Distribution, Abundance, and Habitat Use of the Saltmarsh Topminnow (Fundulus jenkinsi)

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Table of Contents

List of Figures

List of Tables

Executive Summary

The Saltmarsh Topminnow (*Fundulus jenkinsi*) occurs sporadically in tidal marsh habitat along the U.S. coast of the Gulf of Mexico, from Florida to Texas. Little is known about the exact extent of their range, distribution, or abundance but previous studies have shown a link between *Spartina alterniflora* marsh habitat and *F. jenkinsi* occurrences. Historically, the Galveston Bay population represents the western most extent of this species' range with a few occurrences reported as far west as the Rio Grande. Additional infrequent collections of *this species* have been made in Sabine Lake, Cedar Lakes Creek, and Matagorda Bay. Today the Galveston Bay population appears to represent the western most extent of their range although occurrences of the species may be found as far west as San Antonio Bay.

The Saltmarsh Topminnow has been previously found in low to moderate salinities and appears to utilize the edge of the salt marshes and have been primarily found within small intertidal creeks connected to large salt marsh channels. The Texas Parks and Wildlife Department (TPWD) coastal fisheries monitoring program has been unsuccessful in detecting Saltmarsh Topminnow since the mid-1970's based on their standardized monitoring program. It is likely that the current TPWD coastal fisheries monitoring program design which utilizes larger mesh 60 ft. bag seines and trawls deployed in open bay habitats is not selective towards the capture of this species.

The Saltmarsh Topminnow has been listed as a species of concern by the National Oceanic & Atmospheric Administration (NOAA) and in the states of Louisiana, Mississippi, Alabama, and Florida since 2004 (Federal Register 2004a). Collectively, NOAA and the U.S. Fish and Wildlife Service (USFWS) determined that the petition presented substantial scientific information indicating that the petitioned action may be warranted and published a joint 90-day finding in the Federal Register. The USFWS agreed to assume jurisdiction of the species and responsibility for determining whether listing the Saltmarsh Topminnow as threatened or endangered is warranted. The Saltmarsh Topminnow is listed as a species of greatest conservation need for the Gulf Coast Prairies and Marshes ecoregion in the state of Texas.

Due to their apparent rarity there is an urgent need to determine the current population status of *F. jenkinsi* within Texas and across its historical range. Comprehensive data on both the species range, habitat requirements, and demographics is currently lacking within Texas. This data is needed by resource agencies to support ongoing management and conservation of this species and related habitat. Given this species' restricted range in Texas and the recent projections of coastal development, ongoing land subsidence, and projected sea level rise, it is important that the occurrence and habitat associations of this species be carefully delineated as these and other threats can cumulatively degrade saltmarsh habitat and consequently threaten this species of fish.

The primary objectives of this study are:

1) Develop local population abundance estimates of *Fundulus jenkinsi* in Galveston Bay and Sabine Lake, Texas.

2) Evaluate habitat preferences including biological, physical, and water quality attributes of *Fundulus jenkinsi* in Galveston Bay and Sabine Lake, Texas.

3) Estimate demographic parameters including relative size, age, sex distribution, growth, and reproduction characteristics of *Fundulus jenkinsi* in Galveston Bay and Sabine Lake, Texas.

The objectives outlined above were accomplished by: 1) synthesizing and reviewing past literature including agency reports, 2) conducting new surveys using passive and active collection techniques including Breeder traps and seine hauls of areas where Saltmarsh Topminnow have historically been captured, and 3) executing additional surveys in portions of Galveston Bay and Sabine Lake where there is no historic record of the species focusing on wetlands, tidal creeks, with appropriate salinity regimes based on literature derived habitat preferences. During each survey additional biological community data were collected to assess potential interactions between *F. jenkinsi* and co-occurring species of fish.

From February 17, 2014 to November 20, 2014 a total of 135 sites were sampled along the upper Texas coast. Fifty- two sites were sampled in Sabine Lake with *F. jenkinsi* being caught in the upper portion of the bay in the Neches and Sabine River drainages. Eighty-three sites were sampled in Galveston Bay with *F. jenkinsi* being caught in the east and mid-upper portion of the bay in East Bay, Trinity Bay, and Dickinson and Moses Bayou drainage. Analysis of the fish assemblages caught over the course of this study show that *F. jenkinsi* have a strong association with certain fish species. In this study *F. jenkinsi* were always found in association with at least one other species of from the family Fundulidae and often in combination with other marsh edge estuarine fish species (e.g. *P. latipinna*, *G. affinis*, and *C. variegatus*).

Results of this study suggest the existence of a gradient of *F. jenkinsi* density based on geographic location. Not only were a greater percentage of sites found to contain *F. jenkinsi* in Sabine Lake compared to Galveston Bay but *F. jenkinsi* were found, on average, in greater numbers. This pattern of decreasing *F. jenkinsi* frequency and density as one moves further west along the coast is seen within each bay system as well. Our study supports previous literature which states that *F. jenkinsi* seem to prefer lower to mid-salinity ranges. The difference in distribution of *F. jenkinsi* across salinity gradients between bays is most likely due to the degree of freshwater inflow and resulting salinity in each system. This data suggests that while *F. jenkinsi* is able to inhabit a wide salinity range (2-19 ppt) it is equally important to have appropriate marsh habitat available along the existing salinity gradients since large fluctuations in either fresh or saltwater input may result in *F. jenkinsi* actively moving to other locations to stay within their preferred salinity range. Length frequencies of *F. jenkinsi* did not significantly vary by either gender, season, or bay system. An overall trend that we observed in all or our length frequency data was the appearance of two modal peaks, one at about 22 mm and the second at about 35 mm. This data provides strong evidence that these two modes of standard length values represents at least two separate age classes. The GSI (Gonadosomatic Index) and ovary phase analysis conducted during this study supports previous estimates for the *F. jenkins*i spawning season and an existence of an overall seasonal trend in reproductive organ growth. While GSI analysis was incomplete due to a lack of *F. jenkinsi* caught during the summer months a significant rise in the GSI values for both male and females occurred through the spring and early summer months.

Fundulus jenkinsi individuals are likely not as rare as previously thought. Water levels drastically effected marsh inundation and thus our ability to sample effectively with our gear. Furthermore, we collected *F.*

jenkinsi at six sites in the Moses Bayou and East Bay regions of Galveston Bay and seven sites in the Neches River drainage of Sabine Lake for a total of 13 sites were this species has not been reported from historically. More research can be done such as sampling for populations west of Galveston Bay and resurveying sites that were sampled during high water levels in low water condition to gain a more accurate estimation about the locations of viable populations.

Focus areas of future research should include better documentation of oocyte development during spawning season in order to gain a more accurate predictions of spawning times and offspring production. Mark-recapture studies would help establish a more reliable method to determine and validate age and growth estimates for this species as well as facilitate tracking the movement of *F. jenkinsi* individuals and give better estimates of how much inter-marsh migration occurs and the fidelity to certain habitat types.

Introduction

Historic Distribution and Range

The Saltmarsh Topminnow (*Fundulus jenkinsi*) occurs sporadically in tidal marsh habitat along the U.S. coast of the Gulf of Mexico, from Florida to Texas (Peterson et al. 2003). Little is known about the exact extent of their range, distribution, or abundance but previous studies have documented a positive association between *Spartina alterniflora* marshes and *F. jenkinsi* occurrences (Peterson & Turner, 1994). Historically, the Galveston Bay population represents the western most extent of this species' range with sporadic occurrences reported as far west as the Rio Grande River delta (Simpson and Gunter 1956 cited in NatureServe Explorer 2014; Patrick et al. 1998; Hoese and Moore 1998; Jordan and Evermann 1896). Additional sporadic collections of *this species* have been made in Cedar Lakes Creek (Guillen 1996), Matagorda Bay (Akin et al. 2003), and Sabine Lake (Patrick et al. 1998). Based on recent data the Galveston Bay population appears to represent the western most extent of their range although sporadic occurrences of the species may be expected as far west as San Antonio Bay (Nicolau 2001).

Past studies within Galveston Bay watershed have detected *F. jenkinsi* in West Bay, Trinity Bay, Oyster Bayou, East Bay, and the western portion of Galveston Bay including Dickinson Bayou (Hoese and Moore 1998; USGS 2011; Guillen 1996). Prior to this study it has not been reported in other portions of the bay and only rarely in Sabine Lake. Museum records at Texas A&M University and the University of Texas document the collection of this species in the Sabine River and coastal canals near Sabine Pass. Patrick et al. (1998) collected *F. jenkinsi* in the lower Neches River during 1996 using large, fine mesh dip nets but failed using more traditional sampling gear such as seines. The Texas Parks and Wildlife Department (TPWD) coastal fisheries monitoring program has been unsuccessful in detecting Saltmarsh Topminnow since the mid-1970's based on their standardized monitoring program. It is highly likely that the current TPWD coastal fisheries monitoring program design which utilizes large mesh 60 ft. bag seines and trawls deployed in open bay habitats is not selective towards the capture of this species. This is likely attributed to the species relatively small size and observed affinity to moderate to low salinity wetland edge and tidal creek habitats which are typically not included in the current TPWD sampling frame which focuses on open bay sites.

Habitat, Ecology, and Life History Characteristics

The Saltmarsh Topminnow is considered an estuarine species and has been previously found in low to moderate salinities (Peterson & Ross 1991; Lopez et al. 2010; and Griffith 1974). Past research suggests that *F. jenkinsi* have been shown to utilize the edge of the salt marshes (Peterson et al. 2003, Lang et al. 2012, and Lopez et al. 2010) and are primarily found within small intertidal creeks connected to larger channels within the saltmarsh. Access to these small interconnected tidal creeks appears to be an important contributing factor in the Saltmarsh Topminnow's diet and reproduction. High water levels allow *F. jenkinsi* access to larger foraging areas in the inundated marsh and provides refuge from aquatic predators found in deeper water. While little is known about the diet of *F. jenkinsi,* Lopez et al. (2010) found that its diet consists of both small aquatic and terrestrial invertebrates such as Amphipods, Gastropods, Copepods, Diptera, and Hemiptera, and varied according to the age of the individual and season.

The Saltmarsh Topminnow, like other fundulids, are batch spawners and capable of spawning more than once during a single spawning season (Lopez, Peterson, Lang, & Charbonnet, 2010). Monthly gonadosomatic indexes (GSI) and ovarian histological analysis of female *F. jenkinsi* indicate the spawning season occurs from March through August (Lang et al. 2012). Many fish species time their spawning events with regards to the position of the moon and associated tides. Spawning intensity for *F. jenkinsi* appears to increase with the timing of spring tides, when tidal heights are at their greatest, and decrease during neap tides (Lang et al., 2012). The higher water levels allow greater access into the inundated marsh to deposit their eggs on more protected, interior and higher ground. Saltmarsh Topminnow reproduction and diet are strongly linked to inundated salt marsh access. Therefore intertidal creeks embedded within salt marshes are essential to this species as these creeks act as access points for cover, reproduction, and foraging.

Conservation Status

The Saltmarsh Topminnow has been listed as a species of concern by the National Oceanic & Atmospheric Administration (NOAA) and in the states of Louisiana, Mississippi, Alabama, and Florida since 2004 (Federal Register 2004a). These designations were due in part to its sparse populations, lack of information regarding its biology and ecology, and the threat that human activities pose to their essential habitat (Peterson et al. 2003, NOAA 2009). In 2010, the WildEarth Guardians and Sarah Felsen petitioned NOAA and the U.S. Fish and Wildlife Service (USFWS) to list the Saltmarsh Topminnow under the U.S. Endangered Species Act (Felson, 2010). Collectively, NOAA and USFWS determined that the petition presented substantial scientific information indicating that the petitioned action may be warranted and published a joint 90-day finding in the Federal Register (Crabtree 2011; Federal Register 2004b). The USFWS agreed to assume jurisdiction of the species and responsibility for determining whether listing the Saltmarsh Topminnow as threatened or endangered is warranted (Crabtree 2011). Since the Saltmarsh Topminnow is listed as a species of greatest conservation need for the Gulf Coast Prairies and Marshes ecoregion in the state of Texas, responsibility falls to the TPWD for coordinating with their conservation partners to develop initiatives and goals that will monitor and address the needs of *F. jenkinsi* and their related essential habitats within the state (TPWD 2005, TPWD 2011, TPWD 2012b).

Problem Statement

Due to their apparent rarity there is an urgent need to determine the current population status of *F. jenkinsi* within Texas and across its historical range. Comprehensive data on both the species range, habitat requirements, and demographics is currently lacking within Texas. This data is needed by resource agencies to support ongoing management and conservation of this species and related habitat. Recent research confirms that there is a direct link between *F. jenkinsi* abundance, coastal saltmarsh habitat, and specific salinity regimes (Lopez et al., 2010). The link between *F. jenkinsi* abundance and specific habitat requirements to be better quantified to develop meaningful management recommendations for the long-term conservation of this species. Given this species' restricted range in Texas and the recent projections of coastal development, ongoing land subsidence, and projected sea level rise (Warren Pinnacle Inc., 2011; Montagna et al. 2011), it is important that the occurrence and habitat associations of this species be carefully delineated as these and other threats can cumulatively reduce the geographic extent of saltmarshes and consequently potentially threaten the population viability of this species of fish.

Study Objectives

The primary objectives of this study are:

1) Develop local population abundance estimates of *Fundulus jenkinsi* in Galveston Bay and Sabine Lake, Texas.

2) Evaluate habitat preferences including biological, physical, and water quality attributes associated with the occurrence of *Fundulus jenkinsi* in Galveston Bay and Sabine Lake, Texas.

3) Estimate demographic parameters including size, age, sex distribution, growth, and reproduction characteristics of *Fundulus jenkinsi* in Galveston Bay and Sabine Lake, Texas.

The objectives outlined above were accomplished by: 1) reviewing and synthesizing past published literature describing the occurrence of the species in Texas including agency reports, 2) conducting new surveys using passive and active collection techniques including Breder traps and seines in areas where Saltmarsh Topminnows have historically been captured, and 3) executing additional surveys in portions of Galveston Bay and Sabine Lake where there is no historic record of the species focusing on wetlands, tidal creeks, with appropriate salinity regimes based on literature derived habitat preferences. During each survey additional fish community data were collected to assess potential interactions between *F. jenkinsi* and co-occurring species of fish.

Methods

Literature Review and GIS Database

Past records on the occurrence of Saltmarsh Topminnow were obtained from the 1) Fishes of Texas project (Hendrickson & Cohen 2014) and 2) the Texas Parks and Wildlife Department coastal fisheries independent bag seine data, 3) published agency and peer reviewed literature and 4) theses and dissertations. The location and dates of historic occurrences as well as this study's collections were incorporated into an ArcGIS geodatabase and are depicted on sampling site maps (Figure 1-3). A shapefile depicting the distribution of wetland habitats (USFWS 2014) has been overlaid on these maps to highlight the currently available saltmarsh habitat.

Study Area and Sampling Frequency

Sample survey sites were selected in wetland habitats around Galveston Bay and Sabine Lake. Sites chosen were tidally influenced and received some freshwater input that would provide optimal salinity levels (<20ppt) utilized by *F. jenkinsi* (Peterson et al. 2003 and Lopez et al. 2011). Previous studies document a positive link between *S. alterniflora* marsh habitat and *F. jenkinsi* occurrences (Peterson & Turner 1994). We therefore focused our site selection on areas containing *S. alterniflora* or other saltmarsh vegetation. We focused the majority of our sampling on smaller intertidal creeks (Figure 4) but also included a variety of other habitat types including coastal and inland open marsh habitat as well as larger saltmarsh lined tidal channels and streams.

Field sampling was conducted from February 2014 through March 2015. Quarterly biological samples were taken from tidally influenced saltmarsh sites along Galveston Bay and Sabine Lake to estimate the spatial distribution of the *F. jenkinsi* within the region (Figure 1 and 2). During February 28 to November 20, 2014, quarterly samples were collected from a total of 135 individual sites. Additional monthly sampling was conducted from February, 2014 to March, 2015 at two locations within Moses Bayou (Figure 3)[1]. At these sites we found that the population was sufficiently dense to support reproductive and demographic analysis and the investigation of temporal trends.

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[1] The third site was added after September 10, 2014.

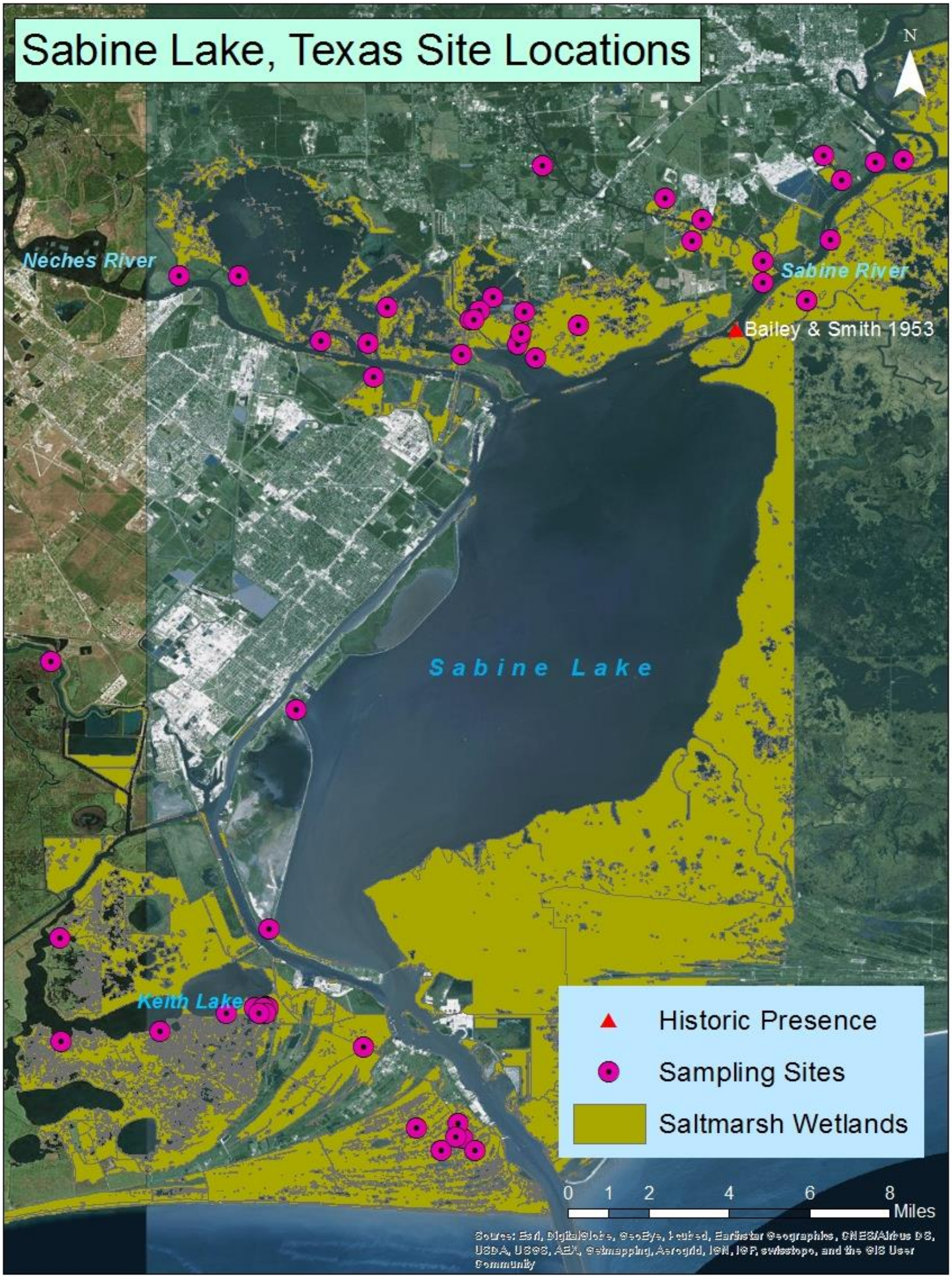

Figure 1 Map of sampling sites located around Sabine Lake TX. Sampling sites are marked by circles. Documented historical occurrences are denoted by solid triangles and are cited. Saltmarsh wetlands based on 2014 USFWS national wetlands inventory data.

