

DISTRIBUTION, HABITAT, AND LIFE HISTORY CHARACTERISTICS OF THE  
SALTMARSH TOPMINNOW (*FUNDULUS JENKINSI*)

By

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## ABSTRACT

### DISTRIBUTION, HABITAT, AND LIFE HISTORY CHARACTERISTICS OF THE SALTMARSH TOPMINNOW (*FUNDULUS JENKINSI*)

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The University of Houston-Clear Lake, 2016

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The Saltmarsh Topminnow (*Fundulus jenkinsi*) occurs intermittently along the coastal saltmarshes of the Gulf of Mexico and is listed as a species of concern by many of the Gulf coast states. Little is known about *F. jenkinsi*'s range, population status, and habitat needs within Texas waters. This information is needed for the successful conservation and management of this relatively rare species. The objective of my study was to fill in the gaps of knowledge about the distribution, habitat characteristics, and life history of *Fundulus jenkinsi* within Texas. During my study multiple sites were sampled quarterly or monthly in order to determine which habitat factors were associated with and influenced the occurrence, growth, reproductive and diet composition of *F. jenkinsi*. Saltmarsh Topminnow were collected at multiple sites where they have been historically documented as well as at new, previously undocumented locations. Salinity and water level are important contributing factors that appear to influence the occurrence of *F. jenkinsi*'s. Analysis of nekton similarity that *F. jenkinsi* are closely associated with other species who share the same habitat. Analysis of both sexes of individuals captured during monthly collections failed to reveal any obvious external sexual dimorphism

between genders. Examination of the size distribution of captured *F. jenkinsi* documented a bi-modal distribution of length frequencies were observed indicating at least two age classes most likely compose the population. Results of gonadosomatic indices (GSI) and ovarian development stage analysis suggest a seasonal progression of gonad maturation with peak spawning activity occurring during the spring and summer months. Diet analysis also documented a seasonal difference in gut content with *F. jenkinsi* feeding on a wide range of both aquatic and terrestrial invertebrates. Evidence from this study shows that both seines and Breder traps can be implemented to catch *F. jenkinsi* but the success of each's implementation is linked to the level of marsh inundation and water level. Findings from my study suggest that *F. jenkinsi* may be more numerous and more widely distributed within Galveston Bay and Sabine Lake than previously thought though more research is needed to better delineate the exact geographic extent of Saltmarsh Topminnow in other estuaries along the Texas coast. The habitat and life history characteristics found in this study aligns with past research on the species but additional research is needed to better define the age structure and reproductive potential of populations within Texas.

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## INTRODUCTION

### **Historic Distribution and Conservation Status**

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; Thompson 1991). 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 represents the western most extent of this species' range with populations being recorded in West Bay, Trinity Bay, Oyster Bayou and western portions of Galveston Bay including Dickinson Bayou (Martin et al. 2012; Guillen 1996; Simpson & Gunter 1956; Evermann 1892). Additional sporadic occurrences have also been reported as far west as the Rio Grande River delta (Simpson and Gunter 1956; Patrick et al. 1998) with other locations occurring 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 still appears to represent the western most extent of their range although sporadic occurrences of the species may occur as far west as San Antonio Bay (Nicolau 2001). The occurrence and year of documented *F. jenkinsi* collections in both Galveston Bay and Sabine Lake are presented in Figure 1. This data was retrieved from the Fish of Texas database (FoTX; Hendrickson et al. 2014) and other published sources. Together they document the occurrence of *F. jenkinsi* within the upper Texas coast over the past 100 years.



NOAA and USFWS came to a decision that a change to *F. jenkinsi*'s listing may be warranted (Crabtree 2011; FR 2004b). The USFWS then assumed jurisdiction of the species and responsibility for determining its listing status (Crabtree 2011). In the state of Texas, the Texas Parks and Wildlife Department (TPWD) lists the *F. jenkinsi* as a species of greatest conservation need for the Gulf Coast Prairies and Marshes ecoregion (TPWD 2011, TPWD 2012b) and, as such, are responsible for coordinating to develop initiatives and goals that monitor and address the needs of *Fundulus jenkinsi* and their related habitats (TPWD 2005).

