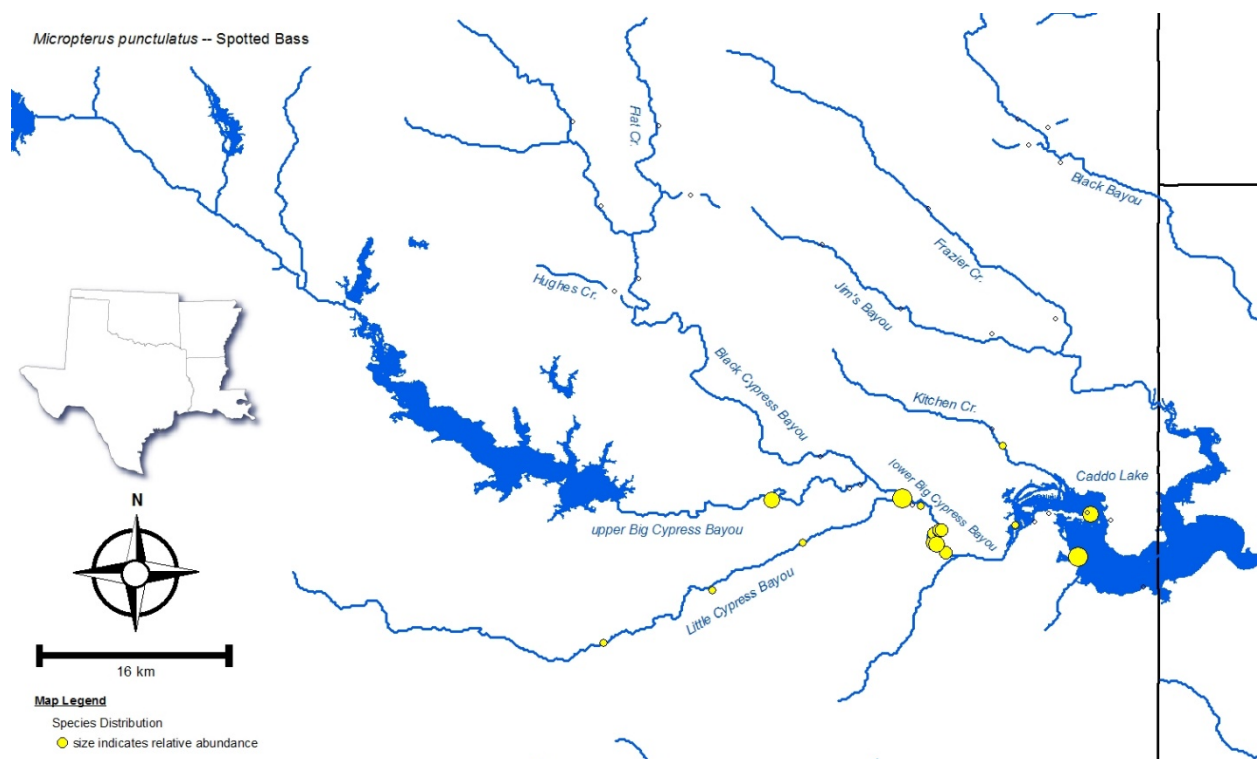
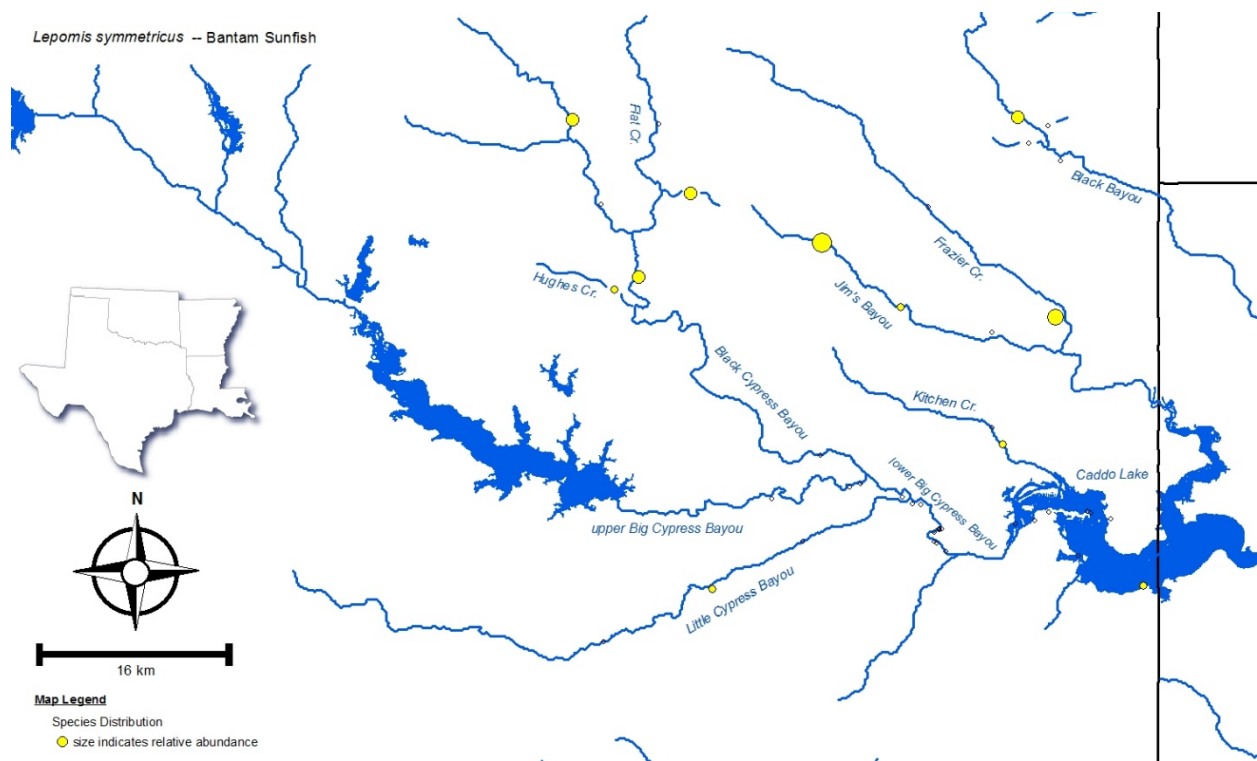