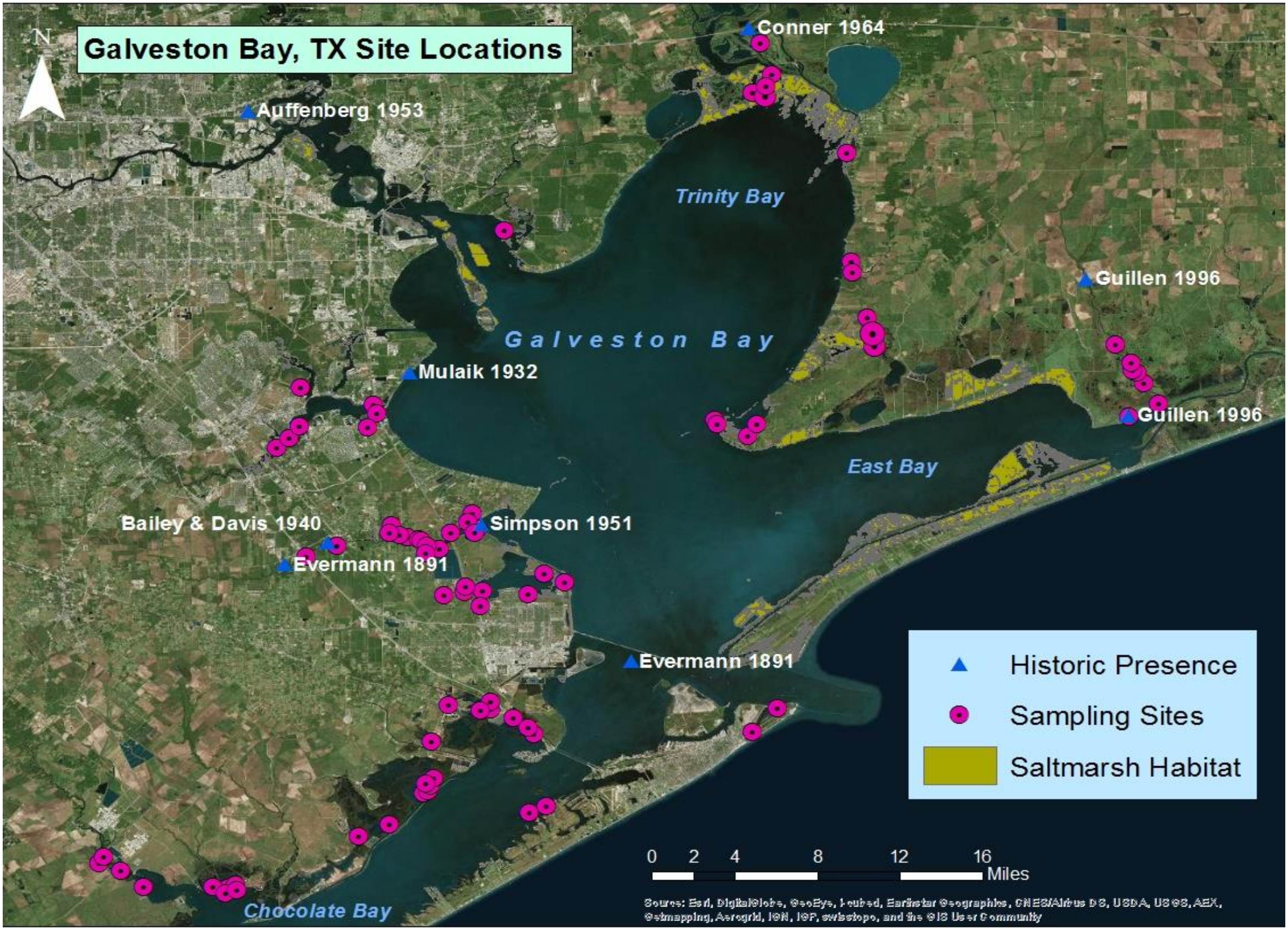

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Figure 2 Map of sampling sites located around Galveston Bay TX. Sampling sites are marked by circles. Documented historical occurrences are denoted by solid triangles and are cited. Saltmarsh wetlands based on 2014 USFWS national wetlands inventory data.

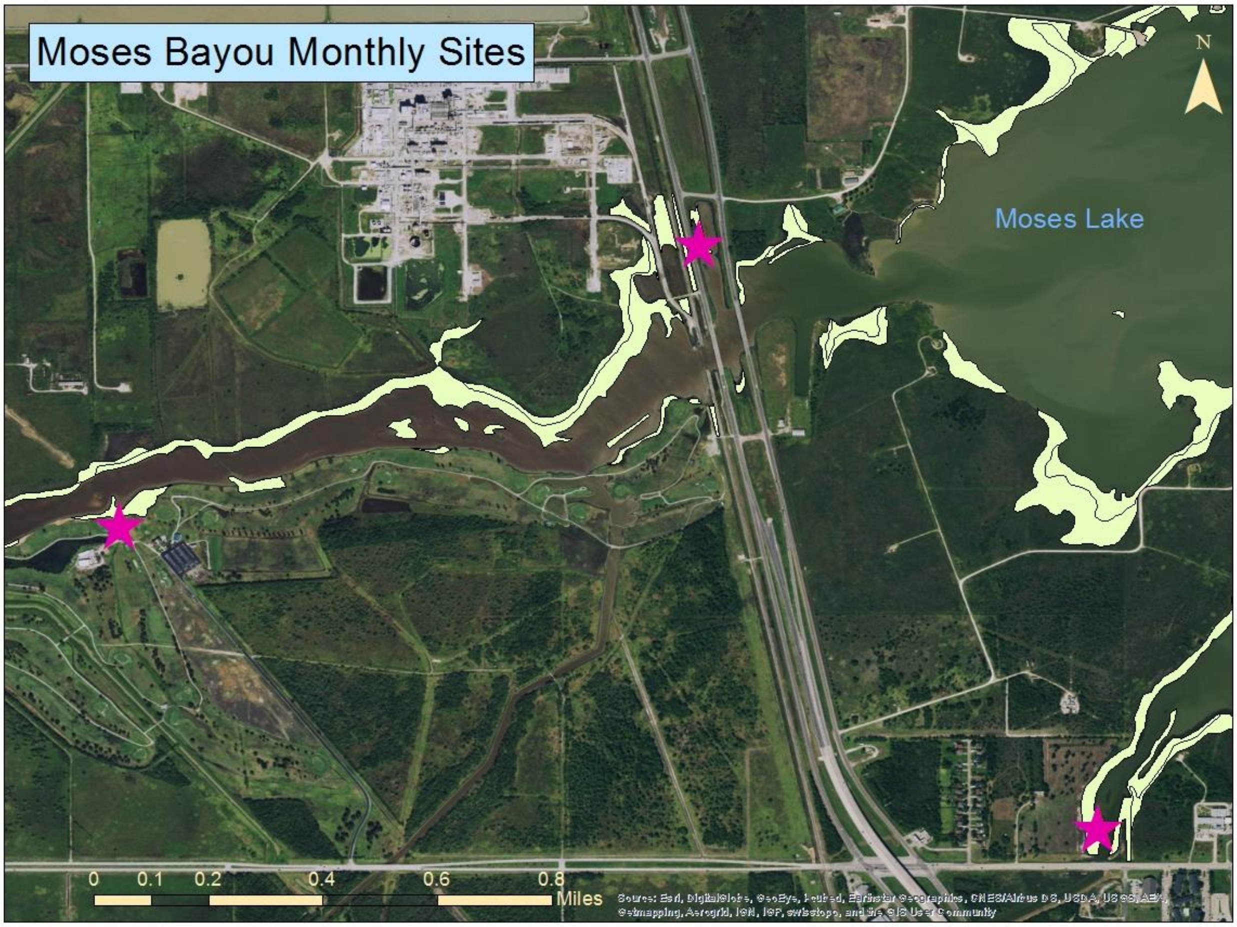

Figure 3 Map of monthly sampling sites located around Moses Bayou in Galveston Bay. Sampling sites are marked by stars. Saltmarsh wetlands based on 2014 USFWS national wetlands inventory data.

Sampling Methods

Fish collections were conducted using a straight seine (15’ x 4’) with ¼” bar mesh and Breder traps (Breder 1960) (Figure 4). Three replicate seine hauls, approximately 10 meters each, were made parallel to the marsh edge at each sampling site. Breder traps were used in conjunction with seine hauls during monthly sampling events and, when possible, during quarterly sampling events. Breder traps were constructed with clear plexiglass (0.08’’ thickness) using the same dimensions (12'' x 6'') as Lopez et al. (2011). Four traps were set at least two meters apart facing the marsh edge at each site at high tide and picked up at the end of low tide. The Breder trap methodology was implemented in the summer of 2014 to supplement the seining efforts in an attempt to collect additional individuals at the designated monthly and quarterly sites. When possible, fish were identified in the field and released. All other specimens were administered a lethal dose of buffered MS-222, fixed in a 10% buffered formalin solution, and then brought back to the laboratory where they were transferred to a 70% ethanol solution, identified to species, counted, and measured (standard length in mm).

Figure 4. (Above) Intertidal creek network within the *Spartina alterniflora* saltmarsh habitat of Chocolate Bay. (Bottom Left) Breder Trap set facing the marsh edge (Bottom Right). Collection of nekton caught after trap retrieval.

During each sampling event water depth in meters was measured. Tide stage (flood, high slack, ebb, low slack) was also recorded. Water level in reference to mean lower low water (MLLW) (m) was obtained from the closest NOAA tide gage site. Water quality measurements including temperature (°C), dissolved oxygen (mg/L and %), conductivity (µS/cm), pH, and salinity (ppt) were collected using an YSI 600 XLM sonde before seining and upon retrieving traps. Water clarity was also measured at each site with the use of a Secchi tube. A square-meter quadrat was used to quantify dominant vegetation (% cover) in front of each trap and along the banks of each seine haul.

Laboratory Processing

All individual fish caught within each seine haul or trap were identified to species and enumerated. The standard length of all *F. jenkinsi* specimens collected were measured and grouped into length frequency histograms to visually assess density and age structure by month and season. Modal lengths were separated using FISATII (Gayanilo 2005) modal progression analysis conducted on monthly length frequencies to establish relative age classes.

To assess the reproductive condition of *F. jenkinsi*, the standard length (SL, mm) and total weight (TW, grams) were taken from individuals captured from monthly collections. The sex of each individual was determined using the dimorphic characteristics described by Lopez et al. (2010). When external sexual dimorphic features (Figure 5) were not clear sex classification was done via observation of the extracted gonads (Figure 6).

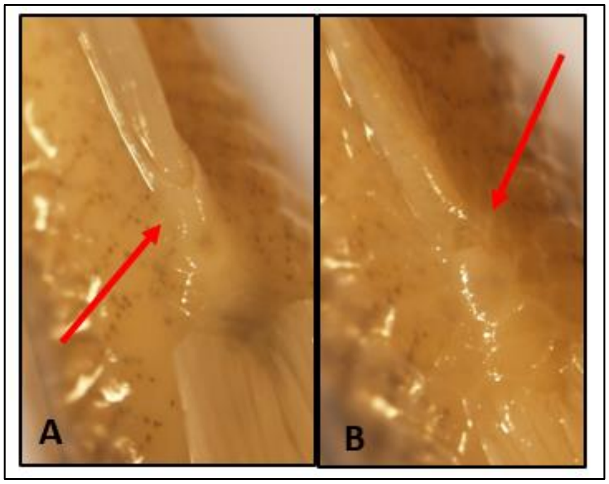

Figure 5 *Fundulus jenkinsi* ventral view. (A) Female genital region showing the anal fin with sheath (arrow). (B) Male genital region showing the anal fin and exposed papilla (arrow).

Figure 6 *Fundulus jenkinsi* gonads extracted from individuals caught in April 2014. (A) Female (B) Male

Once the gonads were extracted they were weighed (GW, g) and the gonadosomatic index (GSI) calculated: [(GW / TW)*100]. The monthly mean GSI was than calculated for both males and females and plotted to assess reproductive condition by month. Reproductive activity of female *F. jenkinsi* were further assessed by classifying the ovarian stage (Figure 7) using methods described Brown-Peterson et al. (2011) and Lopez et al. (2010). Gonads extracted from the females used in GSI analysis were the same ones used in ovary phase analysis.

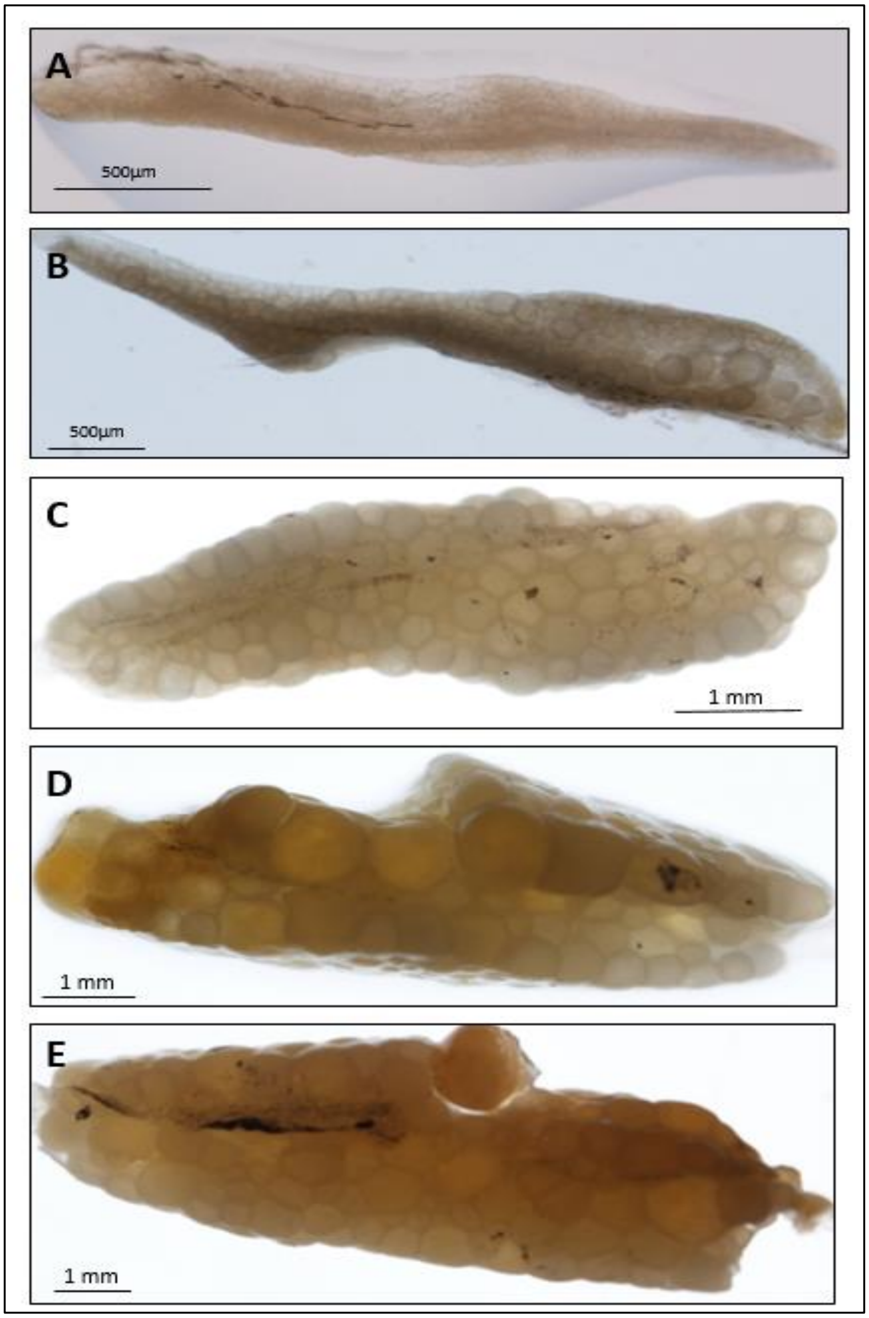

Figure 7 Ovary stages of *F. jenkinsi* females taken from monthly collections. (A) Latent from December 2014 (B) Early maturing from March 2014 (C) Late maturing from February 2015 (D) Mature from April 2014 and (E) Ripe from April 2014.

Data Analysis

Fish community structure was characterized by calculating total species abundance (N), relative abundance (%), richness (S), Shannon-Wiener diversity (H’) and Pielou’s evenness index (J’) (Magurran 2004) and catch per unit effort (CPUE) of *F. jenkinsi* were based on the three replicate seine tows for each site sampled. The diversity (H’) of each quarterly site’s fish community assemblage was calculated in PRIMER and the resulting values were analyzed with T-tests in Minitab 17 (2010) to determine if overall diversity significantly varied between sites where *F. jenkinsi* were present and absent in both bay systems.

Fish assemblage data were 4th-root transformed. A Bray-Curtis similarity index was created using the PRIMER 6 statistical software package (Clarke and Warwick 2001). Site groupings based on similar species assemblages were further investigated using an analysis of similarity (ANOSIM) to test for a pattern in community structure when *F. jenkinsi* was present. Two-way ANOSIM were used to test the influence of season and tidal stage on species assemblages within each bay system. Sites where both seining and Breder traps were used in fish collection where pooled from both bay systems as well as from quarterly and monthly sampling events in order to run an ANOSIM to analyze fish assemblages across gear types. Multidimensional scaling (MDS) plots of assemblages were also constructed in PRIMER 6 to display assemblage similarities by season and gear type.

Salinity (ppt), temperature (°C), mean lower low water level (MLLW), and bank vegetation (% cover) were compared between sites of *F. jenkinsi* presence and absence within both bay systems. The distribution of each variable was tested for normality (Shapio & Wilks 1965) followed by the appropriate T-test or Mann-Whitney U test (Mann & Whitney 1947) to compare the average level of each variable at sites where *F. jenkinsi* were present and not present.

Similarly, standard length and total weight of *F. jenkinsi* were tested for normality prior to statistical analysis. Depending on the results of the normality tests either parametric or nonparametric statistical analysis was conducted to compare average or median standard length between bay systems, gear type, and gender. Standard length, weight, and GSI data were entered into Minitab 17 (2010) and Analysis of Variance (ANOVA) was run to compare standard lengths across seasons. Two-way ANOVA was also used to compare GSI values across months and seasons while a two-sample T-test compared GSI values across gender. If values were found to be significant, a Tukey’s pairwise comparison was run to analyze where the differences occurred. Linear regression analyses were run to test the association between length and weight and GSI values of both male and females. Length and weight values from both male and female individuals were entered and plotted against each other in Excel and the subsequent trend line calculated to show the relationship between total weight and standard length.

Ovary phases were coded (Latent = 1, Early maturing =2, Late maturing = 3, Mature = 4, Ripe = 4) and linear regression analysis on these ranked scores were conducted using Minitab 17 to evaluate potential relationships between standard length and total weight factors versus female and male GSI values. Linear regression analysis was also used to investigate the relationship between season, standard length, and GSI factors versus ovary development. A α-level of 0.05 was used to determine statistical significance in all tests.