The historically sporadic and rare sightings of *F. jenkinsi* may be due, at least in part, to the difficulty in collecting them. Patrick et al. (1998) collected *F. jenkinsi* in the lower Neches River during 1996 using large, fine mesh dip nets but failed to capture them using more traditional sampling gear such as seines. The Texas Parks and Wildlife Department (TPWD) coastal fisheries monitoring program has been unsuccessful in detecting *F. jenkinsi* since the mid-1970's based on their standardized monitoring program which utilizes large 60 ft. bag seines and otter trawls to sample open bay and adjacent shoreline nekton communities. Based on the gear design and location of deployment it is highly likely that the current TPWD coastal fisheries monitoring program design is highly selective against the capture of this species. This is likely attributed to the species relatively small size and affinity for tidal creek edge habitats which are typically not included in the standard TPWD sampling frame. Previous studies have shown that using different sampling methods and/or equipment does result in a sampling bias for the types of species collected in tidal creeks (Fulling 1999, Sergeant 1987). Rather than relying on traditional methods of capture (large bag seines, netting,

trawling, etc.) I instead incorporated and utilized Breder traps which are specifically designed to capture the small minnows from hard-to-sample inundated marsh habitats (Breder 1960).

### **Habitat, Ecology, and Life History**

The saltmarsh topminnow, *Fundulus jenkinsi*, is a member of the abundant Fundulidae family and lives in saltmarsh habitats along the coast of the Gulf of Mexico. While *F. jenkinsi* is closely related to several other well-known and well-studied killifish including the Mummichog (*Fundulus heteroclitus*) and the Gulf Killifish (*Fundulus grandis*) very little is known about the ecology and life history of *F. jenkinsi*.

*Fundulus jenkinsi* is considered an estuarine species and has been most frequently collected in low to moderate salinities (4-20 ppt) in *Spartina alterniflora* dominated tidal creeks and wetlands (Peterson & Ross 1991; Peterson & Turner 1994; 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 creek complexes connected to larger channels within the saltmarsh. Access to these small interconnected tidal creeks appears to be an important contributing factor in *F. jenkinsi*'s diet and reproduction. During high water levels *F. jenkinsi* has access to larger foraging areas in the inundated marsh which also 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.

*Fundulus jenkinsi*, like other fundulids, are classified as batch spawners and capable of spawning more than once during a single reproductive season (Lopez et al. 2010; Lang et al. 2012). Monthly gonadosomatic indexes (GSI) and ovarian histological analysis of female *F. jenkinsi* indicate that the spawning season appears to fall from March through August (Lang et al. 2012). Many fish species synchronize their spawning events with 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). This pattern is most likely due to higher water levels which facilitate greater access to inundated marsh habitat. *Fundulus jenkinsi* can then deposit their eggs in the more protected, interior higher elevation marsh. In summary, *Fundulus jenkinsi* reproduction and diet appear to be strongly linked to the amount of inundated salt marsh access and therefore intertidal creeks embedded within salt marshes appear to be essential to this species viability.

### **Purpose of Study**

Due to their apparent rarity there is need to obtain a reliable estimate of 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, especially within the state of Texas. This data is needed by resource agencies to support ongoing management and conservation of this species and its 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 conditions needs to be better quantified in order to develop meaningful management recommendations for the long-

term conservation of this species. Given this species' restricted range in Texas and the projections of coastal development, ongoing land subsidence, and 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 defined as these and other threats can cumulatively reduce the geographic extent of saltmarsh habitat and consequently threaten the population viability of this species.

### **Study Objectives**

- 1) Develop updated population range and abundance estimates of *Fundulus jenkinsi* in Galveston Bay and Sabine Lake, Texas.
- 2) Evaluate 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 relative size, age, sex distribution, growth, and reproduction characteristics of *Fundulus jenkinsi*.
- 4) Compare the effectiveness of different sampling methods on *Fundulus jenkinsi* capture.

## METHODS

### **Study Location and Sampling Frequency**

Field sampling was conducted from February 2014 through March 2015 at selected sites throughout Galveston Bay and Sabine Lake to estimate the spatial distribution of the *F. jenkinsi* within these regions. From February 28 to November 20, 2014, quarterly samples were collected from a total of 134 individual sites. Additional monthly sampling was conducted from February, 2014 to March, 2015 at three locations within Moses Bayou<sup>1</sup>. *Fundulus jenkinsi* collected from these monthly sampling events were used for reproductive, diet, and demographic analysis.