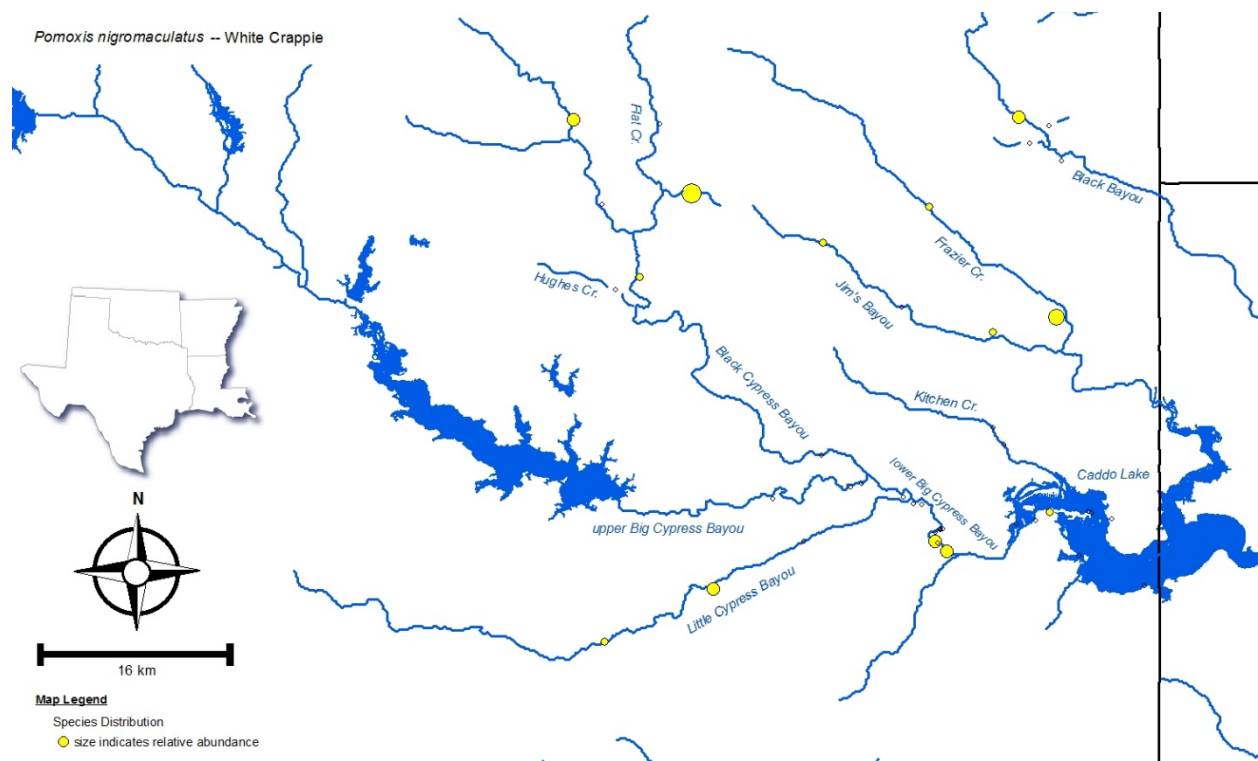




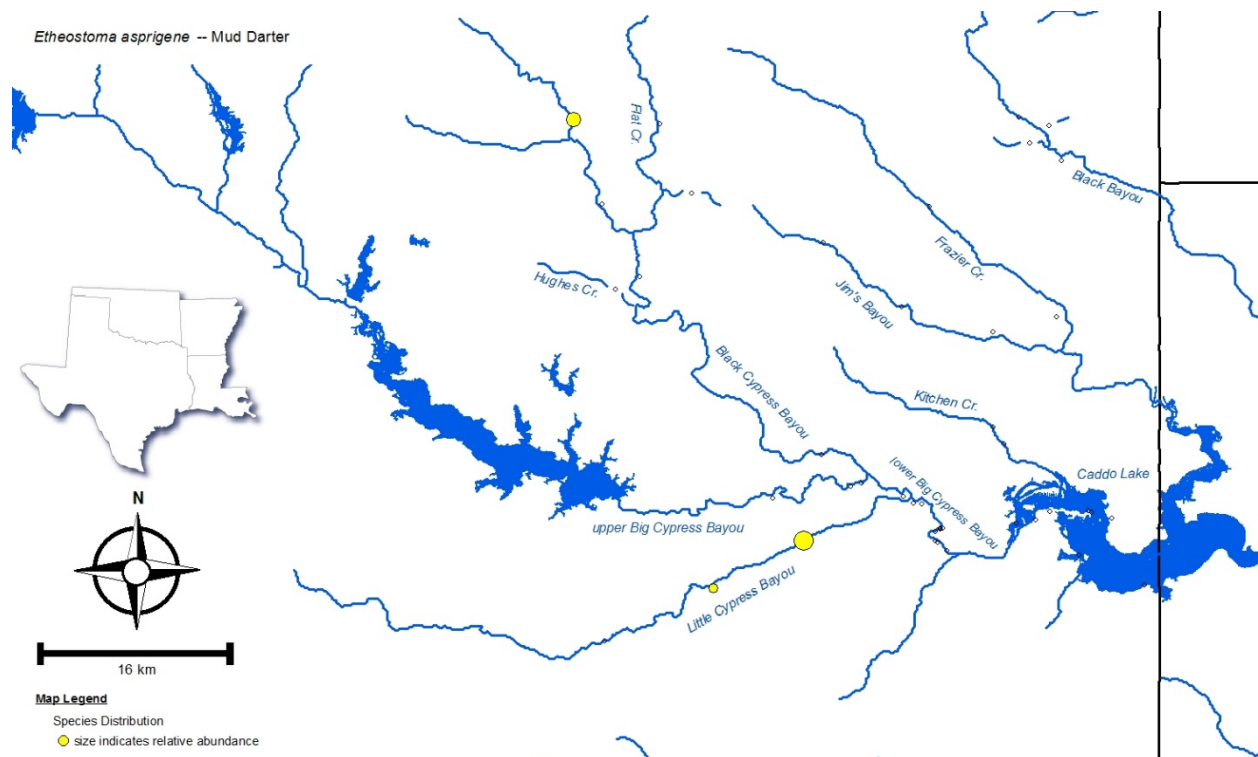
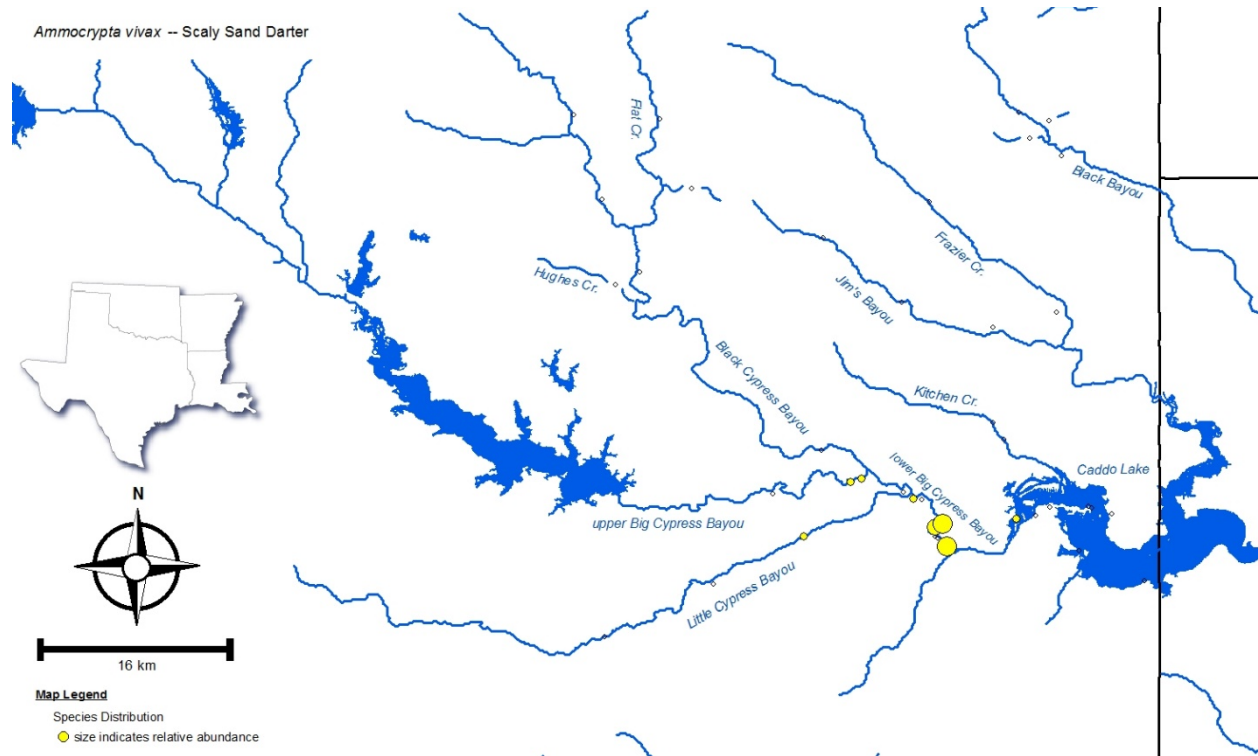




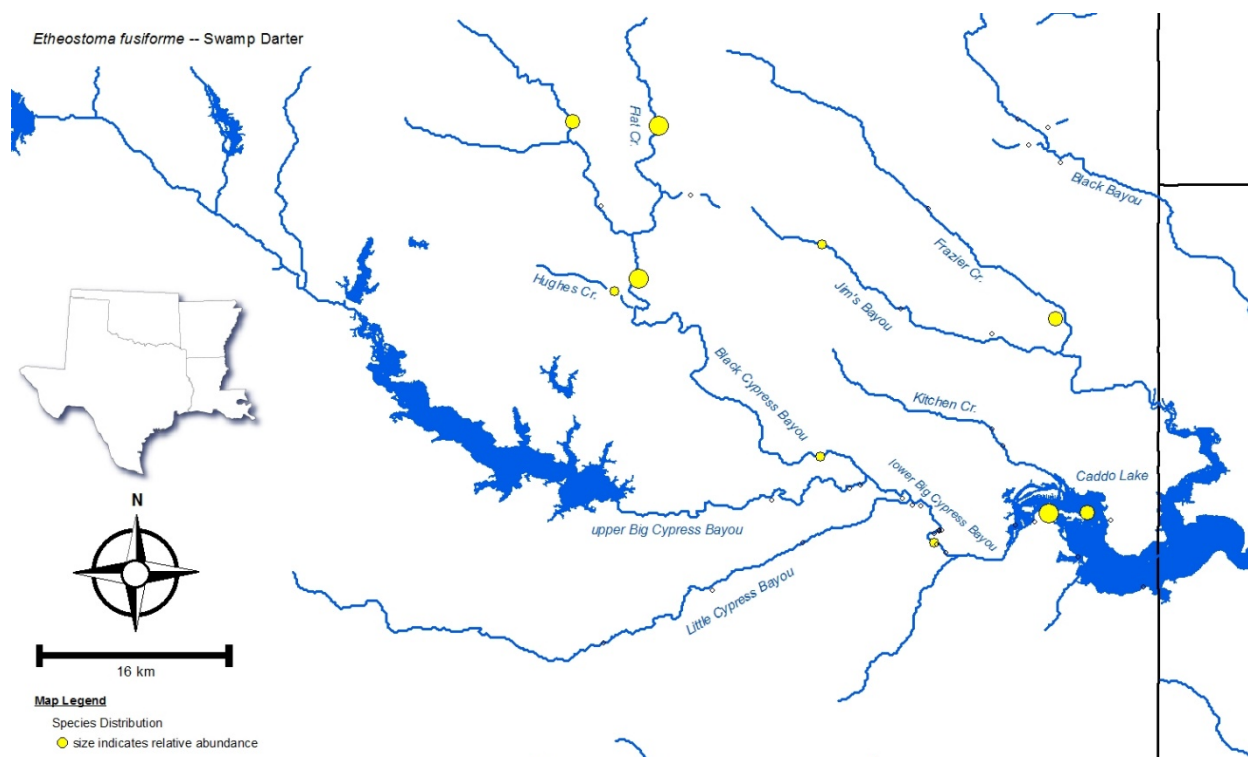
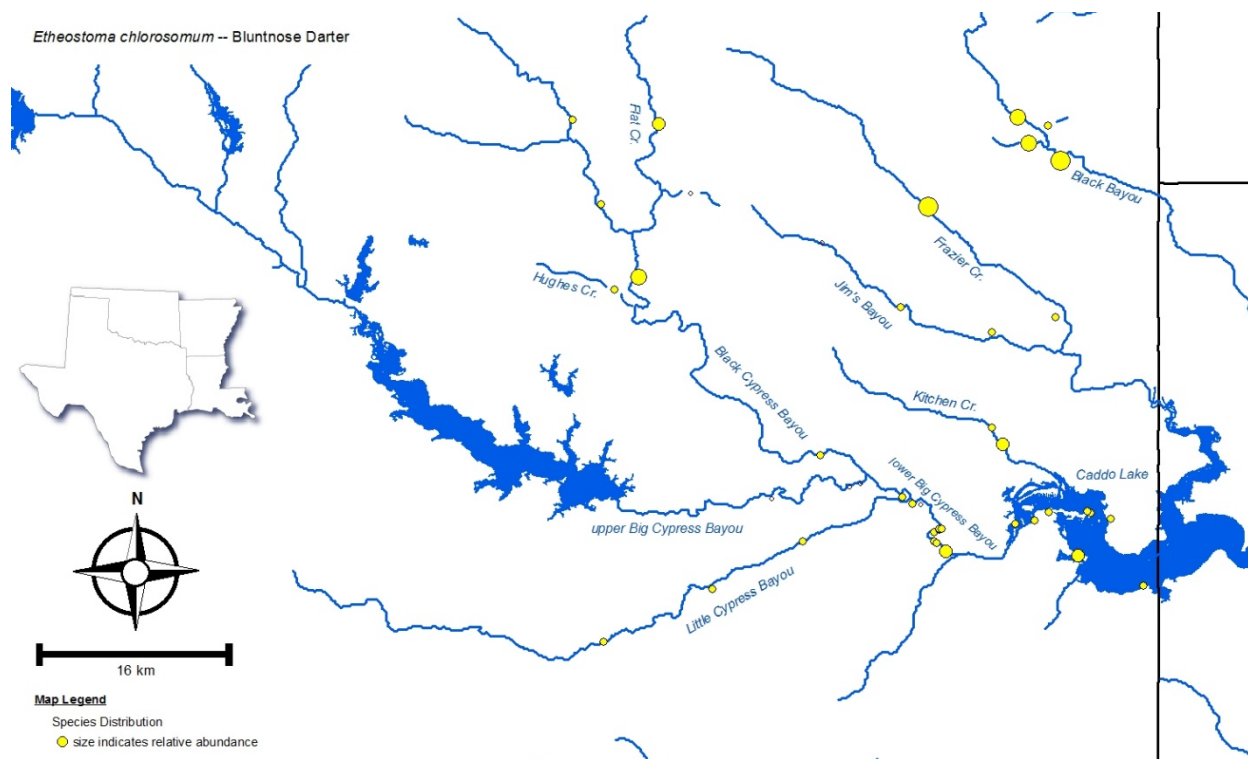