Results

Distribution and Abundance

A total of 135 sites were sampled along the upper Texas coast from February 17, 2014 to November 20, 2014 including 83 in Galveston Bay and 52 in Sabine Lake. The distribution of historical sightings along with the locations and relative abundance where *F. jenkinsi* were captured during this study are depicted on each map. *Fundulus jenkinsi* were caught in the upper portion of Sabine Lake in the Neches and Sabine River drainages (Figure 9). *Fundulus jenkinsi* were also caught in the east and mid-upper portion of Galveston Bay in East Bay, Trinity Bay, and Dickinson and Moses Bayou drainages (Figures 10-12). We collected *F. jenkinsi* at six sites in Moses Bayou and East Bay regions of Galveston Bay, and seven sites in the Neches River drainage of Sabine Lake for a total of 13 sites were this species has not been reported from historically. Locations where surveys were conducted but we failed to detect *F. jenkinsi* are also displayed (Figure 8-12).

The overall percentage of sites where *Fundulus jenkinsi* were found was relatively similar for both Sabine Lake and Galveston Bay systems. Total number of *F. jenkinsi* collected was three times greater in Sabine Lake than in Galveston Bay and the average number of *F. jenkinsi* collected per site in Sabine Lake was also over two times more than the average number of *F. jenkinsi* found per site in Galveston Bay (Table 1).

Within the Sabine Lake system including the Sabine and Neches River drainages, sites where *F. jenkinsi* were found and not found occurred in relatively equal proportions. Catch rates within these three drainages however varied greatly with nearly three times the number of individuals being captured on average at sites within the Sabine River drainage in contrast to the Neches River drainage (Table 1).

Within the Galveston Bay system, the Trinity Bay drainage contained the greatest proportion of sites where *F. jenkinsi* where collected. Sites sampled within the East Bay and Dickinson and Moses drainages contained similar, but smaller proportions of sites containing positive catches of *F. jenkinsi*. Average catch rates of F. jenkinsi varied greatly among Galveston Bay sites with East Bay sites possessing twice the average number of individuals as Trinity Bay and almost six times the average number of individuals captured at Dickinson and Moses Bayou (Table 1).

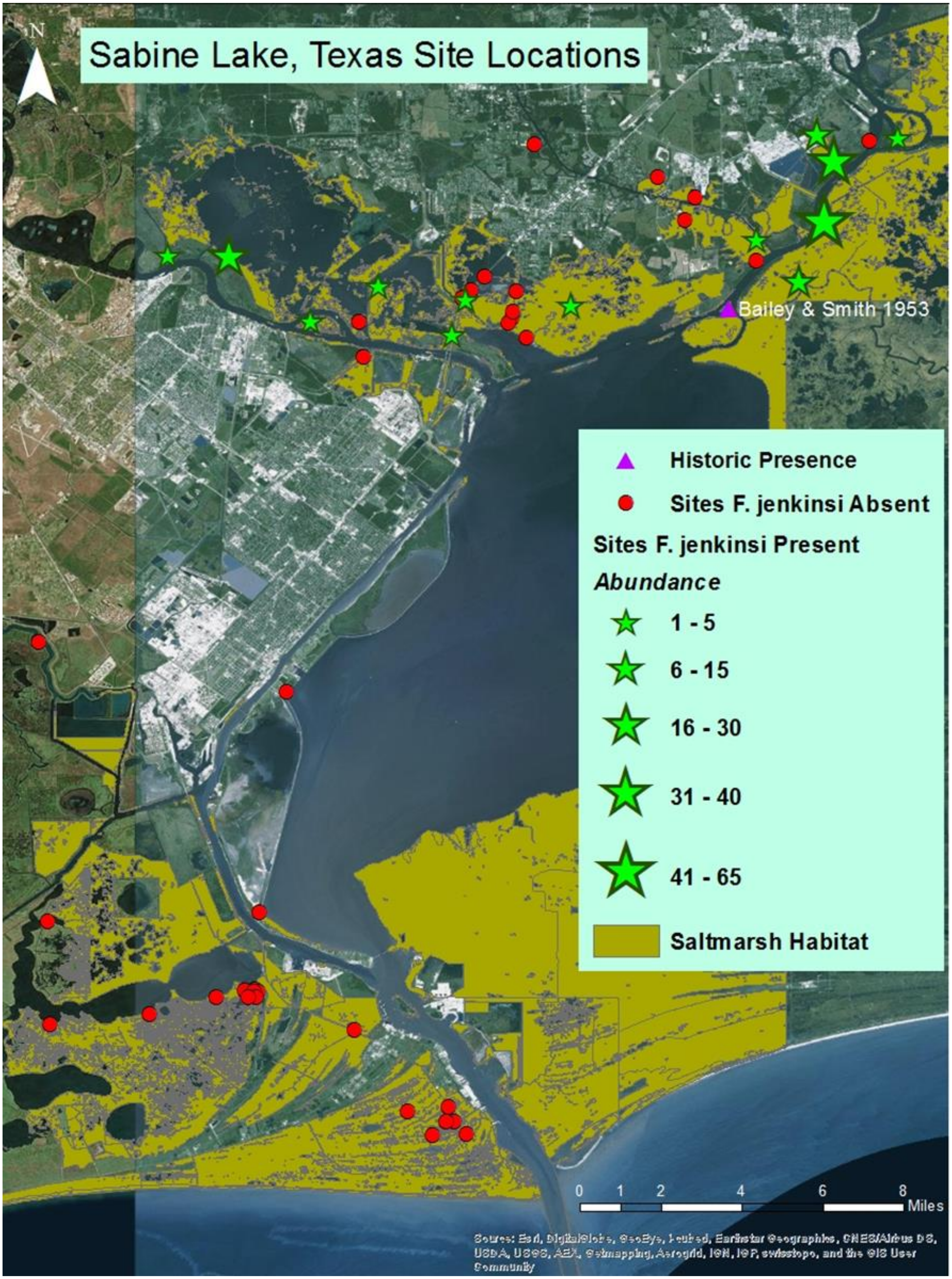

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Figure 8 Map of sampling sites located around Sabine Lake, TX. Green stars and red circles represent sites were *F. jenkinsi* were found and not found respectively. Size of the star corresponds to total number of *F. jenkinsi* collected at that site.

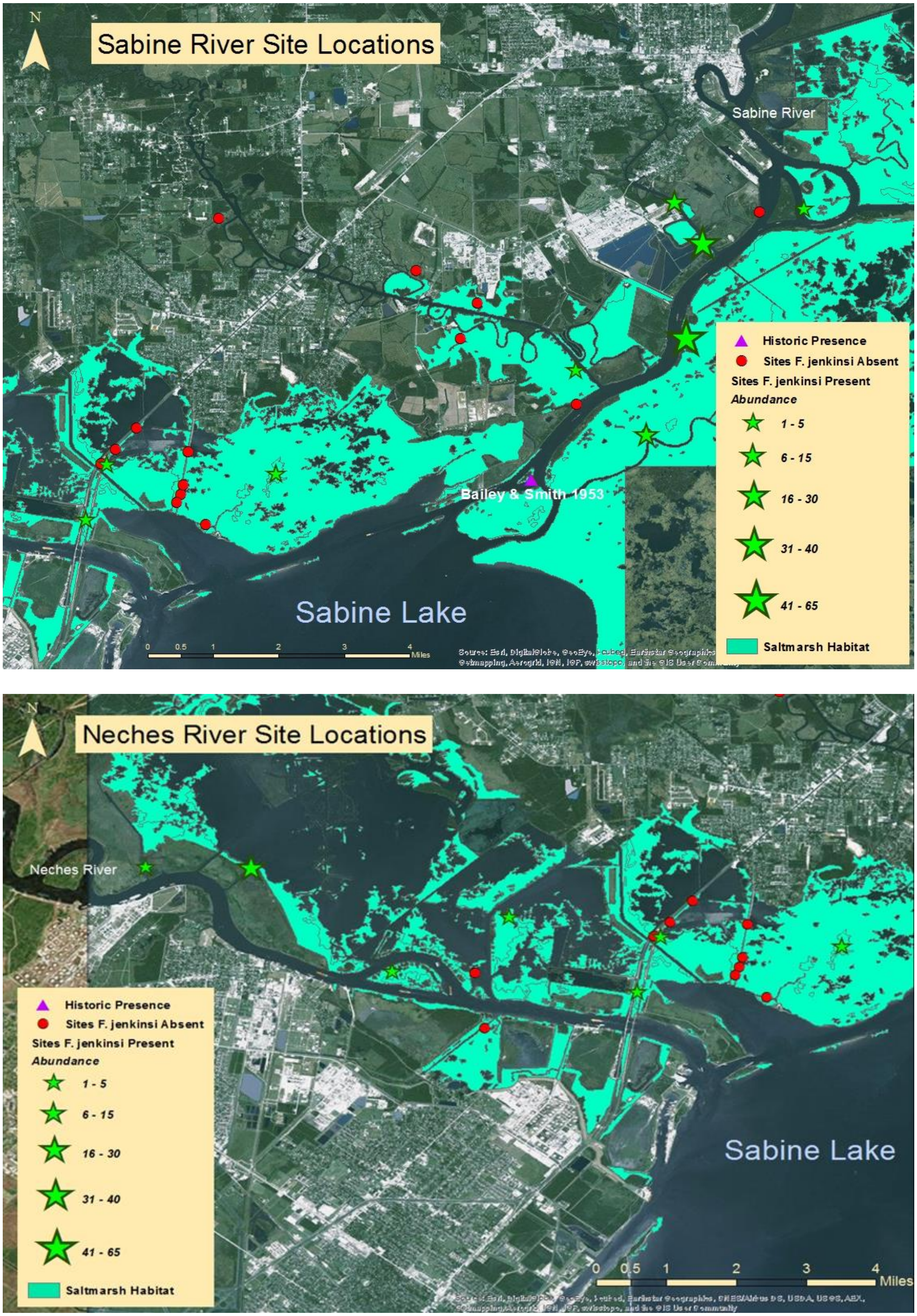

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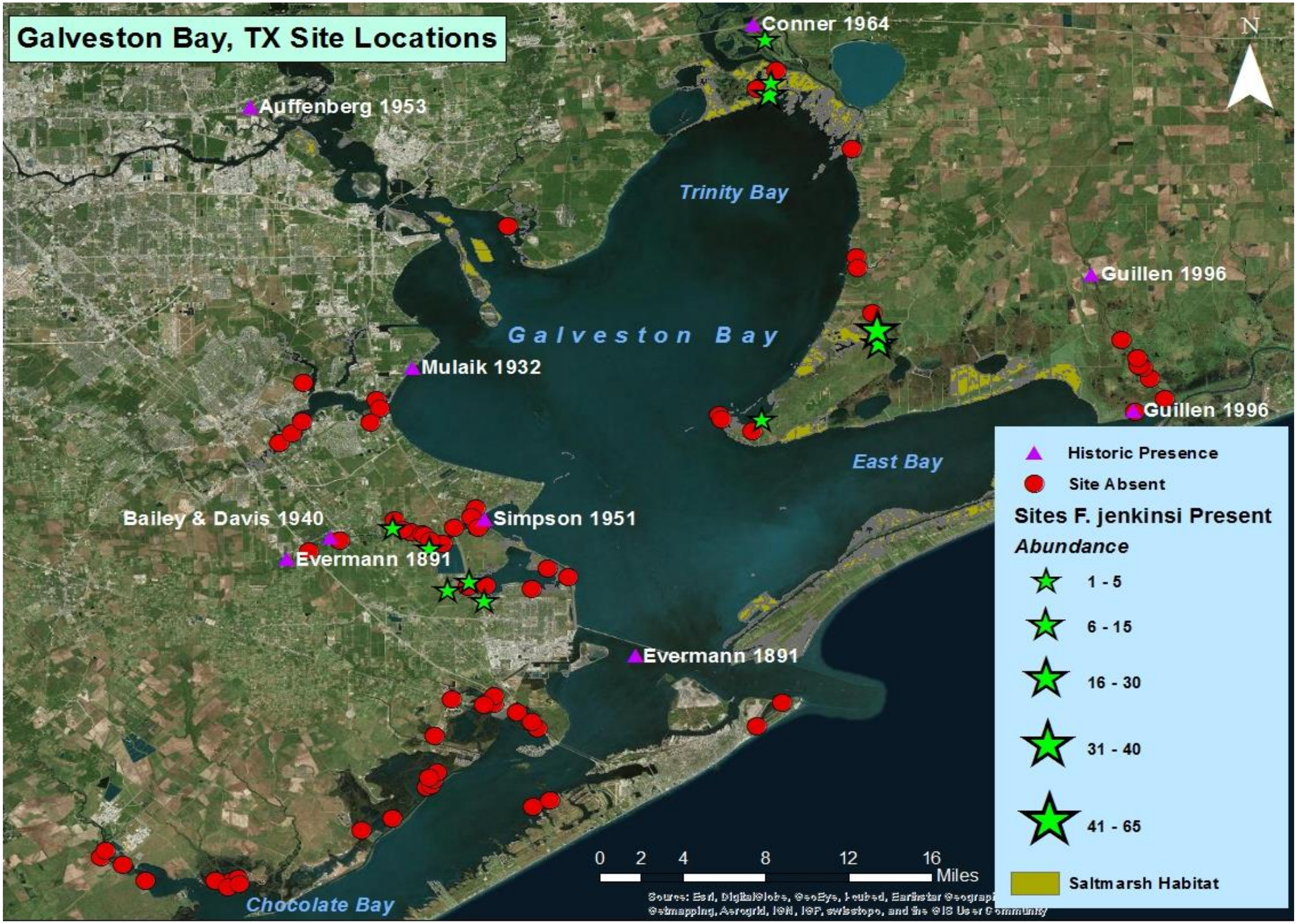
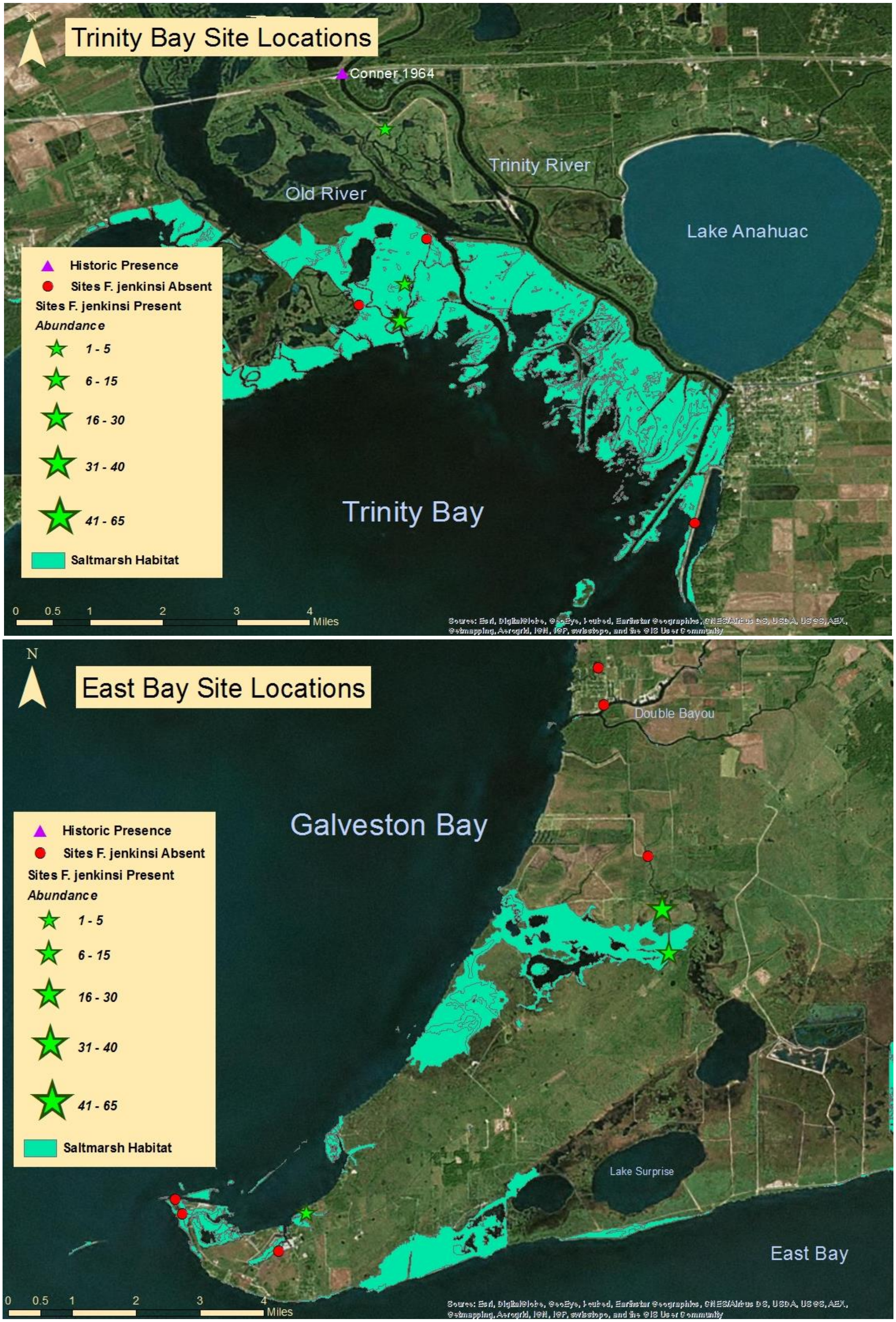
Figure 9 (Above) Map of sampling sites located along the Sabine River, Adams Bayou, and Cow Bayou. (Below) Map of sampling sites located along the Neches River region of Sabine Lake, TX. Green stars and red circles represent sites were *F. jenkinsi* were found and not found respectively. Size of the star corresponds to total number of *F. jenkinsi* collected at that site.



Source: Esri, DigitalGlobe, GeoEye, i-cubed, Earthstar Geographi
Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 10 **Map of sampling sites located around Galveston Bay, TX. Green stars and red circles represent sites were *F. jenkinsi* were found and not found respectively. Size of the star corresponds to total number of *F. jenkinsi* collected at that site.**



Source: Esri, DigitalGlobe, GeoEye, i-cubed, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 11 (Above) Map of sampling sites located around the Trinity Bay region of Galveston Bay. (Below) Map of sampling sites located around the East Bay region of Galveston Bay, TX. Green stars and red circles represent sites were *F. jenkinsi* were found and not found respectively. Size of the star corresponds to total number of *F. jenkinsi* collected at that site.