Based on previous studies most sample sites were selected near tidally influenced wetlands dominated by stands of *S. alterniflora* that receive some degree of freshwater input sufficient to sustain salinity levels (<20ppt). These conditions should provide optimal conditions for *F. jenkinsi* population viability (Peterson et al. 2003; Lopez et al. 2011; Peterson & Turner 1994). The majority of sampling was focused on smaller intertidal creeks (Figure 2) but also included a variety of other habitat types including coastal and inland open marsh habitat and larger saltmarsh lined tidal channels and streams.

The location and dates of historic occurrences as well as this study's collections were input into an ArcGIS geodatabase that was subsequently used to create distribution maps. Additional shapefiles obtained from the National Wetland Inventory (USFWS

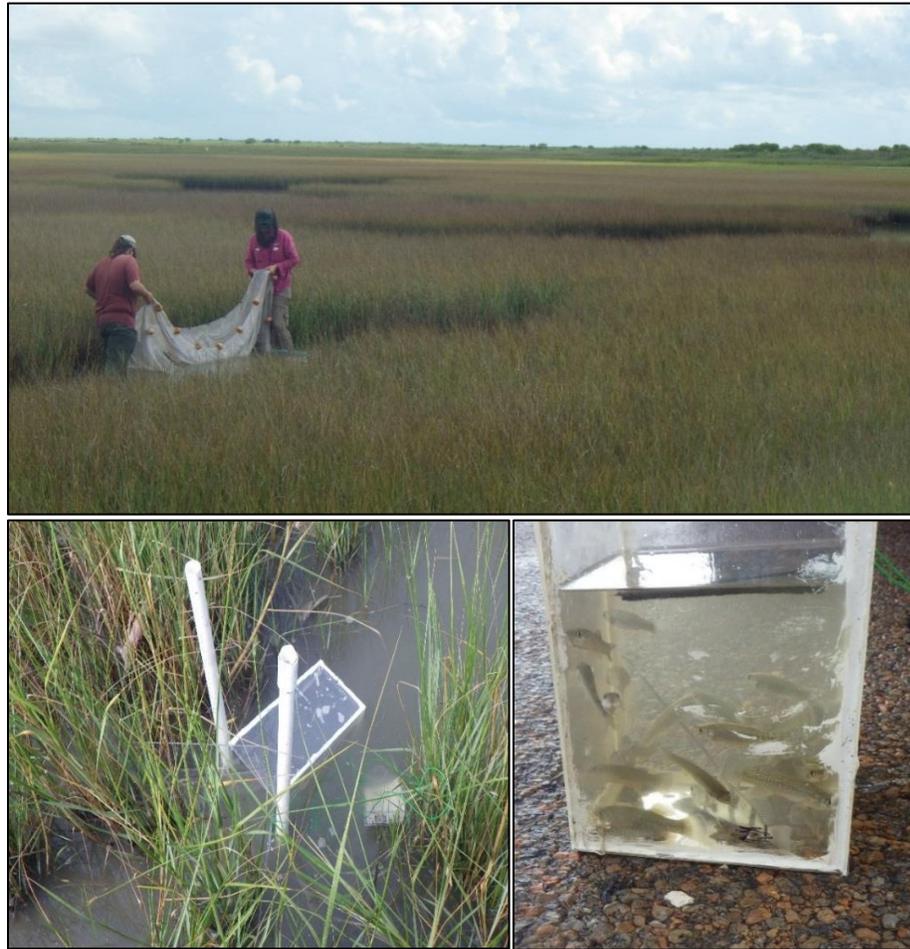
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<sup>1</sup> The third site was added after September 10, 2014.

2014) depict the distribution of recent wetland vegetation was overlaid on these maps to estimate the amount of recently available saltmarsh habitat located near each sample site.

### **Sampling Methods**

Fish were collected using a straight seine (15' x 4') with ¼ inch bar mesh and Breder traps (Breder 1960) (Figure 2). 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. Protocol for the handling of fish species followed the general fish protocol standards in place for the Environmental Institute of Houston (IACUC Protocol #13.005) as outlined by the Institutional Animal Care and Use Committee (IACUC). 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. All fish collected during the course of this study were gathered under a Texas Parks and Wildlife scientific collection permit (TPWD Scientific Permit # SPR-0504-383).