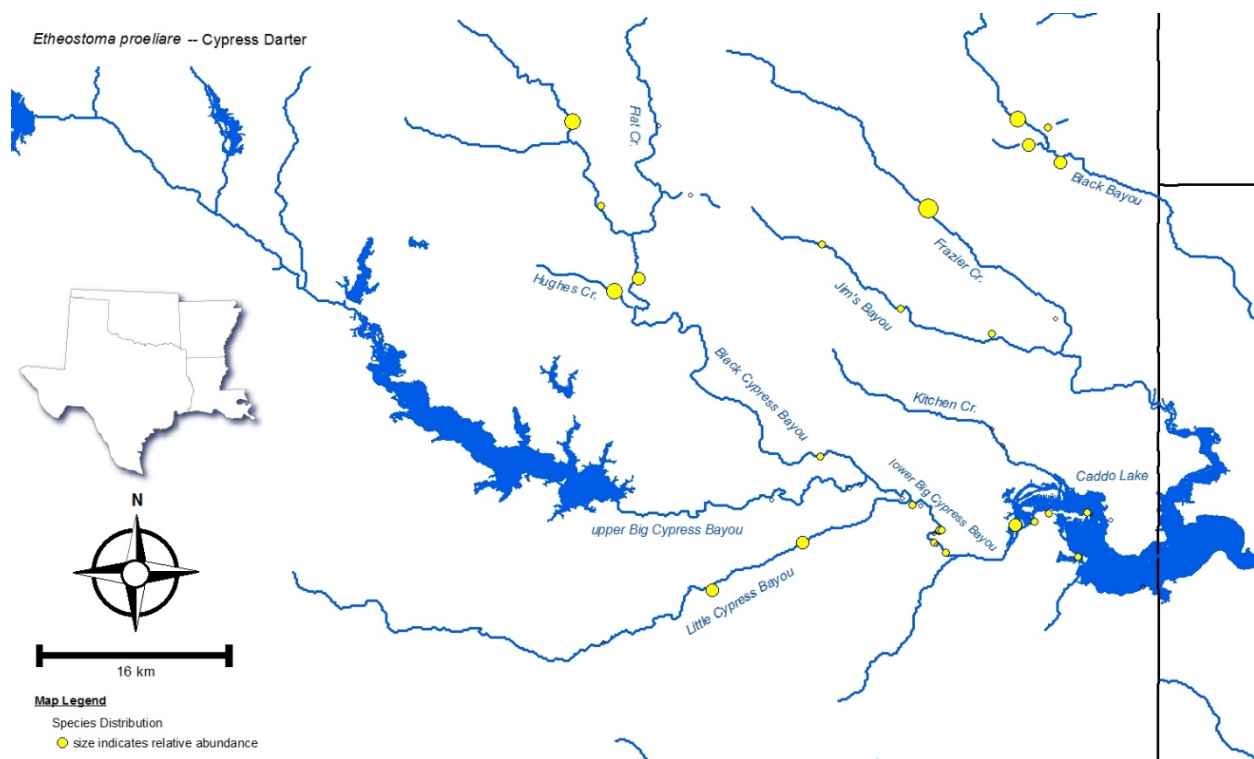
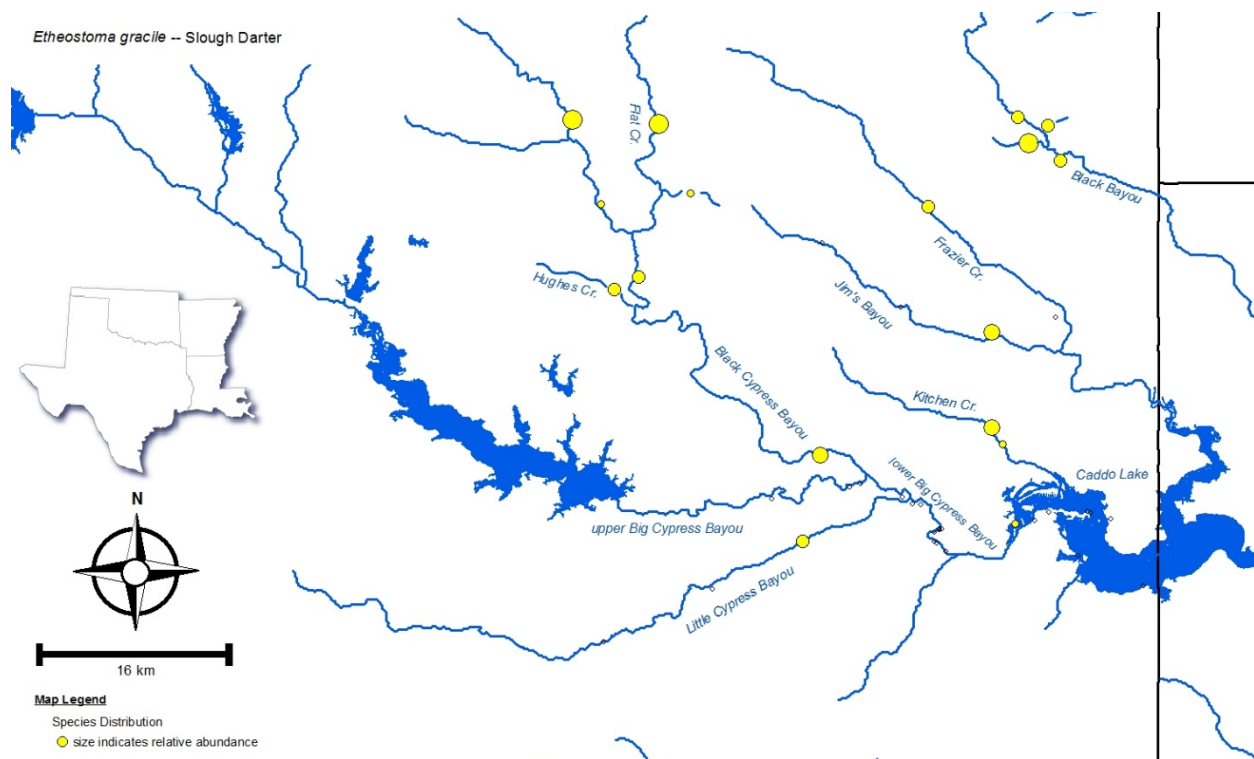


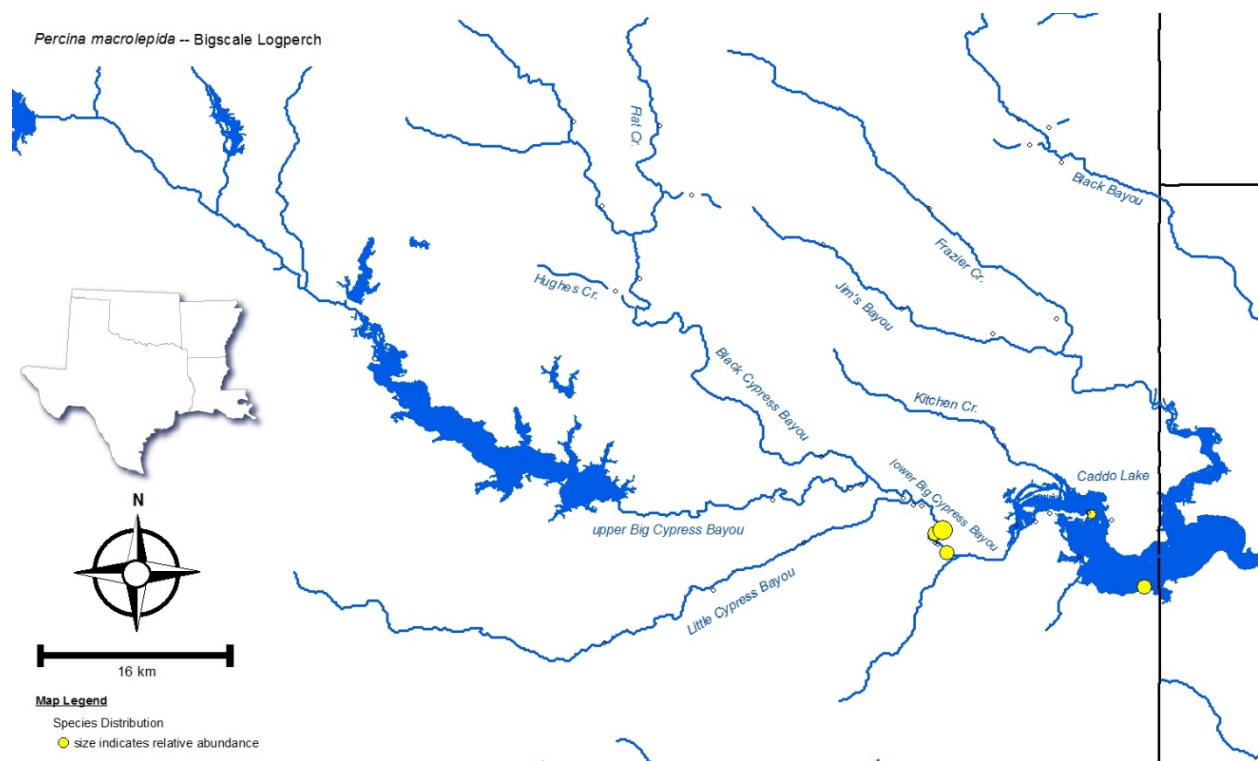
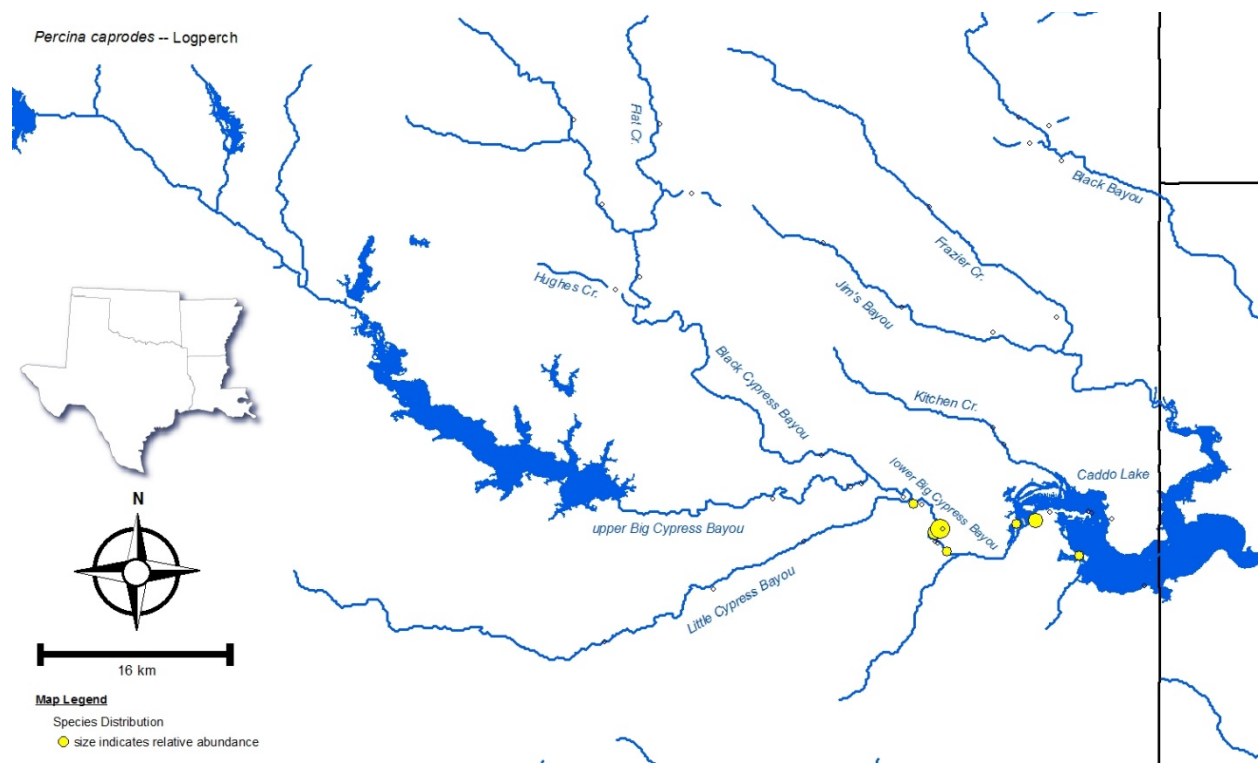
## PERCIDAE – PERCHES