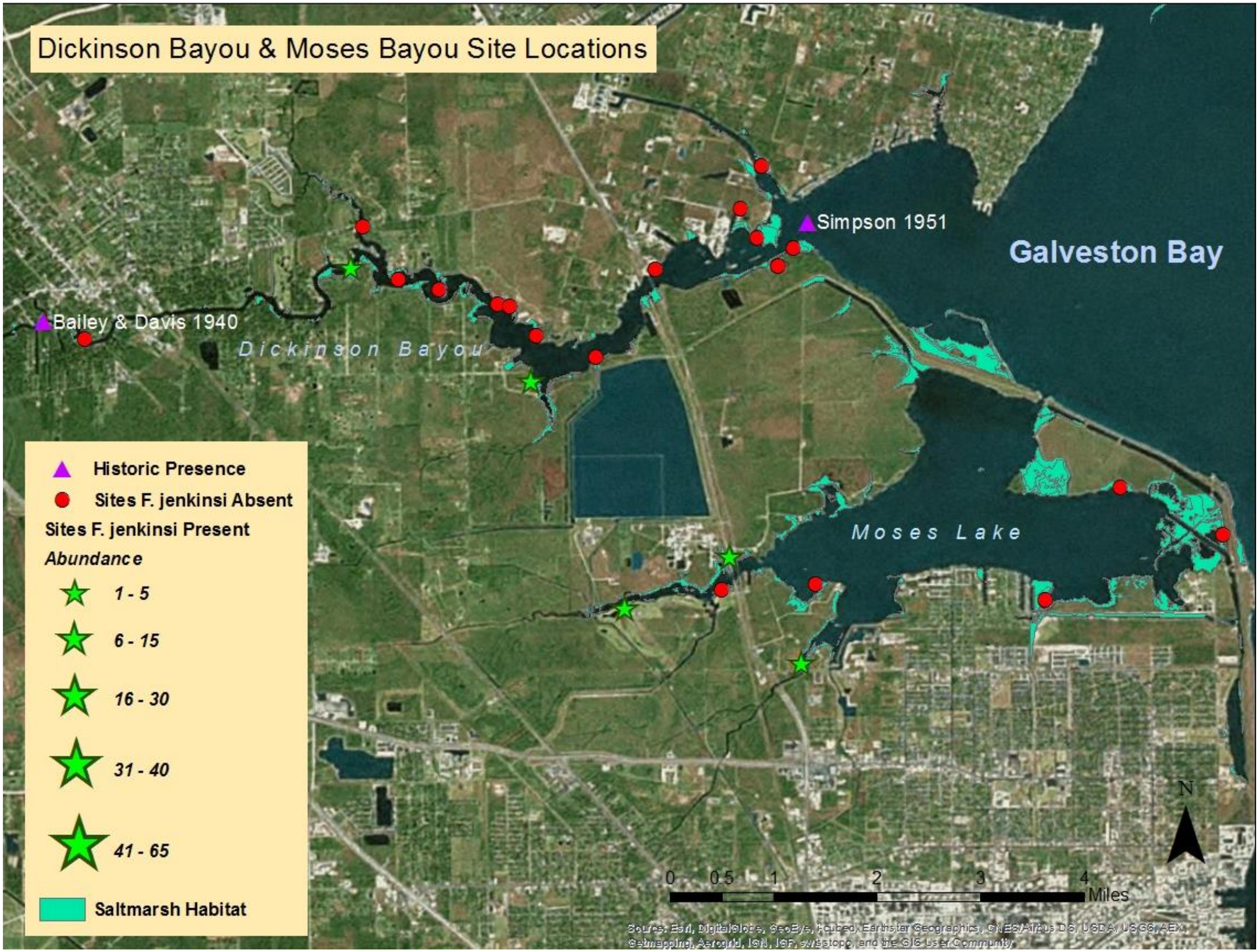


Figure 12 Map of sampling sites located around Moses Bayou and Dickinson Bayou in Galveston Bay, TX. Green stars and red circles represent sites were *F. jenkinsi* were found and not found respectively. Size of the star corresponds to total number of *F. jenkinsi* collected at that site.

Table 1 Summary of sites for all sampling events from February 2014-March 2015 where *F. jenkinsi* were and were not captured within each bay system and of the number of individuals captured at these sites within each estuary.

Sites				*Fundulus jenkinsi* Abundance		
Site Regions	**% Sites *F. jenkinsi* Present**	**% Sites *F. jenkinsi* Not Collected**	**Total Site N**	**Min.-Max.**	**Average per Site**	**Total *F. jenkinsi* N**
Sabine Lake	26%	74%	53	2-64	12	161
Sabine River	50%	50%	12	5-64	20	122
Neches River	42%	58%	19	2-17	7	39
Galveston Bay	14%	86%	81	1-22	5	54
East Bay	33%	67%	9	3-22	11	32
Trinity Bay	50%	50%	6	4-6	5	14
Dickinson & Moses Bayou	21%	79%	24	1-3	2	8

Fish Community Composition

A total of 63,114 individual fish consisting of 27 families and 53 species were collected during all quarterly sampling events. The total abundance of all species captured across all gear types and sampling events from both Sabine Lake and Galveston Bay is presented in Appendix A. The five most abundant families and the five most abundant species captured during all quarterly sites from both Sabine Lake and Galveston Bay is presented in Table 2. The families Clupeidae, Sciaenidae, and Engraulidae cumulatively represented 75% of the total abundance. Four species, including *Brevoortia patronus*, *Leiostomus xanthurus*, *Anchoa mitchilli*, and *Cyprinodon variegatus* represented the top 75% of all species collected numerically (Table 2).

Table 2 List of the five most abundant Families and 5 most abundant species of fish found across all quarterly sites from both bay systems.

Family	Percent of Total Abundance	Species	Percent of Total Abundance
Clupeidae	41.5%	*Brevoortia patronus*	39.3%
Sciaenidae	19.3%	*Leiostomus xanthurus*	15.8%
Engraulidae	13.9%	*Anchoa mitchilli*	13.9%
Cyprinodontidae	5.9%	*Cyprinodon variegatus*	5.9%
Fundulidae	5.2%	*Menidia beryllina*	4.9%
All Other Families	14.2%	All Other Species	20.2%

A total of 835 *F. jenkinsi* individuals were caught over the course of this study from both Galveston Bay (n= 674) and Sabine Lake (n= 161) (Table 3). Of the total count of *F. jenkinsi* individuals, 211 of them were caught during quarterly sampling (Galveston Bay= 50, Sabine Lake= 161) and 624 caught during the monthly collections conducted at Moses Bayou (Table 3). *Fundulus jenkinsi* were collected during every seasonal sampling event except summer in Galveston Bay and every season except summer and fall for Sabine Lake (Table 3). *Fundulus jenkinsi* were collected during every monthly sampling event except for the months of May, June, and September (Table 3).

Table 3 Total number (N) of *F. jenkinsi* individuals caught by monthly and quarterly sampling events from February 2014 – March 2015.

Collection Events	Monthly N	Quarterly N (Sabine Lake)	Quarterly N (Galveston Bay)	Total N
Feb- 14	3	15	7	**25**
March- 14	3	--	--	**3**
April- 14	8	--	--	**8**
May- 14	0	--	--	**0**
June- 14	0	0	0	**0**
July- 14	1	--	--	**1**
Sept- 14	0	0	1	**1**
Oct- 14	36	--	--	**36**
Nov- 14	40	146	42	**228**
Dec- 14	4	--	--	**4**
Jan- 15	139	--	--	**139**
Feb- 15	365	--	--	**365**
March- 15	25	--	--	**25**

The mean species diversity (H’) of sites containing *F. jenkinsi* did not differ significantly from the mean species diversity of sites lacking *F. jenkinsi* within Galveston Bay ($t_{13} = 0.36; p = 0.752$). Meanwhile, the mean diversity of site containing *F. jenkinsi* did significantly differ ($t_{25} = 5.15; p < 0.001$) from the mean diversity of sites lacking *F. jenkinsi* within Sabine Lake.

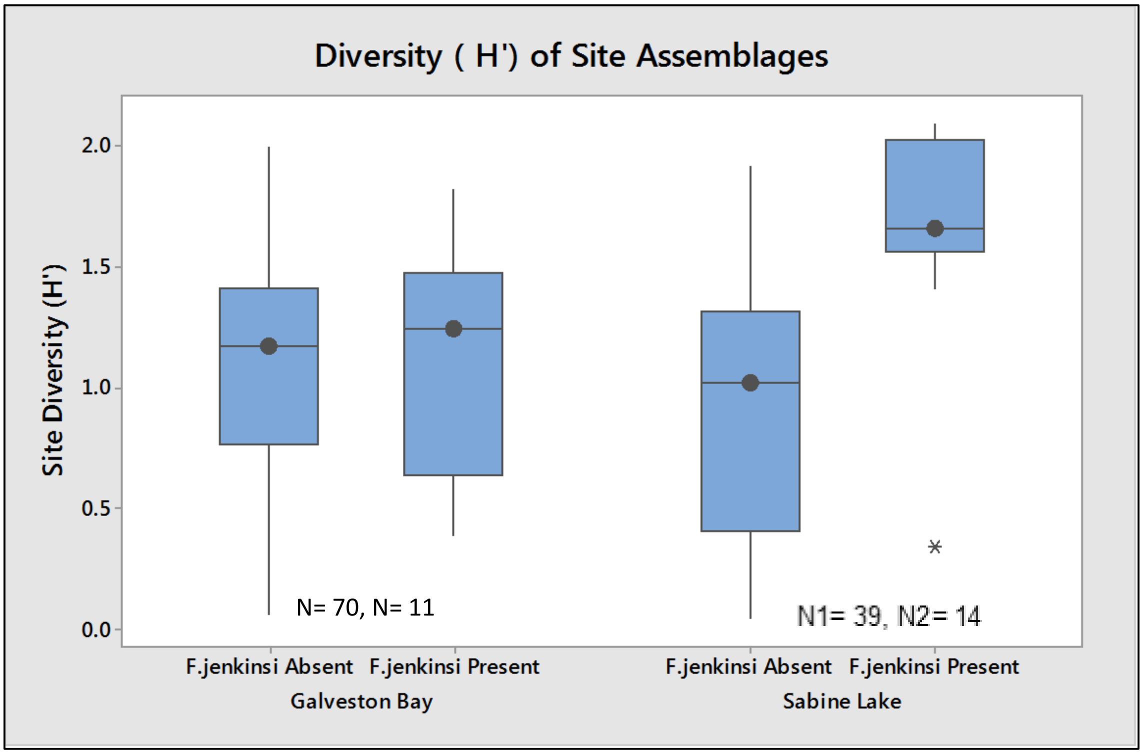


Figure 13 Boxplot of fish species diversity (H') between sites where *F. jenkinsi* were collected and not collected in both Galveston Bay and Sabine Lake.

Kruskal-Wallis H test comparing CPUE of *F. jenkinsi* across bay systems showed no significant difference in CPUE of *F. jenkinsi* between bay systems ($H_1 = 3.06, p = 0.08$). Additional analysis also showed no significance in CPUE of *F. jenkinsi* between drainages within bay systems (Galveston: $H_3 = 2.70, p = 0.440$; Sabine: $H_1 = 0.48, p = 0.489$).

One-way ANOSIM on fish assemblages collected from all quarterly sampling sites documented a significant difference in the fish community assemblages by bay system (Global R = 0.066, *p*=0.003). One-way ANOSIM also documented significant differences in assemblages where *F. jenkinsi* were present versus assemblages where *F. jenkinsi* were not collected (Global R=0.168, *p*=0.001). Based on results of additional ANOSIM analysis, we concluded that assemblages within Sabine Lake with *F. jenkinsi* present were significantly different from assemblages where *F. jenkinsi* were not collected (Global R= 0.174, *p*= 0.006). Similarly, based on ANOSIMs run on quarterly fish assemblages collected within Galveston Bay, significant differences existed in the fish community assemblages when *F. jenkinsi* were collected versus not collected (Global R= 0.163, *p*= 0.014).

The species most often found at sites where *F. jenkinsi* where collected versus not collected changed considerably between sites of both bay systems (Table 4).

Table 4 The most prevalent six species of fish found in collections containing and not containing *F. jenkinsi* at both Galveston Bay and Sabine Lake quarterly sites.

Sabine Lake Sites			**Galveston Bay Sites**		
	Species	**Percent Occurrence (%)**		**Species**	**Percent Occurrence (%)**
Sites *F. jenkinsi* Collected	*Fundulus grandis*	92.9%	**Sites *F. jenkinsi* Collected**	*Cyprinodon variegatus*	100.0%
	Poecilia latipinna	85.7%		*Fundulus grandis*	90.9%
	Fundulus pulvereus	85.7%		*Poecilia latipinna*	81.8%
	Cyprinodon variegatus	78.6%		*Adinia xenica*	72.3%
	Gambusia affinis	78.6%		*Leiostomus xanthurus*	45.5%
	Menidia beryllina	64.3%		*Fundulus pulvereus*	45.5%
	Species	**Percent Occurrence (%)**		**Species**	**Percent Occurrence (%)**
Sites *F. jenkinsi* Not Collected	*Menidia beryllina*	87.2%	**Sites *F. jenkinsi* Not Collected**	*Menidia beryllina*	85.9%
	Anchoa mitchilli	84.6%		*Mugil cephalus*	59.2%
	Leiostomus xanthurus	48.7%		*Leiostomus xanthurus*	54.9%
	Micropogonias undulatus	48.7%		*Anchoa mitchilli*	53.5%
	Mugil cephalus	46.2%		*Fundulus grandis*	49.3%
	Brevoortia patronus	41.0%		*Cyprinodon variegatus*	46.5%

In both bay systems, with a few exceptions, the composition of the most common six species collected at sites where *F. jenkinsi* were found was different from the top ranking species collected at sites where *F. jenkinsi* were not captured. Within both bay systems, *Fundulus grandis*, *Poecilia latipinna*, and *C. variegatus* each occurred in at least 70% of the sites where *F. jenkinsi* were also collected (Table 4). In contrast, *Menidia beryllina*, *Mugil cephalus*, *L. xanthurus*, *Anchoa mitchilli*, and *B. patronus* each appeared in at least 40% of sites where *F. jenkinsi* were not collected (Table 4). Within Sabine Lake, *M. beryllina* occurred over 60% of time at sites where *F. jenkinsi* were both captured and not captured (Table 4). In Galveston Bay *F. grandis*, *L. xanthurus*, and *C. variegatus* were found over 45% of time at all sites regardless of the presence of *F. jenkinsi* (Table 4).

A two-way ANOSIM on site assemblages from Sabine Lake showed that assemblages differed significantly between all seasonal groups (Global R= 0.474; *p*= 0.001) and that assemblages where *F. jenkinsi* were present differed significantly from assemblages where *F. jenkinsi* were not collected across all seasons (Global R= 0.388; *p*= 0.001). Similarly, a two-way ANOSIM on fish assemblages from Galveston Bay also showed a significant difference in assemblages between seasonal groups (Global R=

0.488; *p*= 0.001) and that assemblages with *F. jenkinsi* present differed significantly from assemblages without *F. jenkinsi* across all seasons (Global R= 0.302; *p*= 0.003).

MDS plots of fish assemblages across seasons for both Sabine Lake and Galveston Bay sites show a clear gradient in fish assemblage similarity between seasons as well as show that *F. jenkinsi* were found more often in the winter and fall within both bay systems (Figure 14).

Another two-way ANOSIM on fish assemblages at sites within Sabine Lake revealed that fish assemblages did not significantly differ between tidal stages (Global R= 0.055; *p*= 0.171) and that assemblages where *F. jenkinsi* were present did not differ significantly from assemblages where *F. jenkinsi* were not collected across all tidal stage groups (Global R= 0.119; *p*= 0.134). Galveston Bay fish assemblages also did not show a significant difference in composition across tidal stages (Global R= 0.041; *p*= 0.148) or a significant difference when *F. jenkinsi* was present or absent across tidal stage groups (Global R= 0.145; *p*= 0.103).

Fundulus jenkinsi were found in fish assemblages sampled by both seines and Breder traps (Figure 15). A one-way ANOSIM showed that fish assemblages chosen for gear analysis did not significantly differ from each other by bay system (Global R= 0.055; *p*= 0.259). For this reason, no distinction was made between bay systems in subsequent analyses. A one-way ANOSIM showed that fish assemblages did differed by collection method (Global R= 0.206; *p*=0.001). A subsequent two-way ANOSIM showed a significant difference in fish assemblages when *F. jenkinsi* were present versus absent across both gear types (Global R= 0.159; *p*= 0.024) and a significant difference in fish assemblages between gear types regardless of *F. jenkinsi* presence (Global R= 0.266; *p*= 0.001).

The average standard length of *F. jenkinsi* caught via seining was 26.0 mm (± 6.9 mm) with a range of 13-50 mm and the average standard length of *F. jenkinsi* caught via Breder trap was 26.3 mm (± 7.4 mm) with a range of 18-42 mm. A two-sample T-test revealed that the average standard length of *F. jenkinsi* did not vary significantly by gear type ($t_7 = 0.08; p = 0.939$).

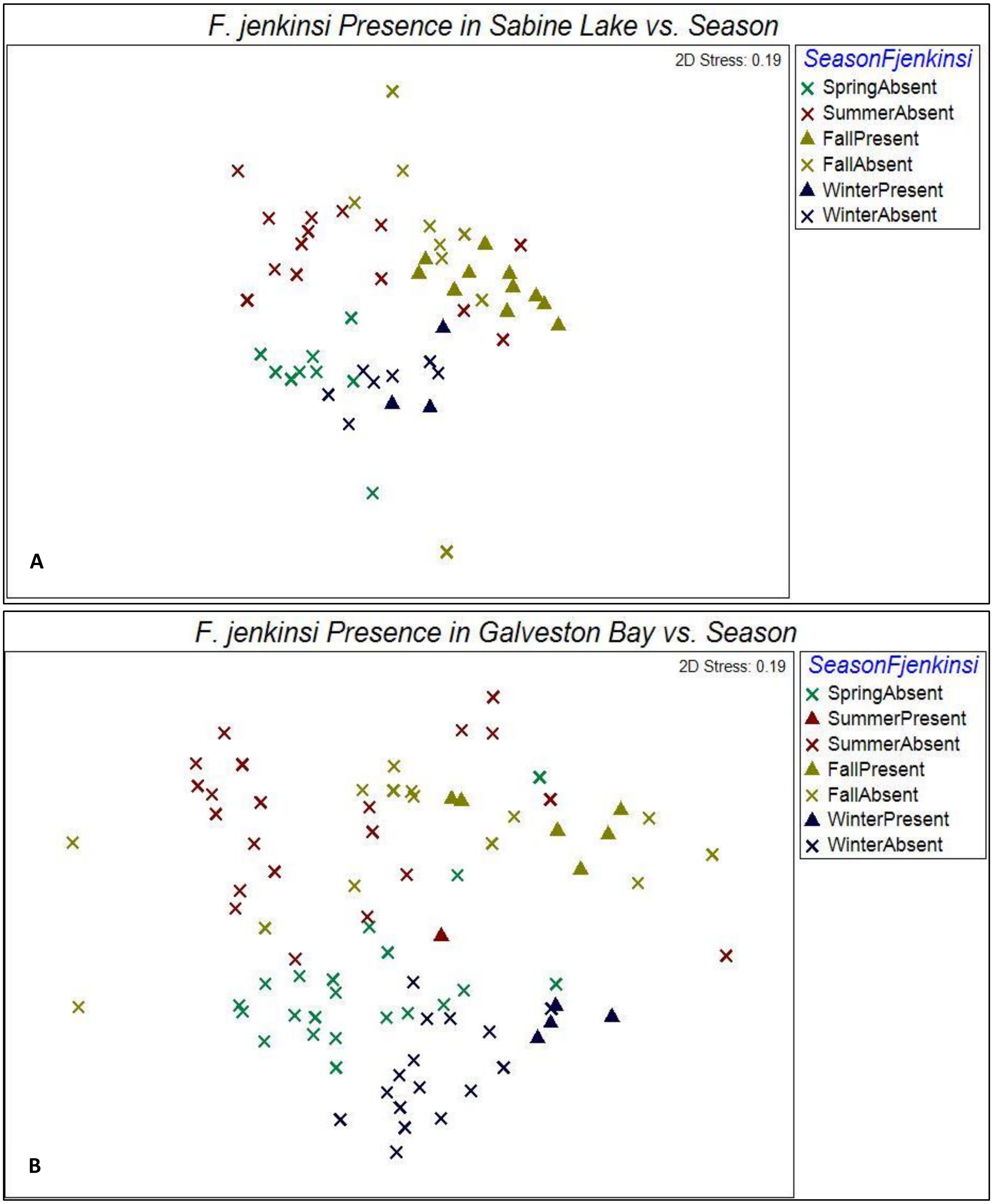


Figure 14 MDS plot of fish assemblage data illustrating the similarity of fish assemblages at sites where *F. jenkinsi* were collected versus not collected by season sampled for (A) Sabine Lake and (B) Galveston Bay. Assemblages are labeled by season (color) as well as *F. jenkinsi* occurrence (shape). Filled triangles represent assemblages containing *F. jenkinsi* while crosses represent assemblages they are lacking from.