**Figure 2. (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 (m) was recorded and tide stage (flood, high slack, ebb, low slack) as well as water level in reference to mean lower low water (MLLW) (m) were obtained from the closest NOAA tide gage (Table 1).

**Table 1. NOAA tidal gage and ID number with corresponding site regions for Galveston Bay and Sabine Lake.**

Site Region	Station Name	NOAA Station ID
Neches River & Sabine River	Rainbow Bridge	8770520
Port Arthur	Port Arthur	8770475
Sabine Pass	Sabine Pass North	8770822
East Bay	Rollover Pass	8770971
Trinity Bay	Round Point	8770559
Northern Galveston Bay	Morgan's Point	8770613
Clear Lake	Clear Lake	8770933
Dickinson & Moses Bayou	Eagle Point	8771013
Galveston	Galveston Pier 21	8771450
Highland Bayou & Greens Lake	Galveston Railroad Bridge	8771486
Chocolate	San Louis Pass	8771972

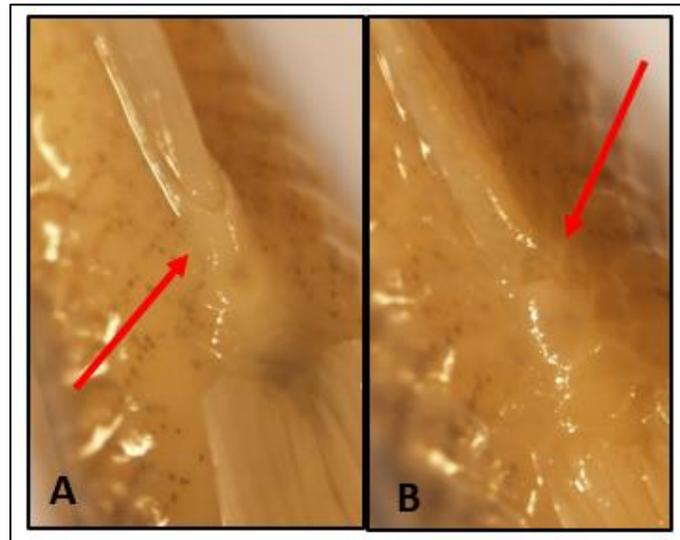
Water quality measurements including temperature ( $^{\circ}\text{C}$ ), dissolved oxygen (mg/L and %), conductivity ( $\mu\text{S}/\text{cm}$ ), pH, and salinity (ppt) were collected using an YSI 600 XLM sonde before seining and upon retrieving traps. Water clarity was also measured at each site with 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 tallied. The standard length (SL, mm) and total weight (TW, grams) 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 FiSAT II (Gayaniilo 2005) and NORMSEP's modal progression analysis conducted on monthly length frequencies to establish relative age classes.

The entire intestinal tract (esophagus to anus) of *F. jenkinsi* individuals were excised, weighed (g) and preserved in 70% ethanol. Contents of each stomach were removed under a dissection scope and individual prey items were identified to lowest possible taxa (Heard 1982; Smith & Johnson 1996; Arnett 2000; Thorp & Covich 2010), photographed, and counted. When possible, the sex of each individual was determined using the dimorphic characteristics described by Lopez et al. (2010). When external sexual dimorphic features (Figure 3) were not clear, sex classification was done via observation of the extracted gonads (Figure 4).