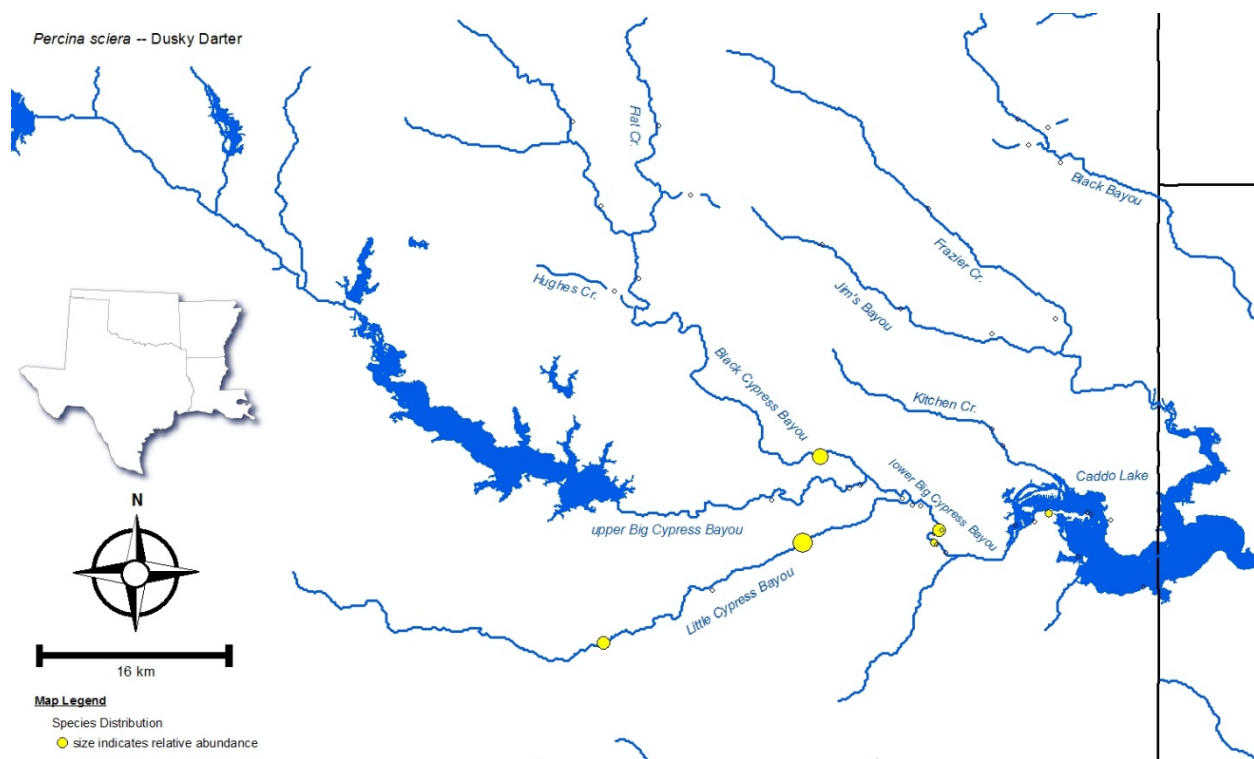
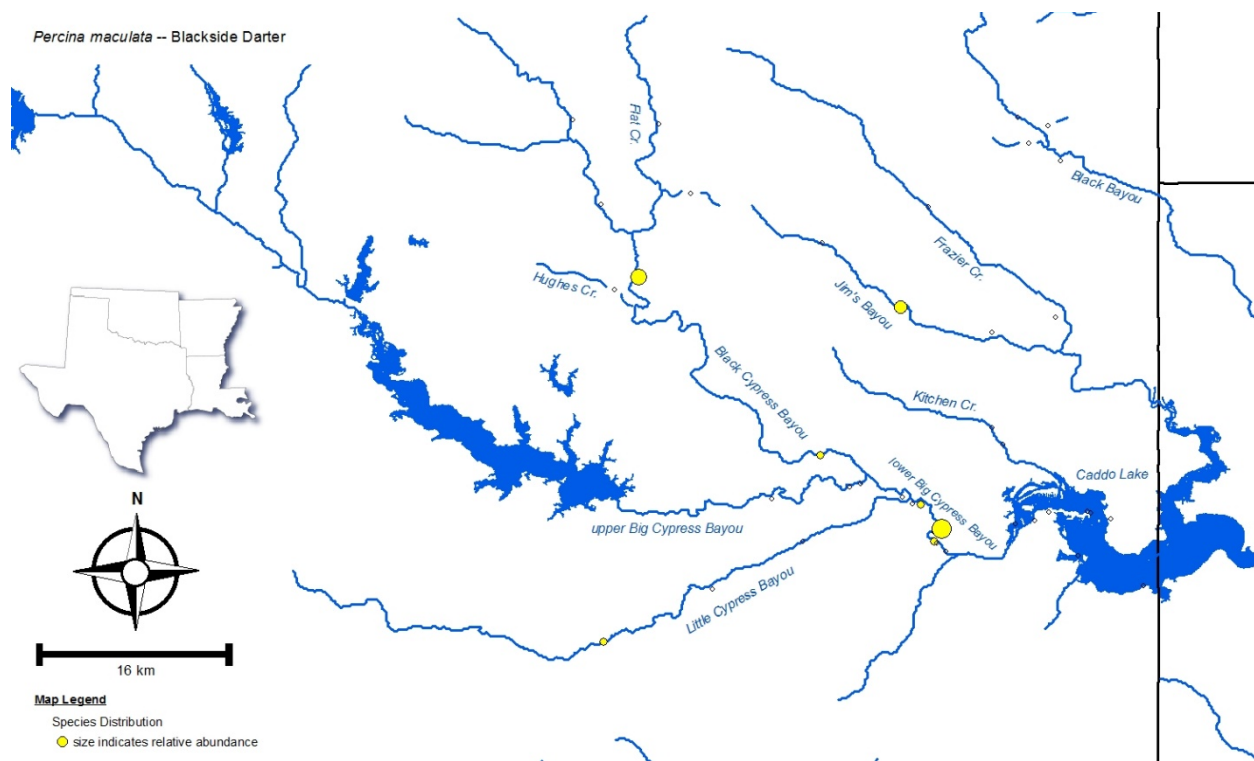