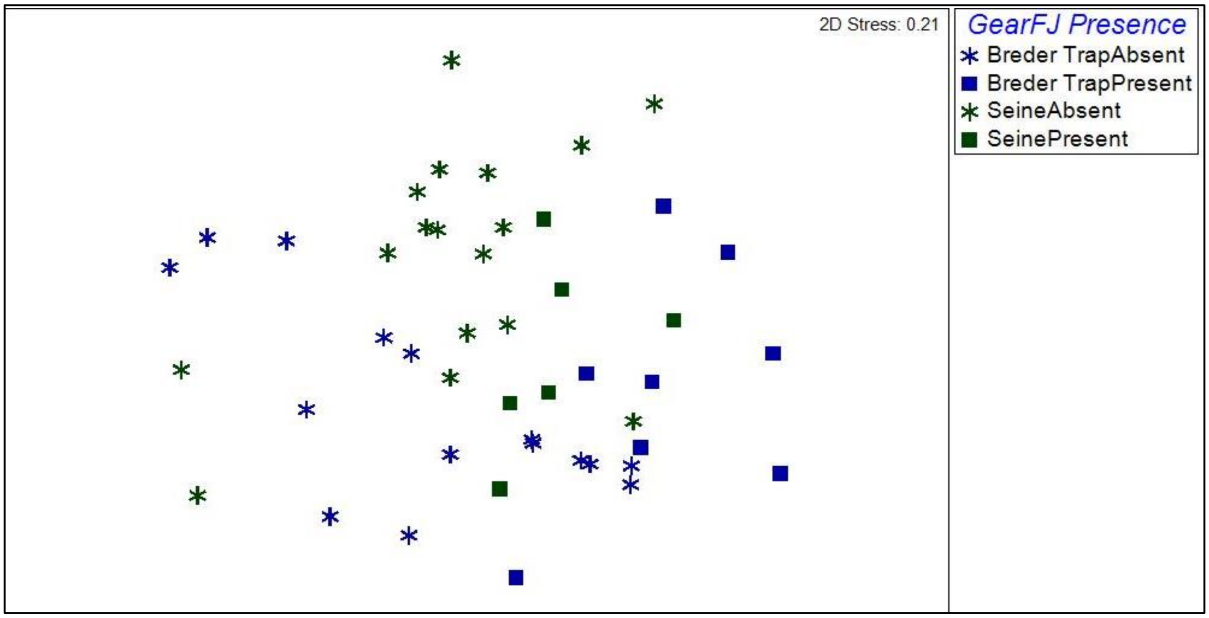


Figure 15 MDS plot of fish assemblage data illustrating the similarity of fish assemblages at sites where *F. jenkinsi* were collected versus not collected by gear type pooled from both bay systems. Assemblages are labeled by capture method (color) as well as *F. jenkinsi* occurrence (shape) and where pooled from all gear replicates of a site. Green markers represent assemblages collected via seine and blue markers represent assemblages collected via Breder trap. Filled squares represent assemblages containing *F. jenkinsi* while stars represent collection where they were not collected.

Habitat Characteristics

Scatterplots showing *F. jenkinsi* abundance by site for each environmental factor described below is presented in Appendix B. Salinity (ppt), temperature (°C), mean lower low water level (MLLW), and bank vegetation (% cover) values were compared between sites where *F. jenkinsi* were collected and not collected within both bay systems (Figure 16). Only salinity ($t_{21} = 2.5; p = 0.021$) and MLLW ($U_{23} = 187; p = 0.017$) showed a significant difference in sites containing *F. jenkinsi* between bay systems. Sabine Lake sites where *F. jenkinsi* were collected had, on average, both lower salinities (8.9ppt vs. 13.5ppt) and MLLW (0.10 m vs. 0.23m) compared to sites where *F. jenkinsi* were collected in Galveston Bay (Figure 16).

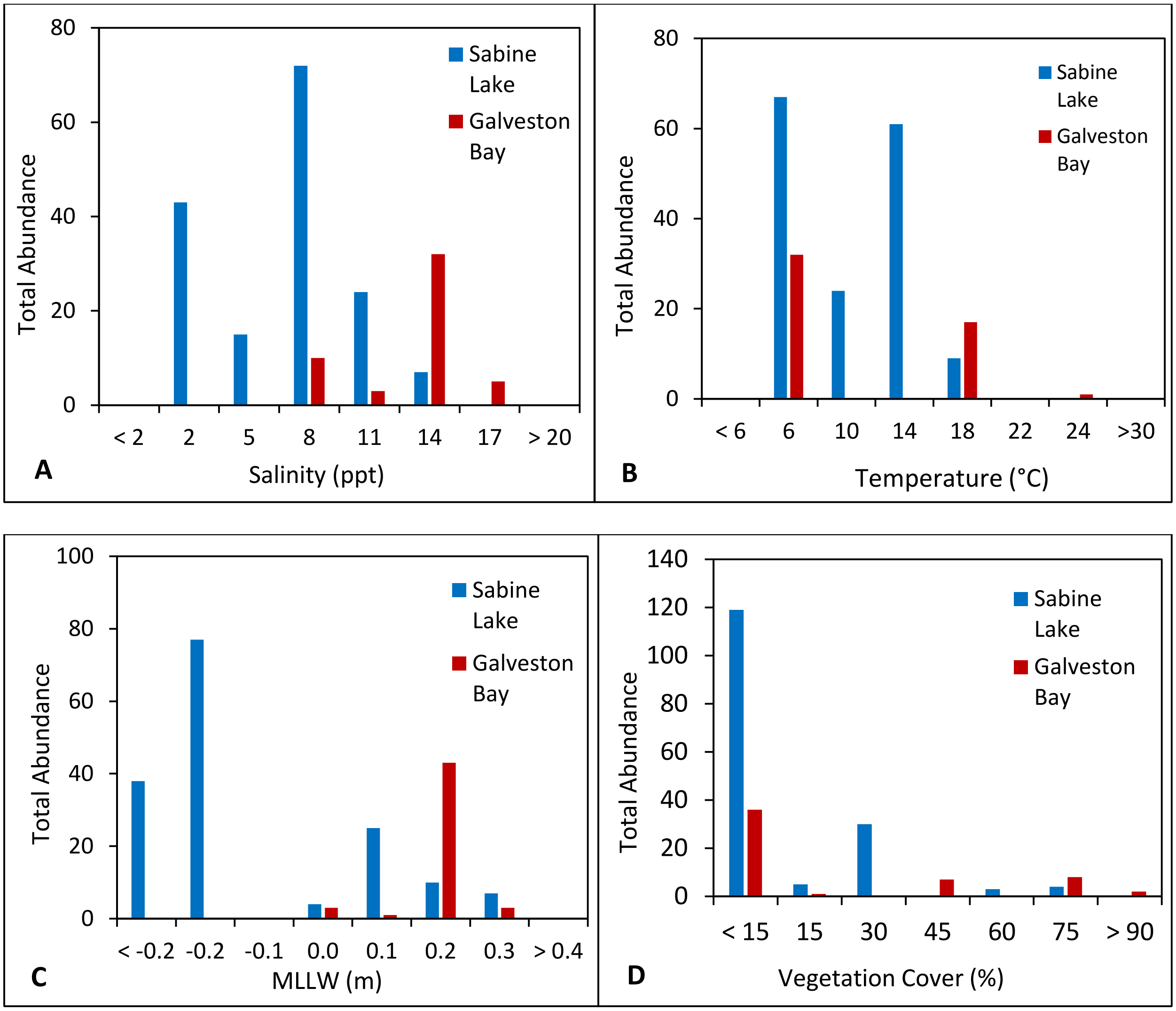


Figure 16 Total number of *F. jenkinsi* collected in both Sabine Lake (n=215 individuals) and Galveston Bay (n= 54 individuals) by (A) salinity, (B) temperature, (C) MLLW level, and (D) percent vegetative cover categories pooled across all seasons.

Within Sabine Lake sites, temperature $(U_{51} = 169.0; p < 0.001)$ and MLLW $(U_{51} = 173.5; p < 0.001)$ significantly differed between sites where *F. jenkinsi* were collected versus where they were not collected. Temperature (14.3°C vs 28.2°C) and MLLW (0.10 m vs. 0.38 m) were, on average, lower at sites where *F. jenkinsi* were collected versus sites where they were not captured.

Within Galveston Bay sites, temperature $(U_{79} = 247; p = 0.005)$ and salinity $(t_{21} = 3.07; p = 0.006)$ significantly differed between sites where *F. jenkinsi* were collected versus where they were not collected. Temperature (19.5°C vs. 28.2°C) and salinity (13.5 ppt vs. 18.5 ppt) were found to be, on average, lower at sites where *F. jenkinsi* were captured versus sites where they were not captured.

The percent vegetation cover was also compared across Sabine Lake and Galveston Bay sites. Within both systems the mean percent vegetation cover did not significantly vary $(\text{Sabine } U_{51} = 342.0; p =$

$0.557;$ Galveston $U_{79} = 526.0; p = 0.394$) between sites where *F. jenkinsi* were present and sites where *F. jenkinsi* were not found. *Spartina alterniflora* represented the most often occurring vegetative species among sites where *F. jenkinsi* were present and at sites *F. jenkinsi* were not found (Table 5). In general, *S. alterniflora*, *Phragmities australis* and *Typha latifolia* were prevalent at sites were *F. jenkinsi* were captured. In contrast, *Junus roemarianus, Batis maritima, Salicornia* spp., *Taxodium distichum*, and *Vallisneria americana* only occurred at sites where *F. jenkinsi* were not collected. *Halodule wrightii* was the only species found at sites were *F. jenkinsi* were captured but was not found at any site where *F. jenkinsi* were not collected.

Table 5 Percent occurrence of the most common plant species at sites where *F. jenkinsi* were captured and not captured. Data is pooled from quarterly sites in both Galveston Bay and Sabine Lake.

Primary Vegetation	Percent Occurrence at Sites *F. jenkinsi* Collected	Percent Occurrence at Sites *F. jenkinsi* Not Collected
Spartina alterniflora	56%	82%
Phragmities australis	48%	10%
Typha latifola	20%	3%
Spartina patens	8%	7%
Iva frutescens	4%	4%
Ruppia maritima	4%	3%
Halodule wrightii	4%	0%
Juncus roemarianus	0%	12%
Batis maritima	0%	5%
Salicornia spp.	0%	4%
Taxodium distichum	0%	1%
Vallisneria americana	0%	1%

Life History Characteristics

Size Distribution

The standard lengths of *F. jenkinsi* individuals caught in Galveston Bay and Sabine Lake were not normally distributed. The standard lengths of *F. jenkinsi* captured ranged from 14-50 mm with an average length of 26.0 mm (± 7.3 mm SD) and did not differ by bay system, season, or sex. Sizes of *F. jenkinsi* caught in Sabine Lake (n= 161; range = 14-44 mm) were not significantly different from specimens caught in Galveston Bay (n= 674; 13-50 mm) ($U_{835} = 68537.5; p = 0.546$). Therefore, standard length measurements from both bay systems were pooled to graph the overall distribution of length frequencies (Figure 17).

For seasonal analysis, summer collections only contained 2 individuals (SL= 29 and 33) and were therefore excluded. One-way ANOVA determined that standard length did not significantly differ between seasons ($F_{3,831} = 1.08; p = 0.385$); however, individuals collected during the winter had the longest range from 13-50 mm (Figure 18).

Individuals selected for GSI analysis were also used to assess standard length distribution by gender (Figure 19). On average, females were 30.3 mm (± 6.8 mm SD) with a range of 13-50 mm and males were an average of 32.2 mm (± 9.2 mm SD) with a range of 20-46 mm. Mean standard length of *F. jenkinsi* was not significantly different between genders ($U_{150} = 6242.2; p = 0.195$).

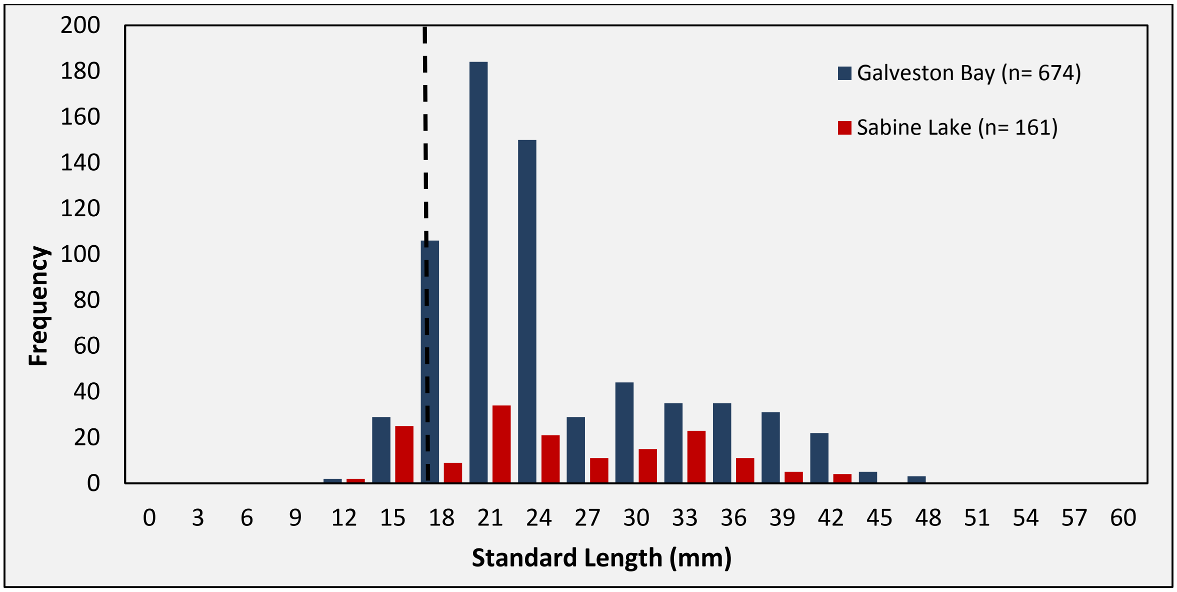


Figure 17 Standard length (mm) distribution of all *F. jenkinsi* individuals collected across all seasons, gear types, and bay systems. Dotted vertical line represents the size break between juveniles and adults (Ross 2001).

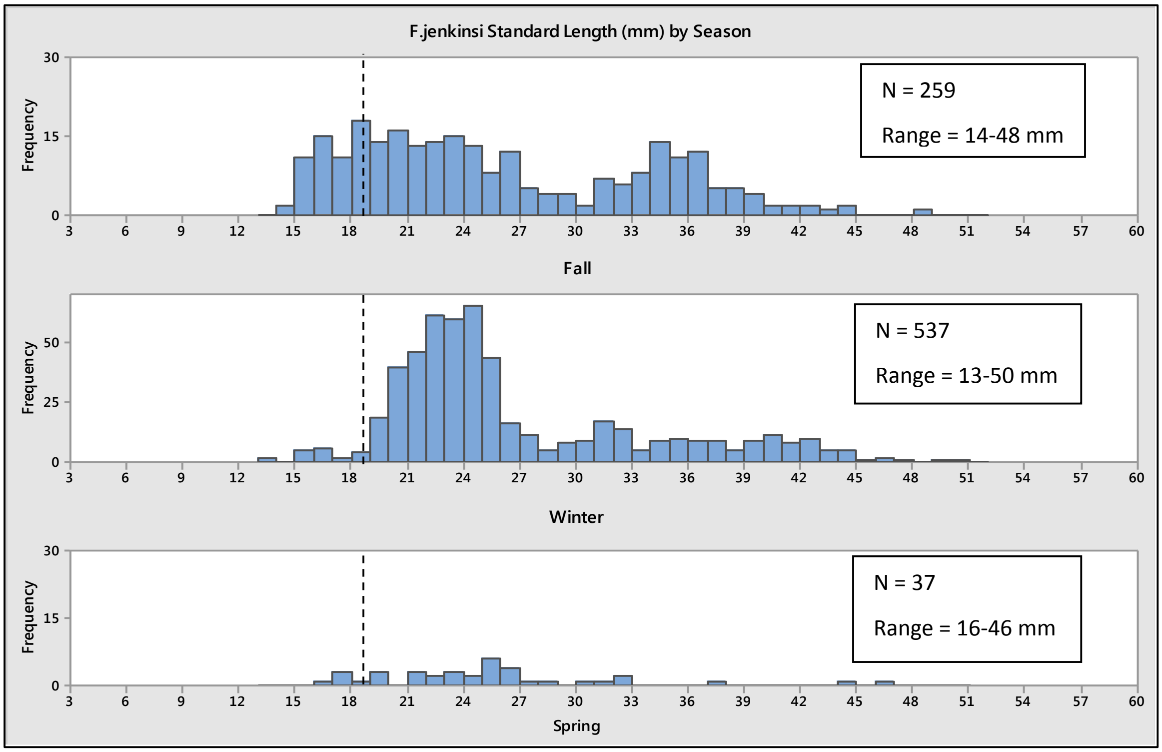


Figure 18 Standard length (mm) distribution of *F. jenkinsi* for fall, winter, and spring across all quarterly sampling events. Summer collections contained only 2 individuals (SL = 29, 33) and are not presented above. Individuals were pooled from both bay systems and gear types. Dotted vertical line represents the size break between juveniles and adults (Ross 2001).

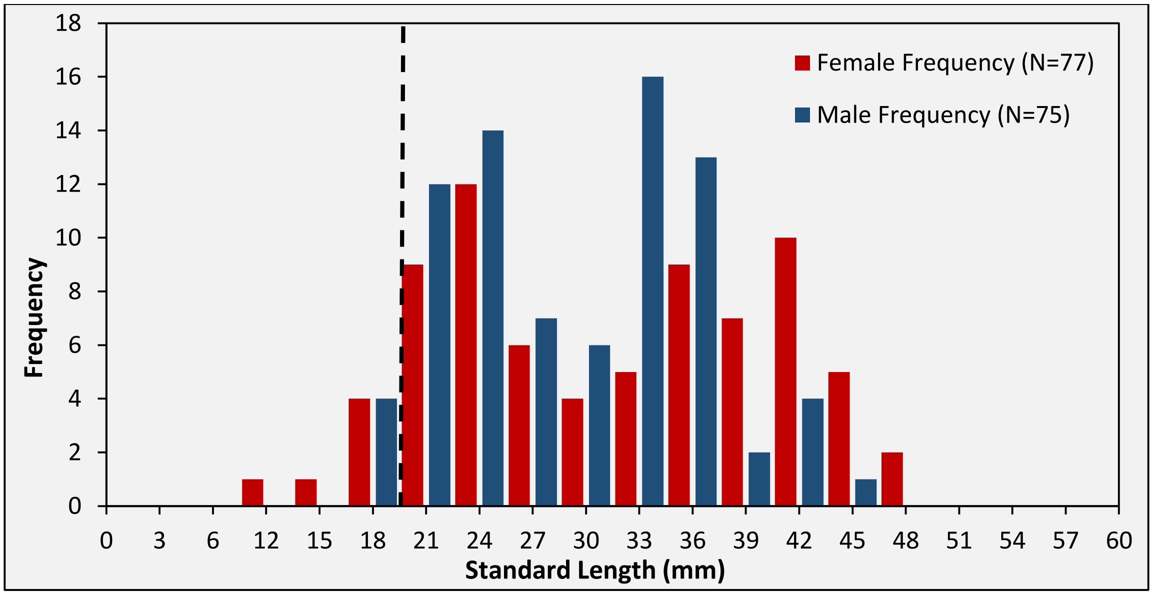


Figure 19 Standard length (mm) distribution of *F. jenkinsi* for both males (n= 75) and females (n=77) across all monthly sampling events. Dotted vertical line represents the size break between juveniles and adults (Ross 2001).

The mean total body weight of *F. jenkinsi* did not significantly differ between genders ($U_{150} = 5369.5; p = 0.176$). Data was therefore pooled from both genders to plot the association of *F. jenkinsi* length and total weight (Figure 20). The best-fit model for the relationship between length and weight in our data series was a non-linear polynomial equation ($r^2 = 0.987$) where for every unit increase in standard length (*x*) total body weight would also increase by $0.00005x^{3.0976}$.

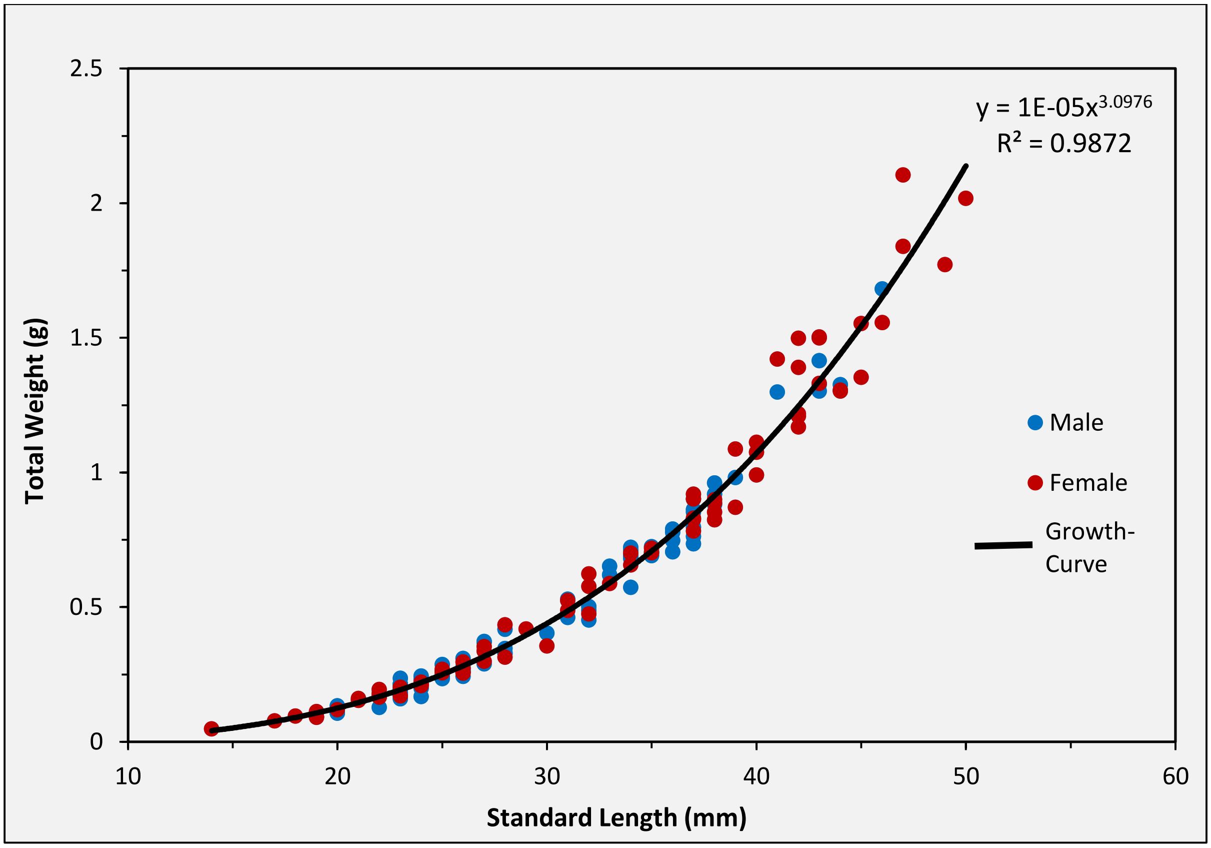


Figure 20 Scatter plot of *F. jenkinsi* standard length (mm) versus total body weight (grams). Each data symbol represents one individual with (female = red; male = blue).

Due to a low sample size within and among months, we were unable to conduct modal progression analysis utilizing length frequency data to estimate relative age classes. The months of November 2014 (n= 40) and February 2015 (n= 362) did possess an adequate number of individuals and produced accurate enough length frequencies histograms for partial analysis. Both months possessed bi-modal distributions and showed two very distinct age classes. Average size classes for November were 20.8 mm (± 3.6 mm SD) and 35.0 mm (± 4.0 mm SD). February's size classes followed a very similar pattern at 23.3 mm (± 1.6 mm SD) and 33.7 mm (± 7.3 mm SD).

Reproduction

A total of 152 individuals (77 females and 75 males) were collected for GSI analysis with the largest female measuring 50 mm and the largest male measuring 46 mm (Table 6). Due to limited catch, the preferred sample size of 30 individuals per month (15 females and 15 males) was not achieved. Elevated GSI values were observed during April and September for females and February-April for males (Figure 21). GSI values were significantly different between females and males ($t_{77} = 5.39; p < 0.001$) with females possessing higher GSI means than males across all months.

Table 6 Number, standard length (mm, mean ± standard error), total weight (grams, mean ± standard deviation), and range of *F. jenkinsi* individuals processed from each month for GSI analysis.

Year	Month	Total (N)	Mean SL (mm) ± SD	Min – Max SL(mm)	Mean TW (g) ± SD	Min. – Max TW(g)
2014	February	15	23.5 ± 3.5	20 -32	0.2 ± 0.1	0.1 – 0.5
	March	3	29.3 ± 4.2	26 - 34	0.4 ± 0.2	0.3 – 0.7
	April	8	32.4 ± 5.8	26 - 44	0.6 ± 0.3	0.3 – 1.3
	May	0	-	-	-	-
	June	0	-	-	-	-
	July	1	29	29	0.4	0.4
	August	0	-	-	-	-
	September	1	33	33	0.6	0.6
	October	18	26.7 ± 7.3	17 - 37	0.4 ± 0.3	0.1 – 1.4
	November	31	35.5 ± 6.7	25 - 47	0.8 ± 0.4	0.1 – 1.5
	December	4	18.3 ± 4.2	14 - 22	0.1 ± 0.03	0.05 – 0.1
2015	January	21	29.4 ± 5.1	23 - 42	0.5 ± 0.3	0.2 – 0.9
	February	30	40.6 ± 4.7	31 - 50	1.2 ± 0.4	0.7 – 2.0
	March	20	25.1 ± 5.2	23 - 46	0.3 ± 0.3	0.2 – 1.7

Regression analysis revealed that body weight of both males and females was not a predictor of GSI value (Females r^2=0.026, p= 0.085; Males r^2=0.022, p= 0.108). However, while male standard length was not a predictor of GSI value (r^2=0.019, p= 0.122) female standard length did show a significant relationship to GSI value (r^2=0.065, p= 0.025).

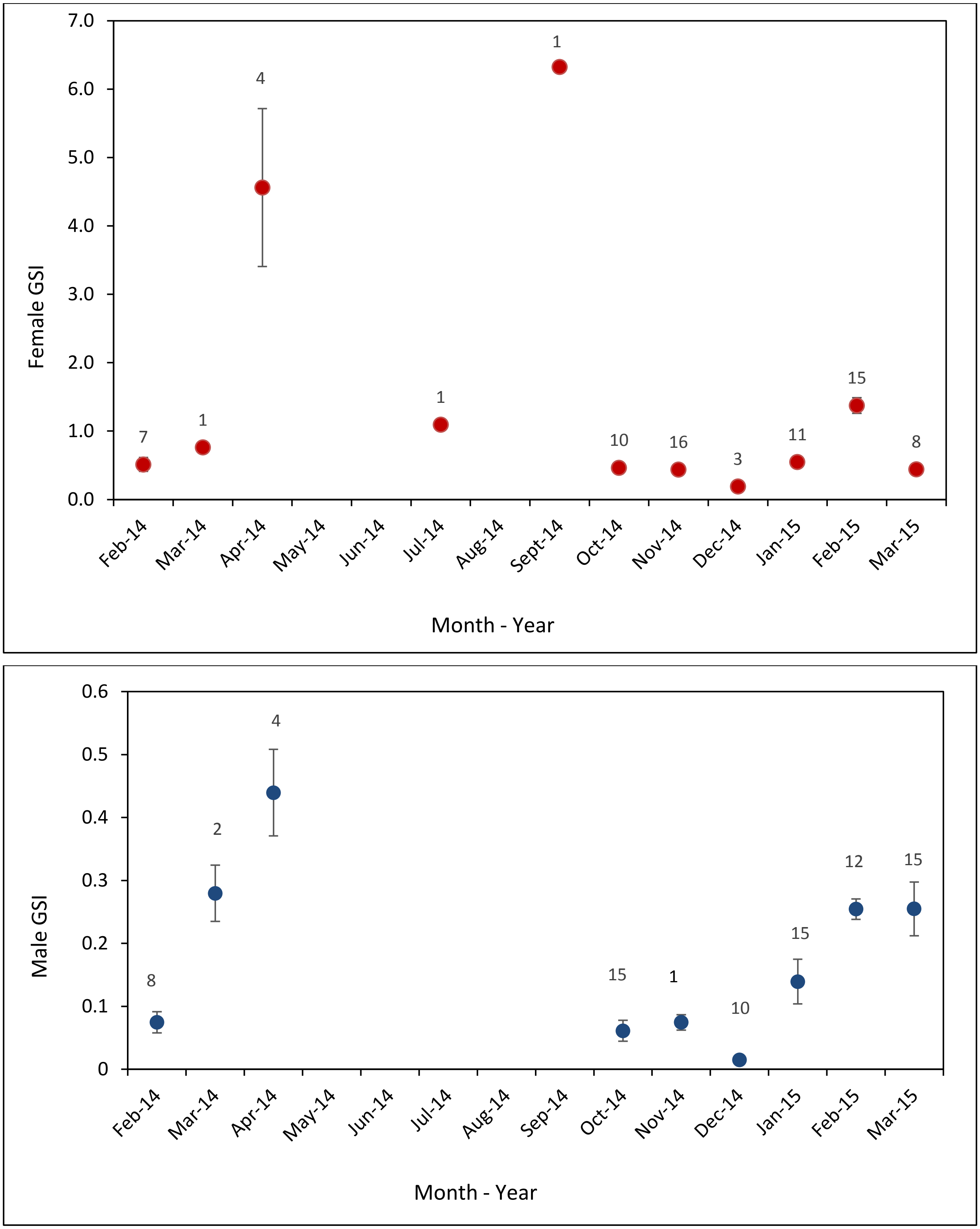


Figure 21 **(Above) Plot of female and (Below) male mean gonadosomatic index by month for *F. jenkinsi*. Bars represent ± 1 SE. Numbers above data points represent that month's sample size (n). In some months SE bar values are very small consequently hidden by the data point.**

Monthly mean GSI values were significantly different for both females ($F_{10,76} = 31.58; p < 0.001$) and males ($F_{8,74} = 13.11; p < 0.001$). February 2015 female GSI value was significantly higher for all months (*p*-values< 0.05) except April 2014 and September 2014 when it was significantly lower (p-values < 0.05) and March 2014 which it was neither significantly higher or lower (*p*= 0.992). Female GSI values for September and April 2014 were significantly higher from all other months (all *p*-values <0.001) except each other (*p*= 0.166). April 2014 male GSI value was significantly higher from all other months (all *p*-values< 0.05) except for March 2014 (*p*= 0.511). Male GSI for the month of February 2014 was significantly lower from GSI values for February 2015 (*p*= 0.001) and March 2015 (*p*= 0.001).

GSI values were pooled by season for each gender and mean GSI values significantly differed by season for both females ($F_{3,76} = 8.36; p < 0.001$) and males ($F_{2,74} = 22.4; p < 0.001$). Females had the highest ranking mean GSI values in summer (mean = 3.7) followed by spring (mean = 1.73), winter (mean = 0.85) and then fall (mean = 0.45). A Tukey's pairwise comparison for female season mean GSI values showed fall was significantly lower from spring (p=0.005) and summer (p=0.001) GSI means but not from winter (p=0.475). Female's summer mean GSI value was significantly higher from winter (p=.003) while mean spring GSI did not significantly differ from either summer (p=0.091) or winter (p=0.072) GSI values. A Tukey's pairwise comparison for male seasonal GSI values showed that all male mean seasonal GSI values significantly differed from each other (all *p*-values ≤ 0.003) with the spring season containing the highest GSI values for males (mean = 0.30) followed by winter (mean = 0.17) and then fall (mean = 0.07). Full Tukey pairwise comparison data for monthly and seasonal GSI values for both genders is presented in Appendix C.

The sample sizes of each ovarian phase were latent (n= 37), early maturing (n= 24), late maturing (n= 12), mature (n= 2), and ripe (n= 2). Regression analysis of season versus ovary phase values did not reveal a significant relationship (r^2=0.009, p= 0.422). However, both female standard length (r^2=0.265, p< 0.001) and female GSI values (r^2=0.651, p< 0.001) showed a significant relationship to ovary phase (Figure 22). Ovary maturation phase increased with larger standard length values as well as increased with greater GSI values.

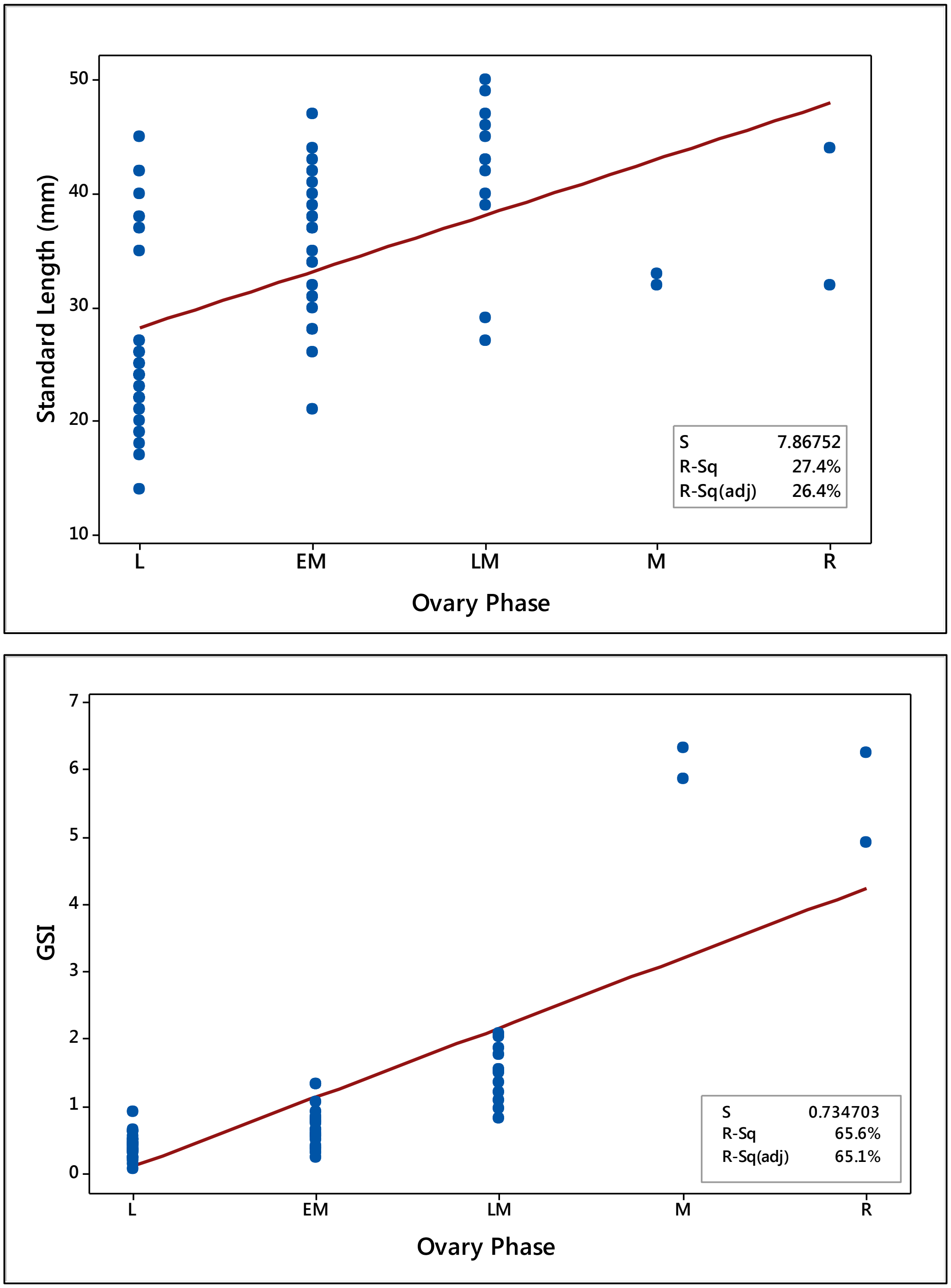


Figure 22 (A) Fitted line plot of ovarian phase distribution by standard length (mm) ($R^2 = 0.264$). Sample sizes are L (n= 37), EM (n= 24), LM (n= 12), M (n= 2), R (n= 2). (B) 23 Fitted line plot of ovarian phase distribution by GSI values ($R^2 = 0.651$). Sample sizes are L (n= 37), EM (n= 24), LM (n= 12), M (n= 2), R (n= 2). (L= latent, EM= early maturing, LM= late maturing, M= mature, R= ripe)

Ovarian phases were pooled by season (Figure 23) in order to assess temporal shifts in the reproductive activity of *F. jenkinsi*. Ovarian development showed signs of seasonal progression. Summer and spring seasons show the greatest percentages of mature and ripe ovaries while none were seen in fall and winter seasons. Contrastingly, the percentage of early maturing ovaries steadily decreased from fall to summer.

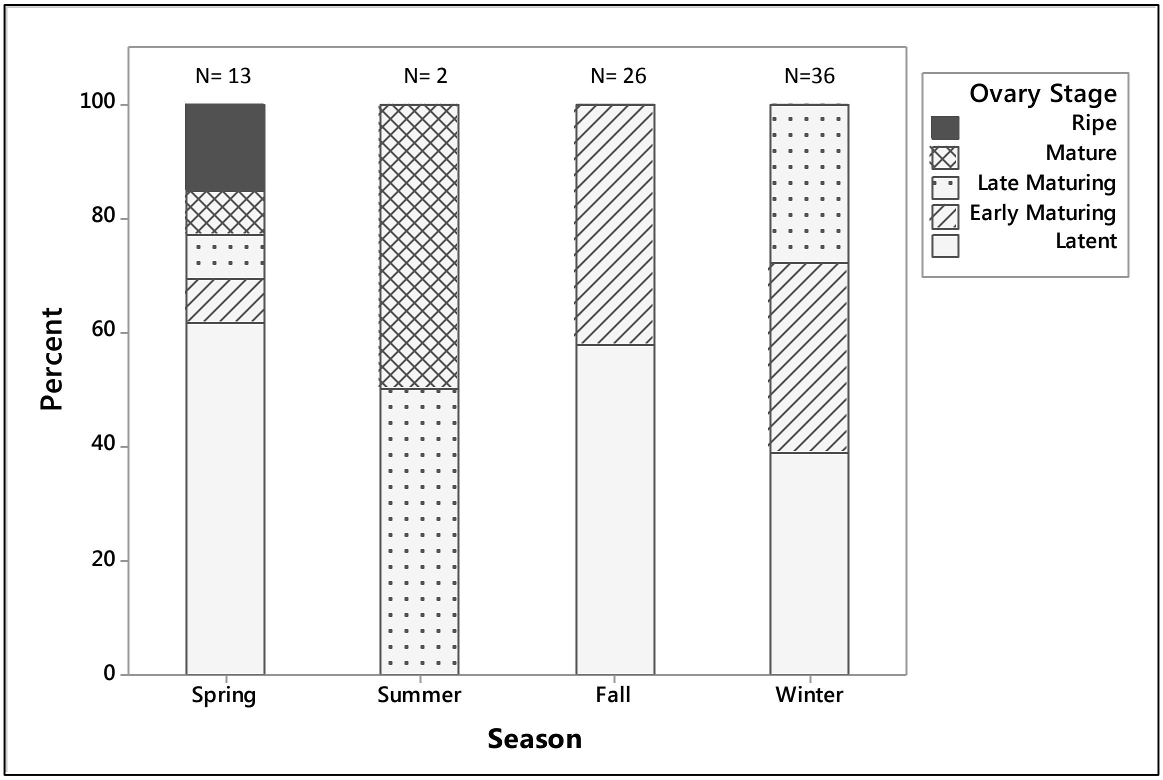


Figure 23 Seasonal (spring, summer, fall, winter) percentages of ovarian phases of female *F. jenkinsi*.