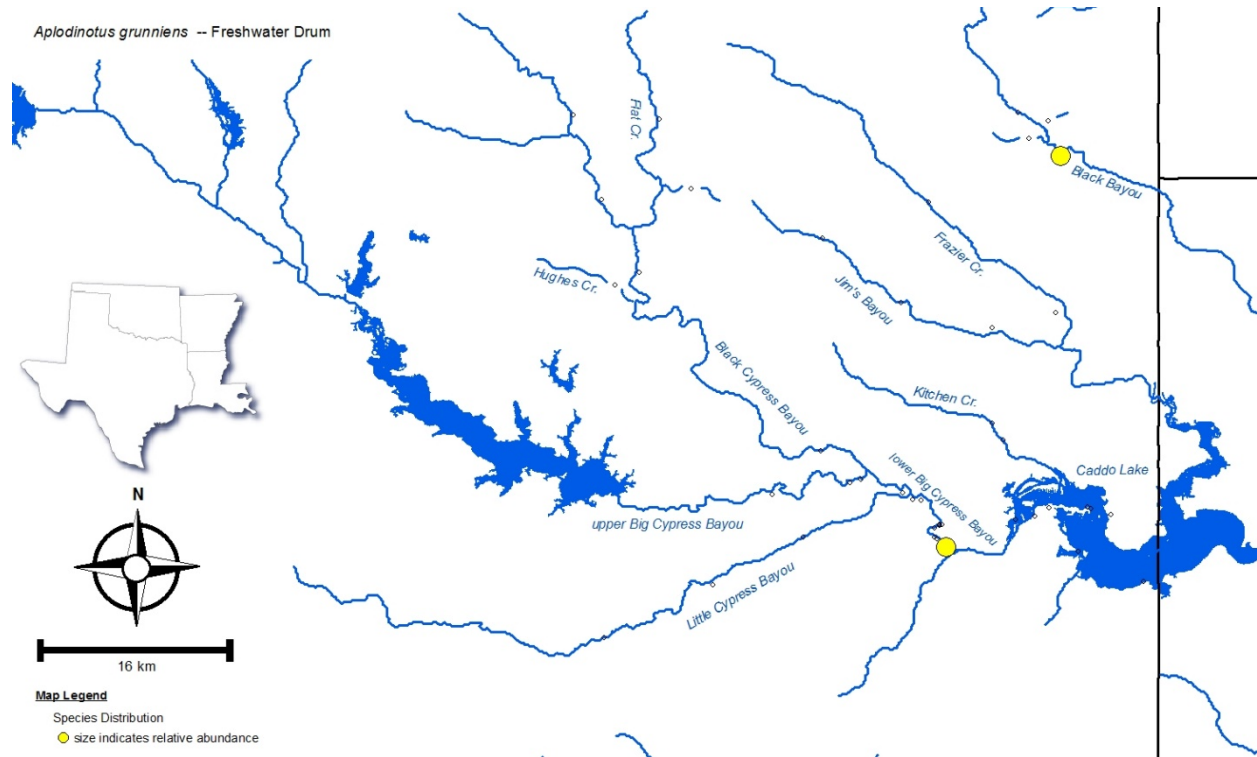








## SCIAENIDAE – DRUMS

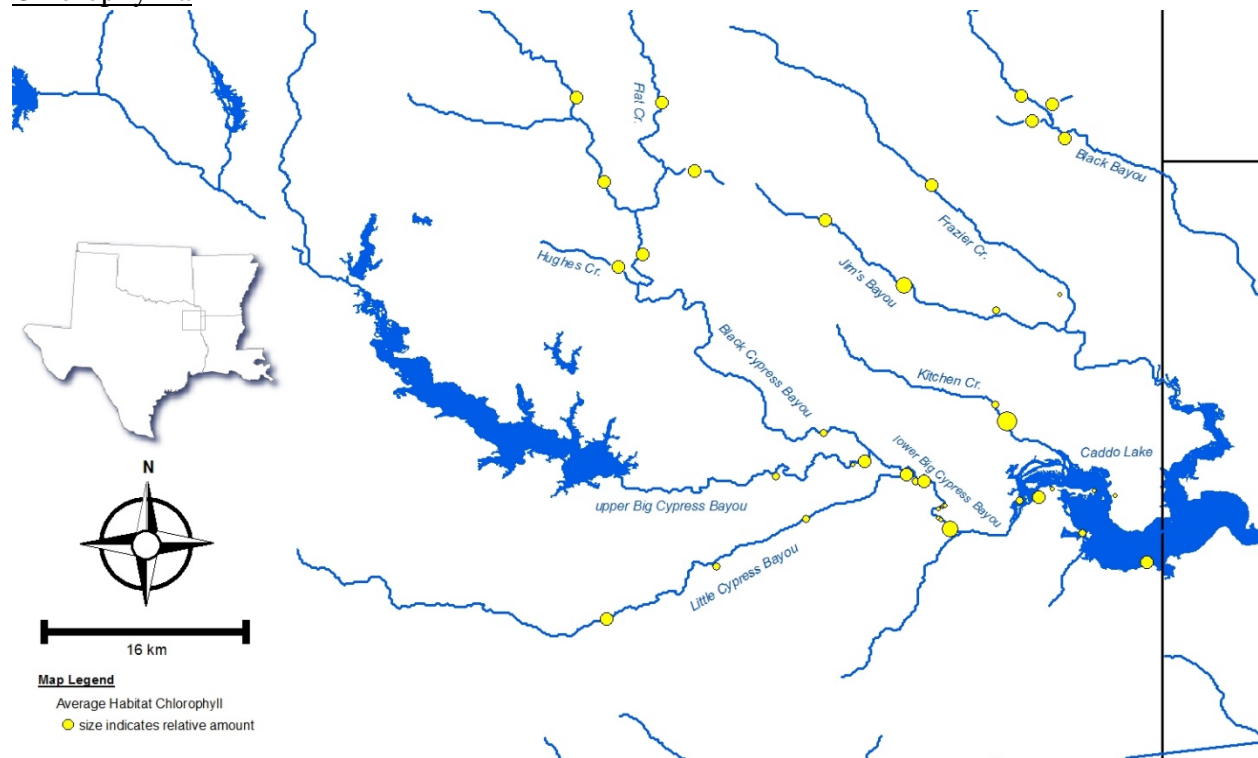




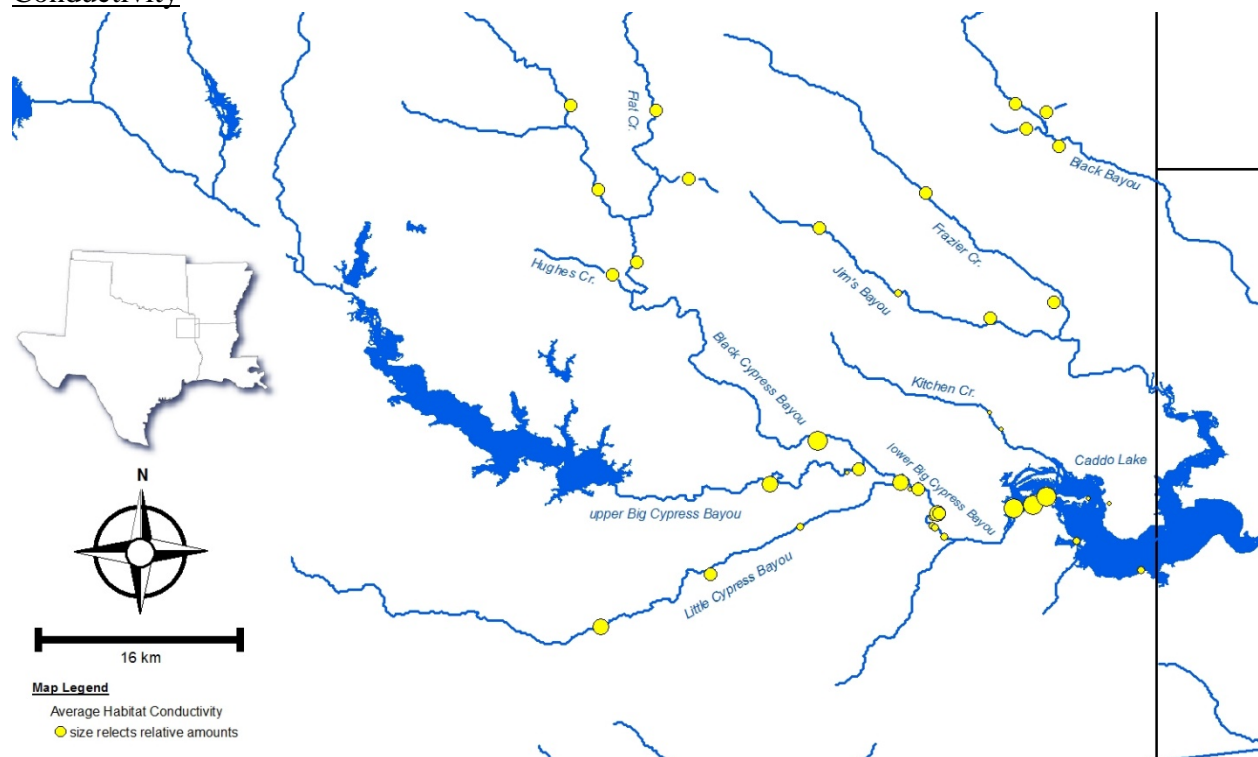
Appendix 2. Spatial variation in mesohabitat and microhabitat variables across the sample basin.

**MESOHABITAT PARAMETERS**

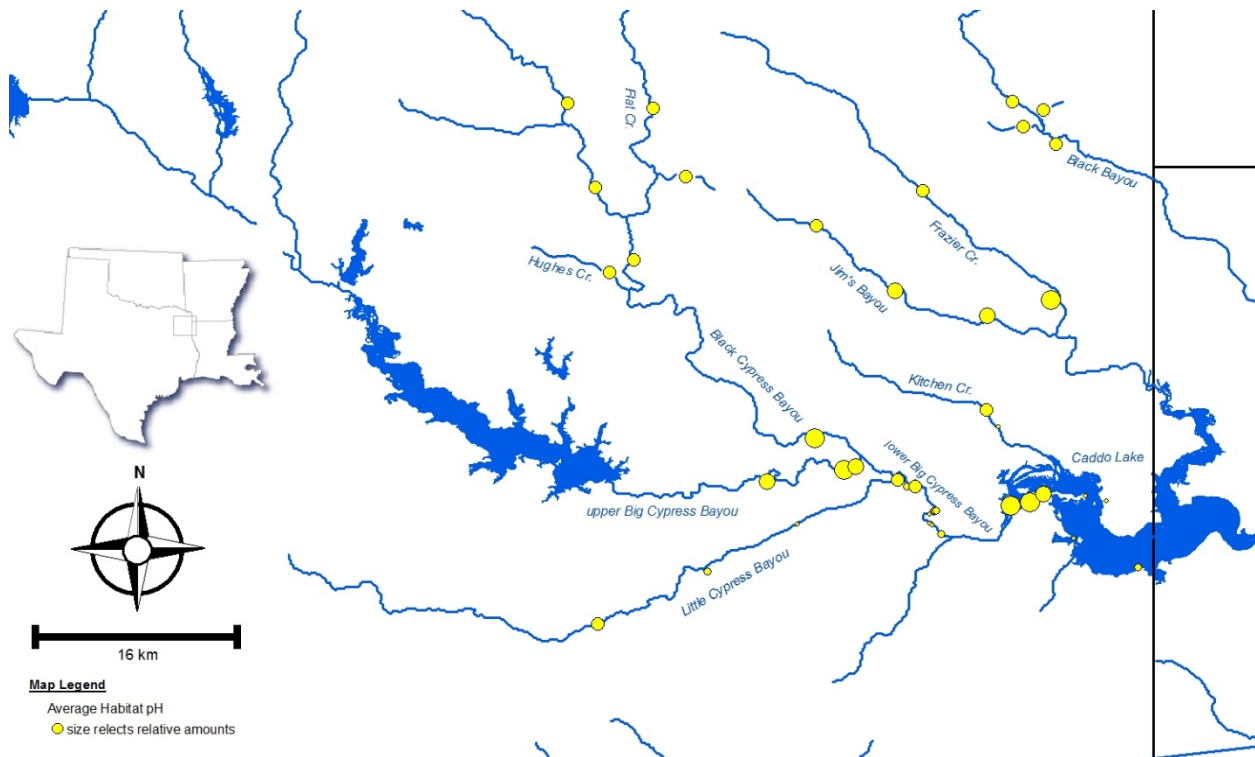
Chlorophyll-a



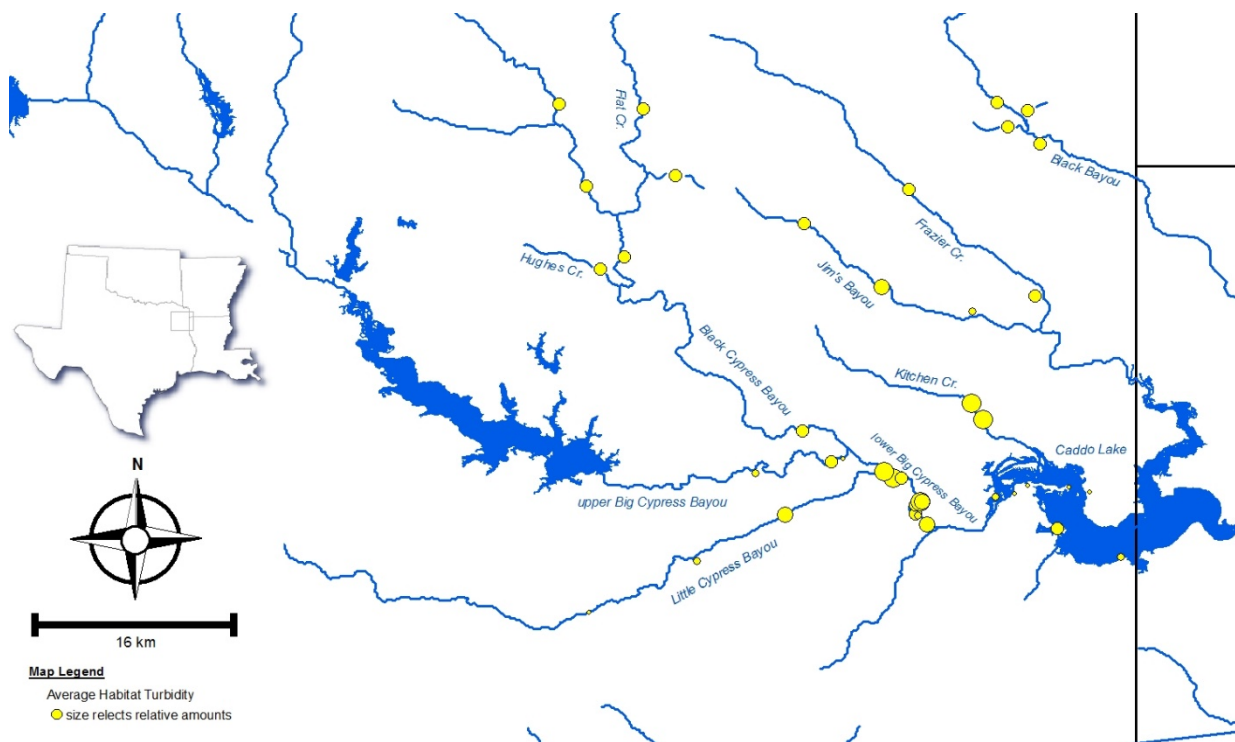
Conductivity



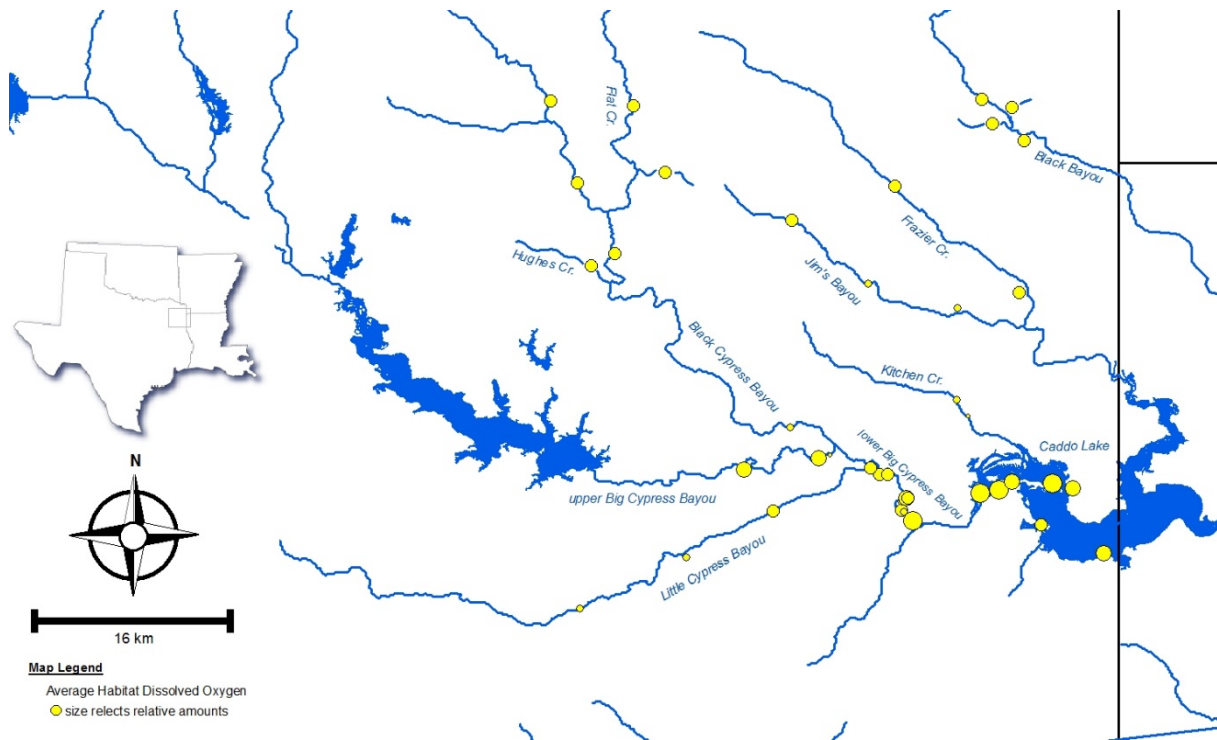
## pH



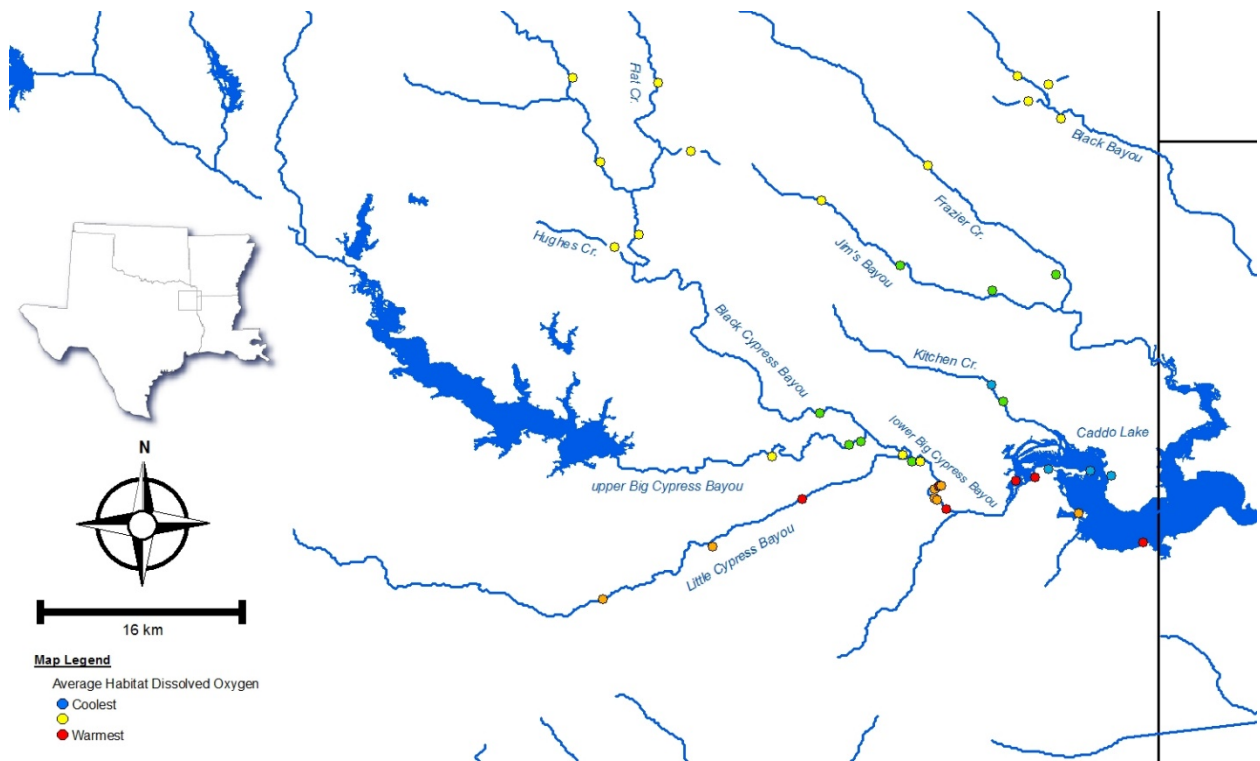
## Turbidity



## Dissolved Oxygen

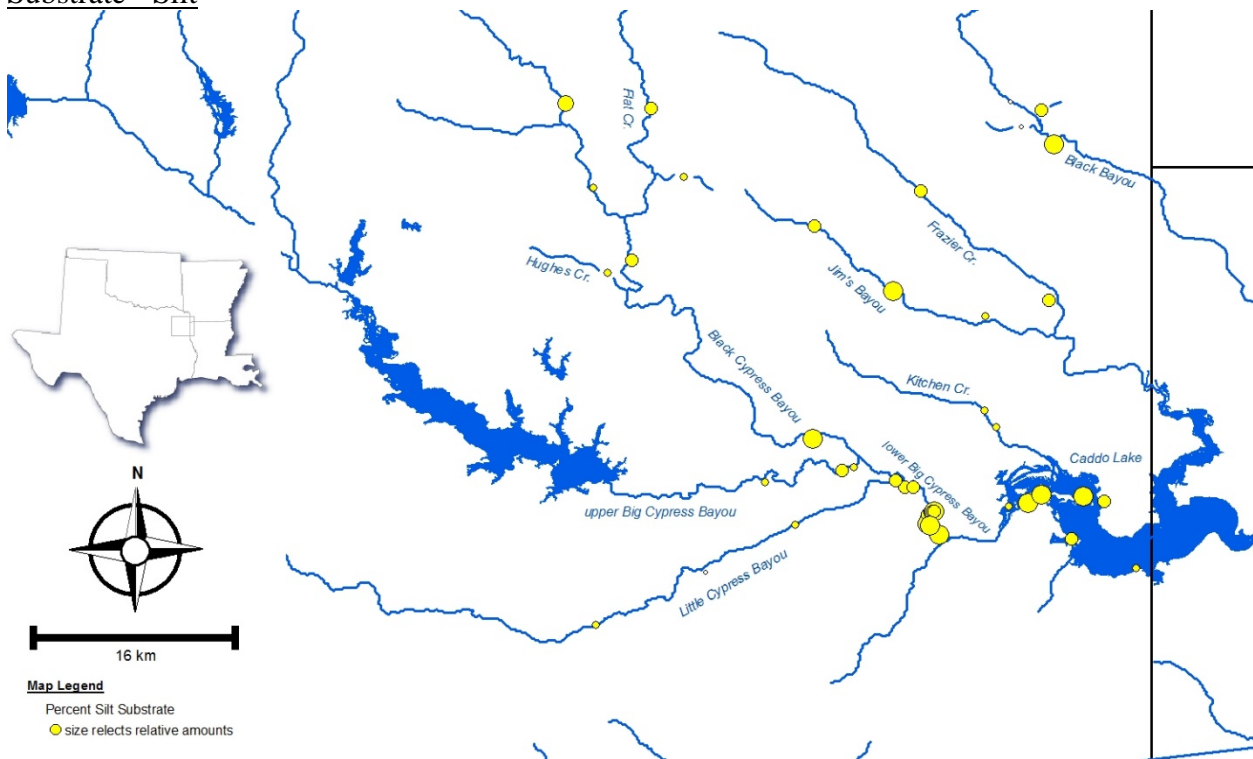