Discussion

Distribution and Abundance

Results of this study suggest the existence of a gradient of *F. jenkinsi* density based on geographic location. This conclusion is based on several lines of evidence. First, a greater percentage of sites contained *F. jenkinsi* in Sabine Lake compared to Galveston Bay (26% vs. 14%). In addition, when collected *F. jenkinsi* were found, on average, in greater numbers as well (12 vs. 5 individuals per site present). This gradient of decreasing *F. jenkinsi* frequency and density from east to west is seen between and within bay systems. Sites sampled along the Sabine River had a higher frequency of *F. jenkinsi* presence (50% vs. 42%) as well as a higher number of individuals found per site present (20 vs. 5) when compared to the more western sites sampled along the Neches River. This trend is also observed within Galveston Bay with the frequency of sites with *F. jenkinsi* present decreasing from 50% (Trinity Bay) to 21% (Dickinson and Moses Bayous) and the average number of individuals found per site present decreasing from 11 (East Bay) to 2 (Dickinson and Moses Bayous).

As the Texas coast represents the western extent of this species' known range (Simpson and Gunter 1956 cited in NatureServe Explorer 2014; Patrick et al. 1998; Hoese and Moore 1998; Jordan and Evermann 1896) the data from this study confirms that the Saltmarsh Topminnow decreases in both occurrence and abundance to negligible levels to the west of Galveston Bay. Recent (<20 years) and past (>20 years) historical records indicate (Akin et al. 2003; Nicolau 2001; Guillen 1996) they may continue to be found intermittently in extremely small numbers further west along the Texas coast but these do not appear to represent large sustainable local populations. However, further surveys are needed in areas and adjacent bay waterways were Saltmarsh Topminnow have been collected including Cedar Lakes Creek and portions of Matagorda Bay to confirm this hypothesis.

Another explanation for the observed east to west gradient in density may be due to the fact that Galveston Bay is significantly more developed and contains less undisturbed habitat than Sabine Lake. As a consequence of habitat fragmentation and loss of wetlands in the western portion of Galveston Bay local populations of Saltmarsh Topminnow have become more isolated from each other and the lack of habitat corridors may prevent the expansion and colonization of adjacent potential habitat in disconnected wetlands. This is in contrast to the Sabine Lake which contains large extensive and continuous wetlands extending along the eastern shoreline and to a lesser extent the western portion of the watershed. This would translate to greater amounts of suitable wetland habitat and corridors for expansion and migration of this species within the Sabine Lake system.

It is possible that the occurrences and numbers of this species were not entirely represented in this study. For example, 293 specimens were captured during one seine haul in February 2015 during monthly sampling. Extremely low water levels at the time of sampling had forced fish and other marsh nekton into a single disconnected pool. Some of the zero and low catches of Saltmarsh Topminnow occurred at sites sampled during the summer when water levels were highest, which allows small nekton to seek refuge in inundated marsh vegetation therefore evading our standard sampling gear. This would results in a high false negative (zero catch; absence) rate since even though the target species is there, it is essentially not vulnerable to our sampling gear. It would be beneficial to revisit sites in both Sabine Lake and Galveston Bay again during winter months to collect additional samples and increase our confidence that these sites likely lack *F. jenkinsi*. In addition, the use of throw traps or high marsh net pens might aid in capturing organisms during high water events.

Fish Assemblages

Analysis of the fish assemblages caught over the course of this study show that *F. jenkinsi* have a strong association with certain fish species. In this study *F. jenkinsi* were always found in association with at least one other species from the family Fundulidae and often in combination with other marsh edge estuarine fish species (e.g. *P. latipinna*, *G. affinis*, and *C. variegatus*). It is not surprising considering that these species share very similar habitat niches (Baltz et al. 1993; Peterson & Ross 1991). Similarly, the assemblages *F. jenkinsi* did not occur in were dominated by more open water species (Table 4) suggesting that the habitat favored by those species is not the type favored by *F. jenkinsi*.

A higher average number of fish species and a higher diversity of fish species were usually found among assemblages where *F. jenkinsi* were present in Sabine Lake. On average, higher numbers of fish species were also found in assemblages where *F. jenkinsi* were present in Galveston Bay although the average diversity of fish assemblages did not significantly vary between assemblages with or without *F. jenkinsi*. It appears that *Fundulus jenkinsi* may prefer saltmarsh habitat that is also able to support a highly diverse array of fish species. Low fish diversity is therefore associated with a lower likelihood of finding *F. jenkinsi* in coastal saltmarshes.

Based on the results of our MDS analysis (Figure 14) it appears that seasonality is a major factor associated with fish community composition. However, *Fundulus jenkinsi* is considered to be a year round resident marsh species (Neill & Turner 1987) and therefore the differences in abundance and occurrence of this species is likely due to other underlying factors. Based on the data collected during this study we conclude that one of the most likely factors is water level, which directly affects our ability to efficiently capture this species. While, statistically, there was no strong evidence in this study to show that tide levels influence fish assemblage composition or *F. jenkinsi* presence this does not rule out water level as an influencing factor in *F. jenkinsi* capture. Tidal stages were not segregated by season and that is mostly likely why no significant difference was discovered. Apparent tidal levels vary greatly by season (Turner 1991) and while the Gulf coast may not experience a large lunar induced tidal fluctuation as some parts of country they can exhibit large fluctuations due to meteorological forcing (Ward 1980) which would influence the amount of saltmarsh inundation, and thus ability to accurately capture *F. jenkinsi*. Extreme high water levels usually occur during warmer months when winds are from the southeast. In contrast, extreme low water levels are more commonly encountered during the colder months of the year when cold fronts with strong northerly winds cause water levels to drop precipitously along Gulf coast estuaries. During these periods water levels recede to deeper tidal creeks and expose most of the marsh surface. Fish would be much more vulnerable to capture during these periods.

Due to varying water levels, the ability to accurately capture *F. jenkinsi* depends not only on the extent of marsh inundation but also the type of gear used. During our study we captured *F. jenkinsi* using both seines and Breder traps. Breder traps were preferred and were better at catching the target assemblage of fish during high water levels when the marsh was inundated while seining was preferred for sites during lower levels of inundation. During high inundation periods seining failed to capture the fish species that are closely associated with habitat edges and tended to capture more open water species. In contrast Breder traps are specifically designed to catch edge species, like *F. jenkinsi*, that reside in inundated marsh vegetation. Based on the results of our study we conclude that surveys designed to collect *F. jenkinsi* should use seines primarily in tidal creeks during low inundation (water level) periods to increase the probability of capturing the target species. We found that during times of low water level or little to no inundation or when bank slope were steep seining was very effective. During these low water periods we were able to seine the entire creek from bank to bank of most sites and at a relatively rapid rate. During these low water periods Breder traps were less effective as a sampling tool since the tidal regime did not facilitate the funneling of fish from the inundated marsh vegetation into the trap. Steep banks also made trap deployment inefficient as the precarious setting often resulted in the traps falling or the inability to place them properly. It is for this reason that future sampling methods would need to take into account a habitat's overall structure and inundation level before selecting one as a primary sampling method.

Habitat

Our study supports previous literature which states (Peterson & Ross 1991; Lopez et al. 2010; and Griffith 1974) that *F. jenkinsi* seem to prefer lower to mid salinity ranges. During our study *F. jenkinsi* were collected within similar salinity ranges of 2-15 ppt for Sabine Lake and 4-19 ppt for Galveston Bay. The mean catch of *F. jenkinsi* did differ between systems with Sabine Lake having a higher number of *F. jenkinsi* in lower salinities (average 9 ppt) than Galveston Bay (average 14 ppt). The difference in distribution of F. jenkinsi across salinity gradients between bays is most likely due to the degree of freshwater inflow and resulting salinity in each system. Sabine Lake has a relatively narrow access point to the ocean and a higher average freshwater inflow and lower average salinity compared to Galveston Bay (Orlando et al 1993). This data suggests that while *F. jenkinsi* is able to inhabit a wide salinity range (2-19 ppt) it is equally important to have appropriate marsh habitat available along the existing salinity gradients.

Mean water temperatures differed significantly at sites where *F. jenkinsi* were collected versus not collected. Higher numbers of *F. jenkinsi* were collected in lower temperatures. This pattern is likely an artifact of the seasonal influence of lower water levels in the winter associated with a higher frequency of positive catches of *F. jenkinsi*. As noted earlier, low water levels resulted in easier and more efficient seine collections of the target species. However, the association of higher catches and occurrence with lower temperatures may also be due to the interaction and influence of water temperature with *F. jenkinsi* physiology. Cold weather typically slows down the metabolism of poikilothermic temperate estuarine species (Clarke & Johnson 1999) and the higher catch rate could be a due to the decrease in swimming activity and gear avoidance caused by the lower seasonal temperatures.

F. jenkinsi were found in marshes of various dominant plant species. While previous studies have stressed the linkage of *F. jenkinsi* presence and *S. alterniflora* (Peterson & Turner 1994) our data suggests that they are also found in association with other marsh vegetation including oligohaline and mesohaline species and not strictly *S. alterniflora*. Past studies have found that several estuarine fish species selectively use microhabitats characterized by varying degrees of *S. spartina* stem density (Baltz et al. 1993) and that higher abundances of *F. jenkinsi* were captured in low to moderate stem densities (<25 stems/ $0.25\mathrm{m}^{-2}$) (Lopez et al. 2011). As *F. jenkinsi* are a species that is heavily connected to inundated marsh habitats our higher catches in low vegetative cover is most likely due to the fact that low vegetation caused less interference with sampling equipment (seining).

Life History

The overall length distribution for *F. jenkinsi* exhibited two modal peaks, one at the 20-22 mm (SL) and the second at the 32-34 mm. These peaks align with the values produced from the limited analysis able to be conducted in FiSAT II. The average length of *F. jenkinsi* did not vary by season but they did follow a similar pattern of distribution with peaks appearing at around the 22 and 35 mark for both fall and winter seasons. The length frequencies of the males and females used for GSI analysis also reflected this trend and possessed peaks at standard length values of approximately 22 mm and 36 mm. This data provides further support and evidence that the two modes of standard length values represents at least two separate age classes.

In addition to length frequency analysis this study does provide a preliminary estimation of growth based on total weight and standard length but as this was calculated lacks the representation of younger (larval and juvenile) individuals that were not collected by our gear it lacks the data needed to make inferences about younger (i.e. smaller) individuals of this species.

There exists very limited data on the growth rates of this species and estimating an individual's age is difficult to do. This is in part due to the difficulty in aging individuals and defining class groups. Since *F. jenkinsi* are considered batch spawners (Lopez, Peterson, Lang, & Charbonnet, 2010) and spawn over a relatively long season (Lang et al. 2012) the sizes of age classes have the potential to vary greatly as individuals in the same class can hatch months apart from each other. Future analysis of growth utilizing methods such as mark and recapture would provide additional data and a method of validating age and growth estimates for this species. Such methods would also have the benefit of allowing the tracking of movement of *F. jenkinsi* individuals and potential residency time in specific habitat types.

Past studies have documented that *F. jenkinsi* are most likely batch spawners, whose spawning season extends from March through August (Lopez et al. 2011; Lang et al. 2012). The GSI analysis conducted during this study supports these estimates. We found that there is a significant rise in the GSI values for both male and females during the spring and summer months. Due to the low catch or total lack of individuals captured during the summer there is a sizable gap for GSI values from the months of May through September. Only two females were caught during the two seasonal collections in July and September which may not be representative of the larger population. The limited data we compiled documents a sharp and steady rise in male GSI values from both February 2014 to April 2014 and from December 2014 to March 2015. Female GSI values follow a similar pattern with values increasing at a high rate from March 2014 to April 2014 with the highest peak GSI being recorded during September 2014. There was a dip in the female GSI values during the month of July 2014 but it is important to keep in mind that the values for both that month and September came from only a single individual. Despite these discrepancies, our data agrees with and supports previously documented patterns and assumptions regarding *F. jenkinsi* reproduction. Further analysis of oocyte stage and development during spring and summer spawning months is warranted. If additional research is conducted, the relationship of tidal stages and gonad development should be evaluated (Lang et al. 2012), to more accurately map the specific spawning dates of this species.

Both GSI value and standard length showed a significant linear relationship with the ovary phase of female *F. jenkinsi*. A greater degree of ovary maturation is expected to be seen more frequently in the older (larger SL) individuals. We would also expect that ovary phase and GSI values to be directly related as the size of the ovary and its maturation are dependent on each other.

Along with the GSI analysis, the data gathered from the ovarian phase analysis in this study supports past assertions (Lopez et al. 2010; Lopez et al. 2011; Lang et al. 2012) that there is a seasonal trend in *F. jenkinsi* reproductive organ growth and development. Ovarian development showed signs of seasonal progression with greater percentages of more developed ovaries being found in spring and summer and more latent and early developing ovaries being found in fall and winter.

Future Research and Management

Further studies are needed to gain a better understanding of the current spatial distribution and abundance, habitat and environmental associations, and reproductive ecology of *F. jenkinsi*. Further surveys are needed in areas west of Galveston Bay to determine if any sizable viable populations of this species occurs in Cedar Lakes, East Matagorda, Matagorda, or San Antonio Bay estuaries. In addition, resurveys of many of the sites visited in Sabine Lake and Galveston Bay should be conducted in the winter when the species is most vulnerable to sampling gear during low tides. Initialization of mark-recapture studies would also help establish a more reliable method to determine and validate age and growth estimates for this species. Such methods would also facilitate tracking the movement of *F. jenkinsi* individuals and give better estimates of how much inter-marsh migration occurs and the fidelity to certain habitat types.

Focus areas of future research should include better documentation of oocyte development and fecundity estimates during the spawning season, and the examination of the influence of high tide stages on gonad development and egg production (Lang et al. 2012). Better documentation of these processes would lead to more accurate predictions of spawning times and estimation of offspring production. Further diet and trophic ecology studies of *F. jenkinsi* are needed along with supporting studies on prey availability and abundance would help define any potential limiting factors associated with diet. Such information would help researchers and managers understand the entire spectrum of habitat and biological needs of this species.

Data from this study provides critical information that USFWS can use to evaluate the need for further research and/or the listing of this species for protection under the ESA. Based on the results of our study, this species is likely more extensive than previously thought and has likely been underestimated due to the selectivity of existing agency fisheries monitoring programs which do not cover the preferred habitat of this species. Based on our findings and previous literature there are many management actions that can be taken currently to promote the conservation of this species. Currently, many of Florida's and Mississippi's management plans concerning the habitat of *Fundulus jenkinsi* can be incorporated into future conservation plans or mitigation within the state of Texas. For example, restored or newly created wetlands should include a network of small interconnecting tidal creeks to maximize edge habitat (Baltz et al. 1993; Peterson et al. 2003; Lopez et al. 2010). These wetlands should also be located in the estuary where there is some degree of fresh water inflow to maintain the preferred salinity range of the Saltmarsh Topminnow (Peterson & Ross 1991; Lopez et al. 2010; and Griffith 1974) as well as other estuarine species. Efforts to prevent large sections of currently established tidal creeks from becoming channelized as well as restoring fish passage should also be goals of future conservation projects, especially in the areas where *F. jenkinsi* have been found to be present.

Appendix A. Total N of species caught using seine (S) and Breder traps (BT) in both Sabine Lake and Galveston Bay across all quarterly and monthly sampling collections Feb 2014 – March 2015.

			Sabine Lake			Galveston Bay			
Family	**Scientific Name**	**Common Name**	**S**	**BT**	**Sabine Total**	**S**	**BT**	**Galveston Total**	**Total N**
Lepisosteidae	*Atractosteus spatula*	Alligator Gar	1	0	1	0	0	0	**1**
Elopidae	*Elops saurus*	Ladyfish	2	0	2	23	0	23	**25**
Engraulidae	*Anchoa hepsetus*	Broad-Striped Anchovy	10	0	10	0	0	0	**10**
	Anchoa mitchilli	Bay Anchovy	4002	0	4002	5813	0	5813	**9815**
Clupeidae	*Brevoortia patronus*	Gulf Menhaden	20777	0	20777	5471	2	5473	**26250**
	Harengula jaguana	Scaled Sardine	1238	0	1238	74	0	74	**1312**
Synodontidae	*Synodus foetens*	Inshore Lizardfish	0	0	0	4	0	4	**4**
Mugilidae	*Mugil cephalus*	Striped Mullet	347	7	354	2808	7	2815	**3169**
Atherinopsidae	*Menidia beryllina*	Inland Silverside	1606	0	1606	2711	15	2726	**4332**
	Membras martinica	Rough Silverside	0	0	0	3	0	3	**3**
Belonidae	*Strongylura notata*	Redfin Needlefish	1	0	1	0	0	0	**1**

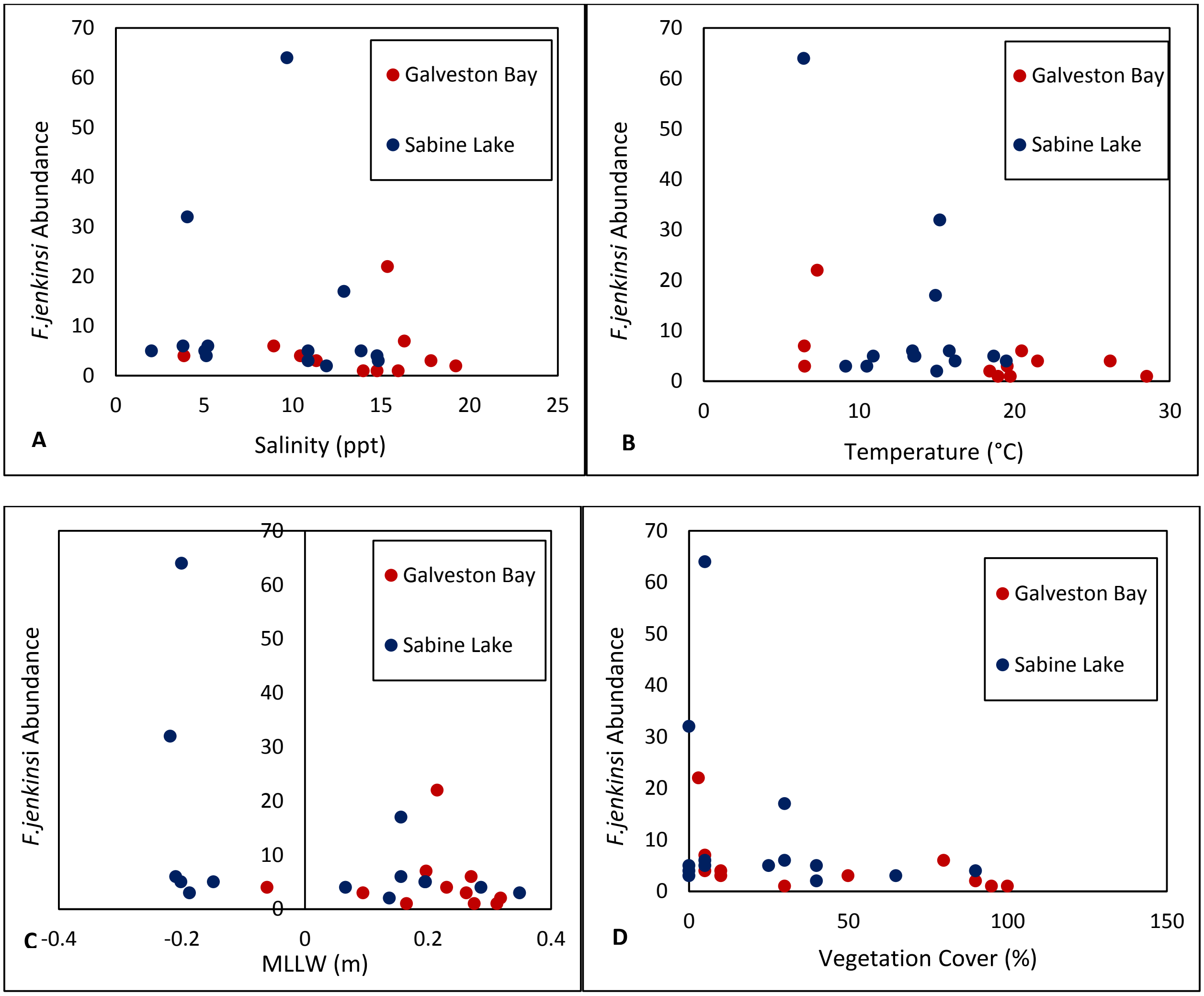
			Sabine Lake			Galveston Bay			
Family	**Scientific Name**	**Common Name**	**S**	**BT**	**Sabine Total**	**S**	**BT**	**Galveston Total**	**Total N**
Fundulidae	*Adinia xenica*	Diamond Killifish	52	5	57	2170	34	2204	**2261**
	Fundulus chrysotus	Golden Topminnow	0	0	0	2	0	2	**2**
	Fundulus grandis	Gulf Killifish	480	53	533	1032	37	1069	**1602**
	Fundulus jenkinsi	Saltmarsh Topminnow	161	0	161	622	9	631	**792**
	Fundulus pulvereus	Bayou Killifish	117	18	135	429	13	442	**577**
	Fundulus similis	Longnose Killifish	0	0	0	43	0	43	**43**
	Lucania parva	Rainwater Killifish	429	3	432	155	0	155	**587**
Cyprinodontidae	*Cyprinodon variegatus*	Sheepshead Minnow	1059	86	1145	3657	12	3669	**4814**
Poeciliidae	*Gambusia affinis*	Mosquito Fish	892	13	905	655	6	661	**1566**
	Heterandria formosa	Least Killifish	1	0	1	0	0	0	**1**
	Poecilia latipinna	Sailfin Molly	1155	113	1268	1699	18	1717	**2985**
Syngnathidae	*Syngnathus louisianae*	Chain Pipefish	1	0	1	24	0	24	**25**
	Syngnathus scovelli	Gulf Pipefish	1	0	1	1	0	1	**2**

			Sabine Lake			Galveston Bay			
Family	**Scientific Name**	**Common Name**	**S**	**BT**	**Sabine Total**	**S**	**BT**	**Galveston Total**	**Total N**
Carangidae	*Oligoplites saurus*	Leatherjack	1	0	1	21	0	21	**22**
Lutjanidae	*Lutjanus griseus*	Grey Snapper	7	0	7	8	0	8	**15**
Gerreidae	*Eucinostomus argenteus*	Spotfin Mojarra	2	0	2	96	0	96	**98**
	Eucinostomus melanopterus	Flagfin Mojarra	3	0	3	142	2	144	**147**
Sparidae	*Lagodon rhomboides*	Pinfish	20	0	20	491	1	492	**512**
Sciaenidae	*Bairdiella chrysoura*	Silver Perch	0	0	0	2	0	2	**2**
	Cynoscion arenarius	Sand Seatrout	22	0	22	38	0	38	**60**
	Cynoscion nebulosus	Spotted Seatrout	42	0	42	48	3	50	**93**
	Cynoscion nothus	Silver Seatrout	0	0	0	1	0	1	**1**
	Larimus fasciatus	Banded Drum	0	0	0	1	0	1	**1**
	Leiostomus xanthurus	Spot	836	0	836	10765	22	10787	**11623**
	Micropogonias undulatus	Atlantic Croaker	778	3	781	1244	13	1257	**2038**

			Sabine Lake			Galveston Bay			
Family	**Scientific Name**	**Common Name**	**S**	**BT**	**Sabine Total**	**S**	**BT**	**Galveston Total**	**Total N**
Sciaenidae	*Pogonias cromis*	Black Drum	1	1	2	0	0	0	**2**
	Stellifer lanceolatus	Star Drum	3	0	3	5	0	5	**8**
	Sciaenops ocellatus	Red Drum	29	0	29	192	7	199	**228**
Centrarchidae	*Lepomis miniatus*	Redspotted Sunfish	2	0	2	0	0	0	**2**
	Micropterus salmoides	Largemouth Bass	1	0	1	0	0	0	**1**
	Lepomis gulosus	Warmouth	1	0	1	0	0	0	**1**
	Lepomis macrochirus	Bluegill	7	0	7	1	0	1	**8**
Eleotridae	*Dormitator maculatus*	Fathead Sleeper	1	1	2	1	0	1	**3**
Gobiidae	*Ctenogobius boleosoma*	Darter Goby	32	0	32	9	0	9	**41**
	Ctenogobius shufeldti	Freshwater Goby	2	0	2	0	0	0	**2**
	Gobiosoma bosc	Naked Goby	96	0	96	20	0	20	**116**
	Gobiosoma robustum	Code Goby	5	0	5	1	0	1	**6**
	Microgobius gulosus	Clown Goby	13	0	13	7	0	7	**20**

			Sabine Lake			Galveston Bay		
Family	**Scientific Name**	**Common Name**	**S**	**BT**	**Sabine Total**	**S**	**BT**	**Galveston Total**	**Total N**
Ephippidae	*Chaetodipterus faber*	Atlantic Spadefish	0	0	0	2	0	2	**2**
Achiridae	*Achirus lineatus*	Lined Sole	0	0	0	1	0	1	**1**
Cynoglossidae	*Symphurus plagiusa*	Blackcheek Tonguefish	1	0	1	8	1	9	**10**
Tetraodontidae	*Sphoeroides parvus*	Least Puffer	0	0	0	2	0	2	**2**

Appendix B. Scatterplots of *F. jenkinsi* abundance in Sabine Lake (n= 14) and Galveston Bay (n= 11) sites by (A) salinity, (B) temperature, (C) mean lower low water level, and (D) percent vegetative cover.



Appendix C Multiple Tukey's Pairwise Comparisons for GSI Monthly and Seasonal Means for both Females and Males.

Appendix C 1. Tukey Pairwise Comparison for Female Monthly GSI Values

```
Grouping Information Using the Tukey Method and 95% Confidence

Date-F     N    Mean  Grouping
Sept-14    1   6.325  A
April-14   4    4.56  A
Feb-15    15   1.373    B
July-14    1   1.095    B C
March-14   1  0.7612    B C
Jan-15    11  0.5468      C
Feb-14     7   0.511      C
Oct-14    10  0.4624      C
March-15   8  0.4399      C
Nov-14    16  0.4380      C
Dec-14     3  0.1897      C

Means that do not share a letter are significantly different.


Tukey Simultaneous Tests for Differences of Means

                      Difference       SE of                             Adjusted
Difference of Levels    of Means  Difference            95% CI          T-Value   P-Value
Dec-14 - April-14         -4.373       0.424  (-5.788, -2.957)   -10.31     0.000
Feb-14 - April-14         -4.051       0.348  (-5.212, -2.890)   -11.64     0.000
Feb-15 - April-14         -3.189       0.312  (-4.232, -2.147)   -10.21     0.000
Jan-15 - April-14         -4.016       0.324  (-5.097, -2.934)   -12.39     0.000
July-14 - April-14        -3.468       0.621  (-5.539, -1.396)    -5.59     0.000
March-14 - April-14       -3.801       0.621  (-5.873, -1.729)    -6.12     0.000
March-15 - April-14       -4.122       0.340  (-5.257, -2.988)   -12.13     0.000
Nov-14 - April-14         -4.124       0.310  (-5.160, -3.089)   -13.29     0.000
Oct-14 - April-14         -4.100       0.328  (-5.196, -3.004)   -12.48     0.000
Sept-14 - April-14         1.763       0.621  (-0.309,  3.835)     2.84     0.166
Feb-14 - Dec-14            0.322       0.383  (-0.957,  1.600)     0.84     0.999
Feb-15 - Dec-14            1.183       0.351  ( 0.011,  2.355)     3.37     0.045
Jan-15 - Dec-14            0.357       0.362  (-0.850,  1.564)     0.99     0.996
July-14 - Dec-14           0.905       0.641  (-1.235,  3.045)     1.41     0.941
March-14 - Dec-14          0.571       0.641  (-1.568,  2.711)     0.89     0.998
March-15 - Dec-14          0.250       0.376  (-1.004,  1.505)     0.67     1.000
Nov-14 - Dec-14            0.248       0.349  (-0.918,  1.414)     0.71     1.000
Oct-14 - Dec-14            0.273       0.365  (-0.947,  1.492)     0.75     1.000
Sept-14 - Dec-14           6.136       0.641  ( 3.996,  8.275)     9.57     0.000
Feb-15 - Feb-14            0.862       0.254  ( 0.013,  1.710)     3.39     0.043
Jan-15 - Feb-14            0.035       0.268  (-0.860,  0.931)     0.13     1.000
July-14 - Feb-14           0.583       0.594  (-1.398,  2.564)     0.98     0.996
March-14 - Feb-14          0.250       0.594  (-1.731,  2.231)     0.42     1.000
March-15 - Feb-14         -0.071       0.287  (-1.030,  0.887)    -0.25     1.000
Nov-14 - Feb-14           -0.073       0.252  (-0.913,  0.766)    -0.29     1.000
Oct-14 - Feb-14           -0.049       0.274  (-0.962,  0.864)    -0.18     1.000
Sept-14 - Feb-14           5.814       0.594  ( 3.833,  7.795)     9.80     0.000
Jan-15 - Feb-15           -0.826       0.220  (-1.562, -0.091)    -3.75     0.015
July-14 - Feb-15          -0.278       0.573  (-2.192,  1.636)    -0.49     1.000
March-14 - Feb-15         -0.612       0.573  (-2.525,  1.302)    -1.07     0.992
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March-15 - Feb-15         -0.933       0.243  (-1.744, -0.122)    -3.84     0.012
Nov-14 - Feb-15           -0.935       0.200  (-1.601, -0.269)    -4.69     0.001
Oct-14 - Feb-15           -0.910       0.227  (-1.667, -0.154)    -4.02     0.007
Sept-14 - Feb-15           4.953       0.573  ( 3.039,  6.866)     8.64     0.000
July-14 - Jan-15           0.548       0.580  (-1.387,  2.483)     0.94     0.997
March-14 - Jan-15          0.214       0.580  (-1.721,  2.150)     0.37     1.000
March-15 - Jan-15         -0.107       0.258  (-0.968,  0.754)    -0.41     1.000
Nov-14 - Jan-15           -0.109       0.217  (-0.835,  0.617)    -0.50     1.000
Oct-14 - Jan-15           -0.084       0.243  (-0.894,  0.725)    -0.35     1.000
Sept-14 - Jan-15           5.779       0.580  ( 3.843,  7.714)     9.97     0.000
March-14 - July-14        -0.334       0.785  (-2.954,  2.287)    -0.42     1.000
March-15 - July-14        -0.655       0.589  (-2.620,  1.311)    -1.11     0.989
Nov-14 - July-14          -0.657       0.572  (-2.567,  1.253)    -1.15     0.986
Oct-14 - July-14          -0.632       0.582  (-2.576,  1.311)    -1.09     0.991
Sept-14 - July-14          5.231       0.785  ( 2.610,  7.851)     6.66     0.000
March-15 - March-14       -0.321       0.589  (-2.287,  1.644)    -0.55     1.000
Nov-14 - March-14         -0.323       0.572  (-2.233,  1.587)    -0.56     1.000
Oct-14 - March-14         -0.299       0.582  (-2.242,  1.645)    -0.51     1.000
Sept-14 - March-14         5.564       0.785  ( 2.944,  8.185)     7.09     0.000
Nov-14 - March-15         -0.002       0.240  (-0.804,  0.800)    -0.01     1.000
Oct-14 - March-15          0.023       0.263  (-0.856,  0.901)     0.09     1.000
Sept-14 - March-15         5.886       0.589  ( 3.920,  7.851)     9.99     0.000
Oct-14 - Nov-14            0.024       0.224  (-0.723,  0.771)     0.11     1.000
Sept-14 - Nov-14           5.887       0.572  ( 3.977,  7.797)    10.29     0.000
Sept-14 - Oct-14           5.863       0.582  ( 3.920,  7.806)    10.07     0.000

Individual confidence level = 99.86%
```

Appendix C 2. Tukey Pairwise Comparison for Male Monthly GSI values

```
Grouping Information Using the Tukey Method and 95% Confidence

Date-M     N     Mean  Grouping
April-14   4   0.4395  A
March-14   2   0.2796  A B C D E
March-15  12   0.2548    B
Feb-15    15   0.2544    B
Jan-15    10   0.1393    B C D E
Feb-14     8   0.0746          E
Nov-14    15   0.0744        D E
Oct-14     8   0.0611      C D E
Dec-14     1  0.01474    B C D E

Means that do not share a letter are significantly different.


Tukey Simultaneous Tests for Differences of Means

                      Difference       SE of                                  Adjusted
Difference of Levels    of Means  Difference        95% CI          T-Value   P-Value
Dec-14 - April-14         -0.425       0.100  ( -0.747,  -0.102)      -4.23     0.002
Feb-14 - April-14        -0.3649      0.0550  (-0.5414, -0.1884)      -6.64     0.000
Feb-15 - April-14        -0.1851      0.0505  (-0.3473, -0.0228)      -3.66     0.014
Jan-15 - April-14        -0.3002      0.0531  (-0.4707, -0.1297)      -5.65     0.000
March-14 - April-14      -0.1599      0.0778  (-0.4095,  0.0898)      -2.06     0.511
March-15 - April-14      -0.1847      0.0518  (-0.3511, -0.0183)      -3.56     0.019
Nov-14 - April-14        -0.3650      0.0505  (-0.5273, -0.2028)      -7.22     0.000
Oct-14 - April-14        -0.3783      0.0550  (-0.5549, -0.2018)      -6.88     0.000
Feb-14 - Dec-14           0.0598      0.0952  (-0.2459,  0.3656)       0.63     0.999
Feb-15 - Dec-14           0.2397      0.0927  (-0.0581,  0.5374)       2.58     0.213
Jan-15 - Dec-14           0.1245      0.0942  (-0.1778,  0.4268)       1.32     0.921
March-14 - Dec-14          0.265       0.110  ( -0.088,   0.618)       2.41     0.297
```

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March-15 - Dec-14         0.2400      0.0935  (-0.0600,  0.5401)     2.57     0.220
Nov-14 - Dec-14           0.0597      0.0927  (-0.2380,  0.3574)     0.64     0.999
Oct-14 - Dec-14           0.0464      0.0952  (-0.2594,  0.3521)     0.49     1.000
Feb-15 - Feb-14           0.1798      0.0393  ( 0.0536,  0.3060)     4.57     0.001
Jan-15 - Feb-14           0.0647      0.0426  (-0.0720,  0.2014)     1.52     0.843
March-14 - Feb-14         0.2050      0.0710  (-0.0229,  0.4329)     2.89     0.111
March-15 - Feb-14         0.1802      0.0410  ( 0.0486,  0.3118)     4.40     0.001
Nov-14 - Feb-14          -0.0002      0.0393  (-0.1264,  0.1260)    -0.00     1.000
Oct-14 - Feb-14          -0.0134      0.0449  (-0.1576,  0.1307)    -0.30     1.000
Jan-15 - Feb-15          -0.1151      0.0367  (-0.2328,  0.0025)    -3.14     0.059
March-14 - Feb-15         0.0252      0.0676  (-0.1918,  0.2422)     0.37     1.000
March-15 - Feb-15         0.0004      0.0348  (-0.1113,  0.1120)     0.01     1.000
Nov-14 - Feb-15          -0.1800      0.0328  (-0.2852, -0.0747)    -5.49     0.000
Oct-14 - Feb-15          -0.1933      0.0393  (-0.3195, -0.0671)    -4.92     0.000
March-14 - Jan-15         0.1403      0.0696  (-0.0830,  0.3636)     2.02     0.537
March-15 - Jan-15         0.1155      0.0384  (-0.0079,  0.2389)     3.00     0.084
Nov-14 - Jan-15          -0.0648      0.0367  (-0.1825,  0.0528)    -1.77     0.702
Oct-14 - Jan-15          -0.0781      0.0426  (-0.2149,  0.0586)    -1.83     0.660
March-15 - March-14      -0.0248      0.0686  (-0.2450,  0.1954)    -0.36     1.000
Nov-14 - March-14        -0.2052      0.0676  (-0.4222,  0.0118)    -3.04     0.078
Oct-14 - March-14        -0.2185      0.0710  (-0.4463,  0.0094)    -3.08     0.070
Nov-14 - March-15        -0.1804      0.0348  (-0.2920, -0.0687)    -5.19     0.000
Oct-14 - March-15        -0.1936      0.0410  (-0.3252, -0.0621)    -4.72     0.000
Oct-14 - Nov-14          -0.0133      0.0393  (-0.1395,  0.1129)    -0.34     1.000

Individual confidence level = 99.80%
```

Appendix C 3. Tukey Pairwise Comparison for Female Seasonal GSI values

```
Grouping Information Using the Tukey Method and 95% Confidence

Season   N    Mean  Grouping
Summer   2    3.71  A
Spring  13   1.733  A B
Winter  36  0.8544    B C
Fall    26  0.4474      C

Means that do not share a letter are significantly different.


Tukey Simultaneous Tests for Differences of Means

Difference of    Difference       SE of                                Adjusted
Levels             of Means  Difference        95% CI       T-Value    P-Value
Spring - Fall         1.286       0.372  ( 0.307,  2.264)      3.46      0.005
Summer - Fall         3.263       0.804  ( 1.148,  5.377)      4.06      0.001
Winter - Fall         0.407       0.282  (-0.335,  1.149)      1.44      0.477
Summer - Spring       1.977       0.832  (-0.212,  4.166)      2.38      0.091
Winter - Spring      -0.879       0.354  (-1.811,  0.054)     -2.48      0.072
Winter - Summer      -2.856       0.796  (-4.949, -0.762)     -3.59      0.003

Individual confidence level = 98.96%
```

Appendix C 4. Tukey Pairwise Comparison for Male Seasonal GSI values

```
Grouping Information Using the Tukey Method and 95% Confidence

Season-Male   N     Mean  Grouping
```

```
Spring       18   0.2986  A
Winter       34   0.1712    B
Fall         23  0.06979      C

Means that do not share a letter are significantly different.


Tukey Simultaneous Tests for Differences of Means

Difference of    Difference       SE of                                Adjusted
Levels             of Means  Difference         95% CI        T-Value   P-Value
Spring - Fall        0.2288      0.0342  ( 0.1471,  0.3105)     6.69     0.000
Winter - Fall        0.1014      0.0293  ( 0.0313,  0.1715)     3.46     0.003
Winter - Spring     -0.1274      0.0317  (-0.2031, -0.0517)    -4.02     0.000

Individual confidence level = 98.05%
```

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