## Temperature

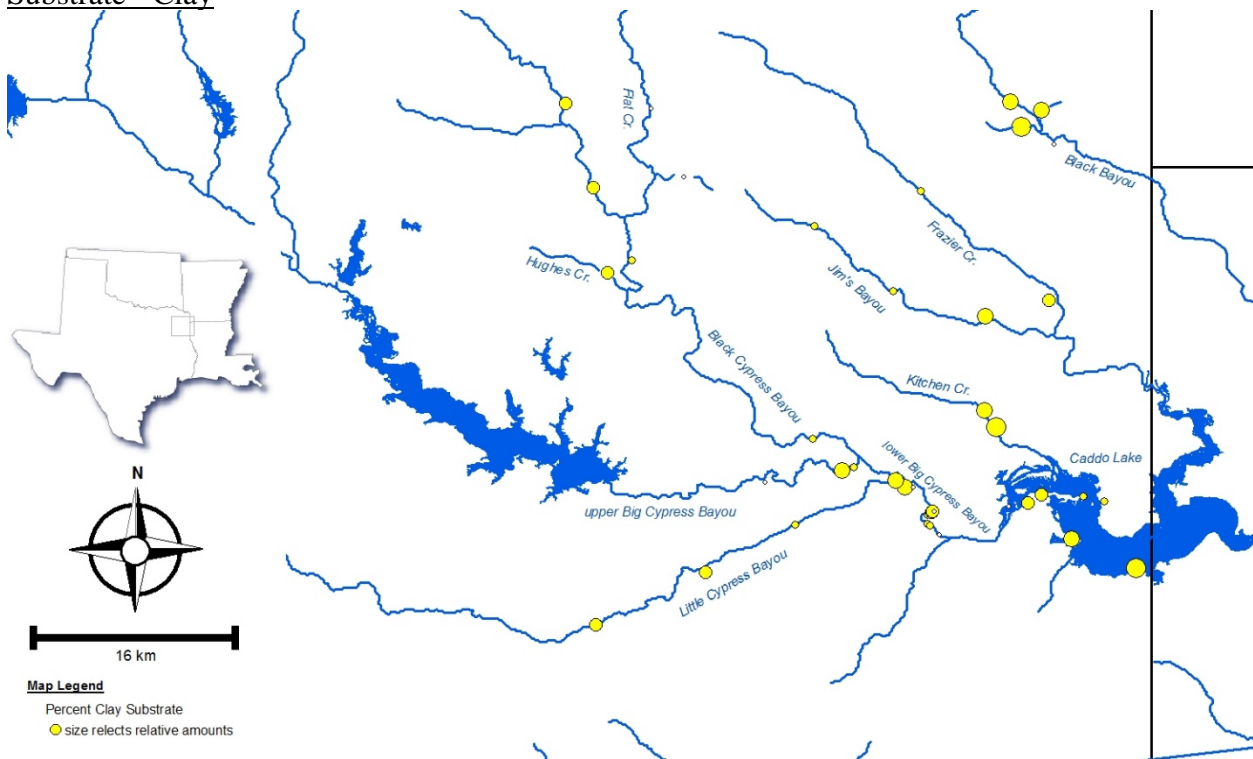


## MICROHABITAT PARAMETERS

### Substrate - Silt

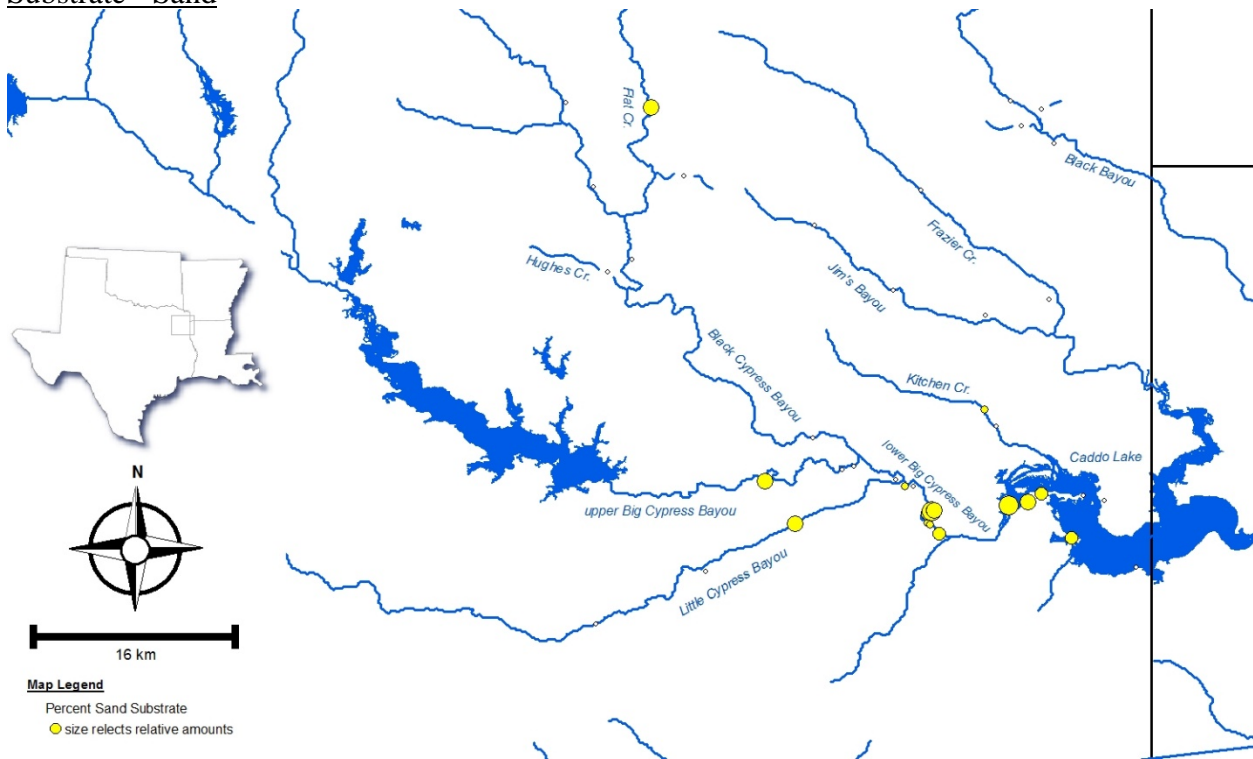


### Substrate - Clay

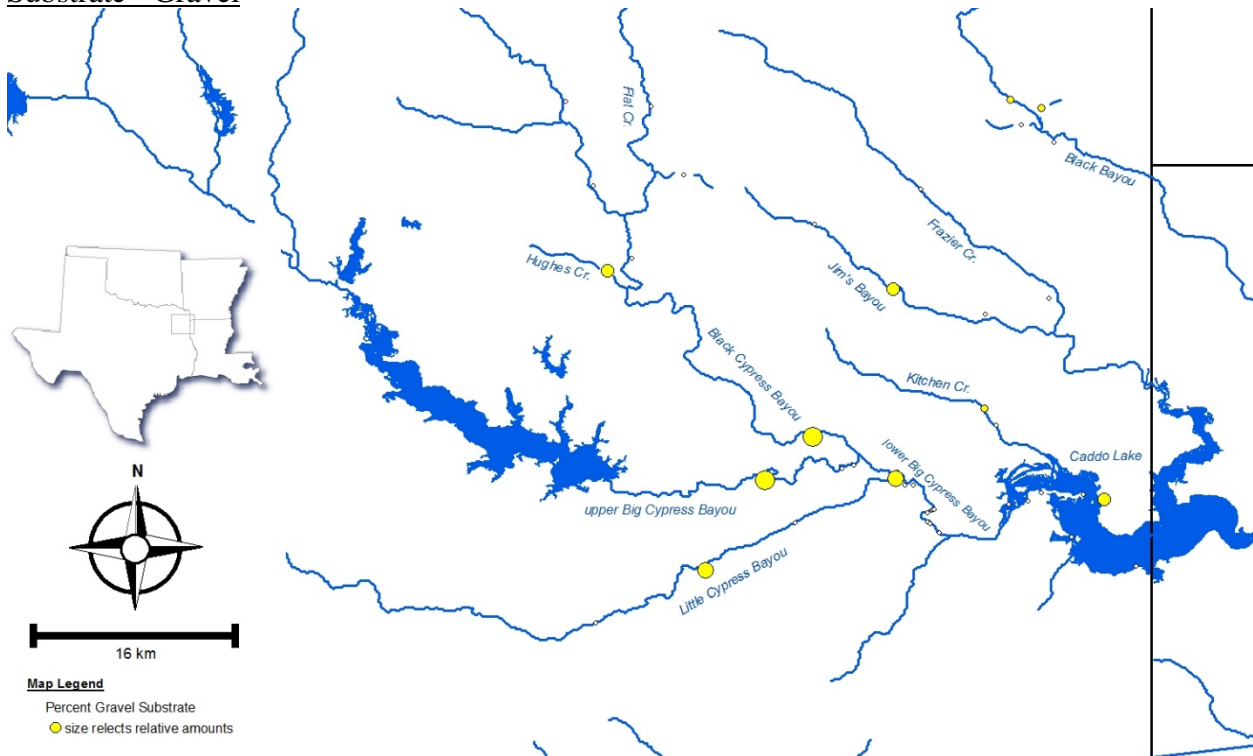




## Substrate - Sand

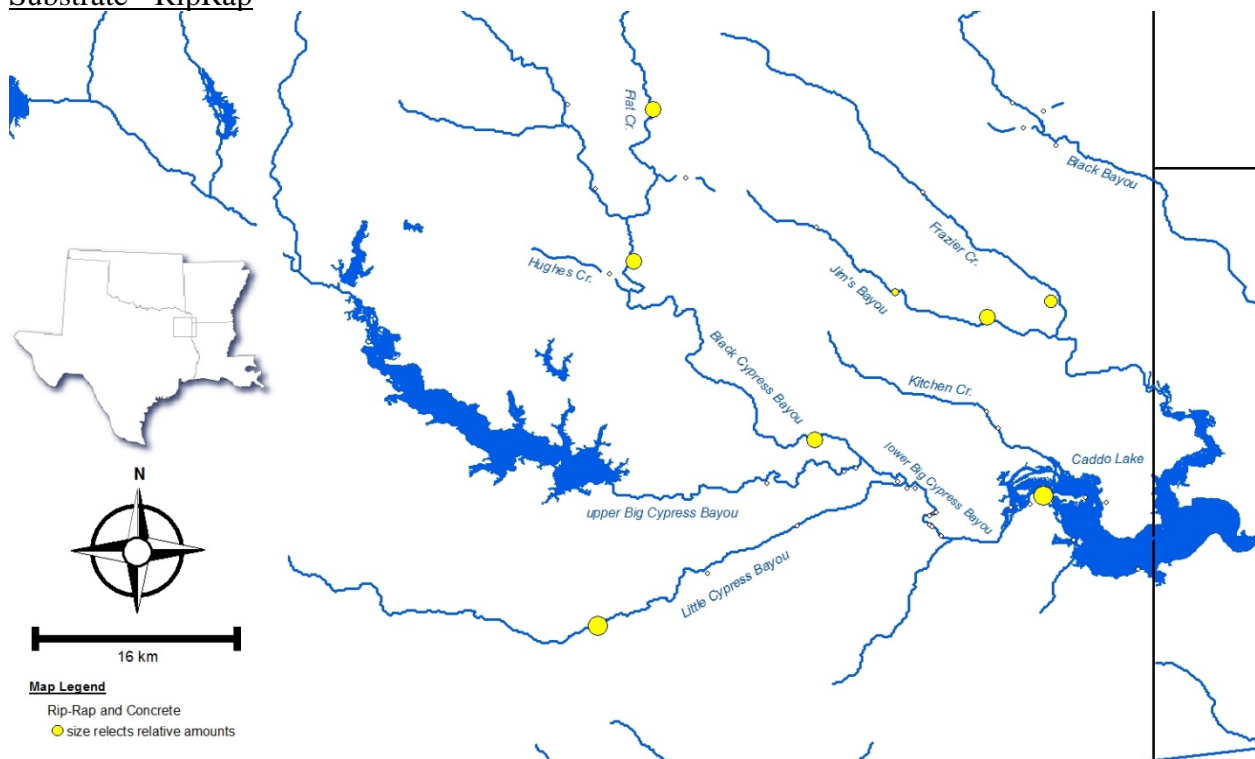


## Substrate - Gravel

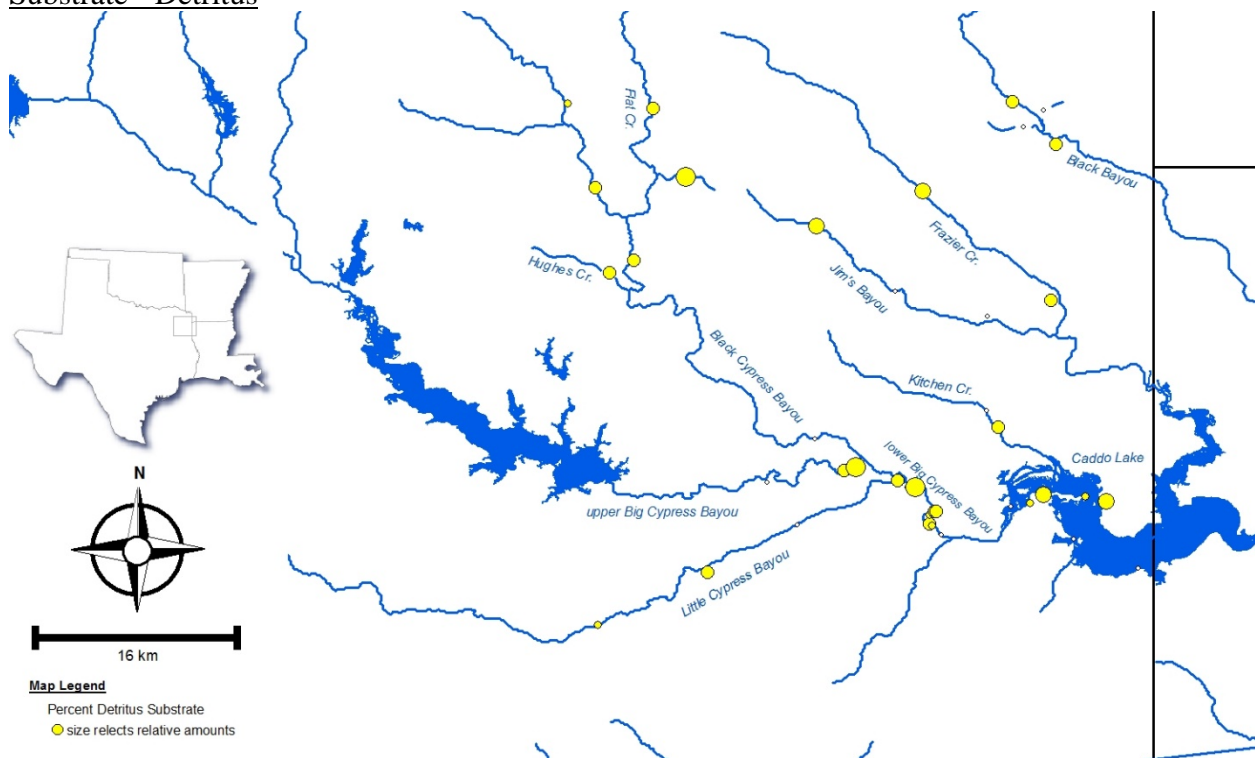




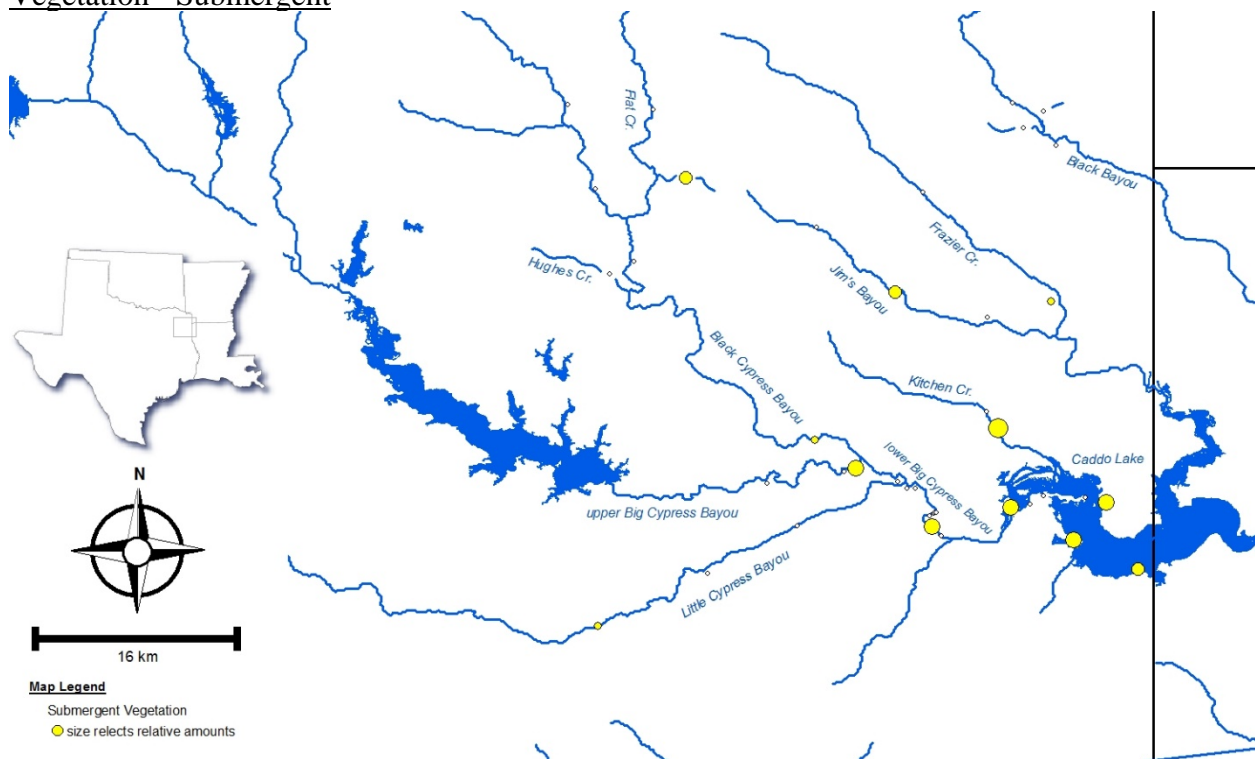
## Substrate - RipRap



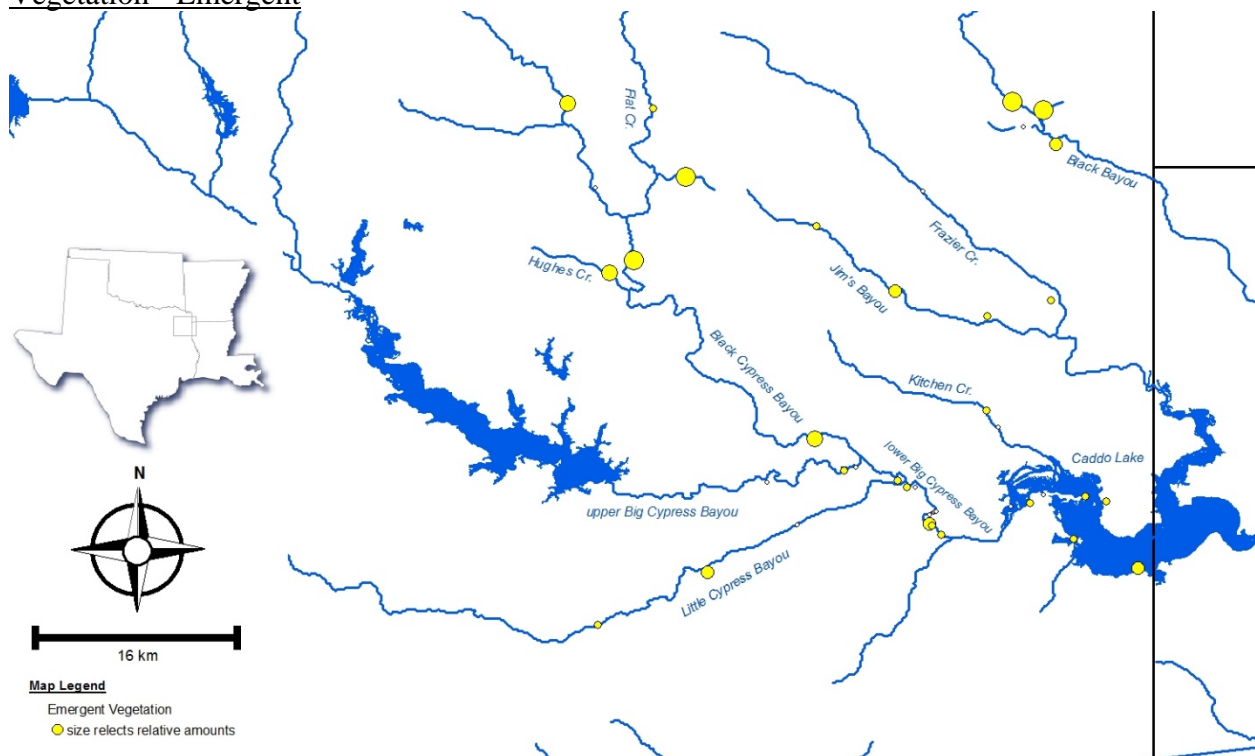
## Substrate - Detritus



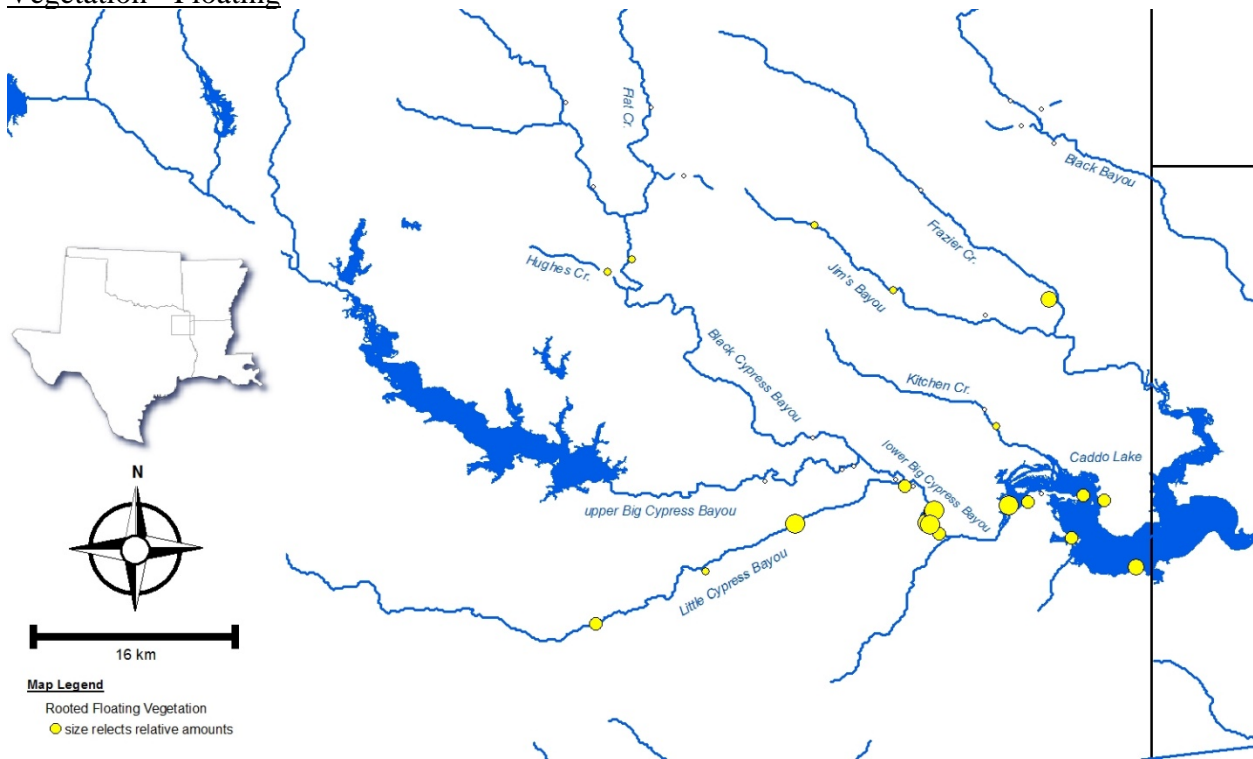
## Vegetation - Submergent



## Vegetation - Emergent



## Vegetation - Floating



## Physical Structure/Habitat Complexity