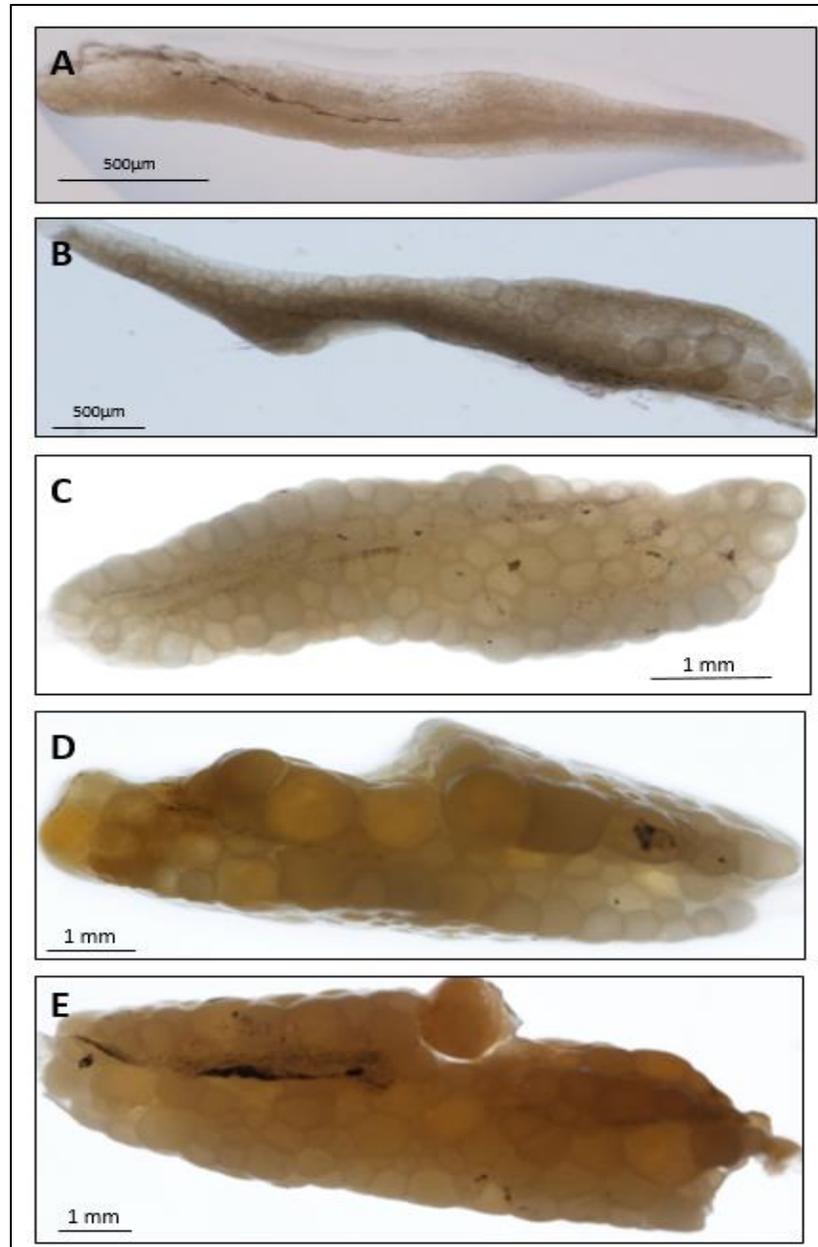


**Figure 3** *Fundulus jenkinsi* ventral view. (A) Female genital region showing the anal fin with sheath (arrow). (B) Male genital region showing the anal fin and exposed papilla (arrow).



**Figure 4** *Fundulus jenkinsi* gonads extracted from individuals caught in April 2014. (A) Female and (B) Male.

To assess the reproductive condition of each *F. jenkinsi* individual, the gonads were extracted, weighed (GW, grams), and the gonadosomatic index (GSI) calculated:  $[(GW / TW) * 100]$ . The monthly mean GSI was 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 5) using methods described by 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 5 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

Salinity (ppt), temperature ( $^{\circ}\text{C}$ ), mean lower low water level (MLLW), and bank vegetation (% cover) were compared between sites within both bay systems. The

distribution of each variable was tested for normality (Shapiro & Wilks 1965) followed by the appropriate parametric (Student's T-test) or nonparametric tests (Mann-Whitney U) to compare habitat variables across sites where *F. jenkinsi* were found and not found.

Fish community structure was characterized by calculating total species abundance ( $N$ ), relative abundance (%), and Shannon-Wiener diversity ( $H'$ ). Catch per unit effort (CPUE) of *F. jenkinsi* were based on either three replicate seine tows or four set Breder traps. The diversity ( $H'$ ) of each quarterly sampling sites' fish assemblage was calculated in PRIMER 6 (Clarke and Warwick 2001) and the resulting values analyzed in Minitab 17 (2010) to determine if overall diversity significantly varied between sites where *F. jenkinsi* were found and not found in both bay systems.

Fish assemblage abundance data was 4<sup>th</sup>-root transformed and a Bray-Curtis similarity index was estimated between sites using the PRIMER 6 statistical software package (Clarke and Warwick 2001). Site groupings based on the similarity of fish assemblages were further investigated using an analysis of similarity (ANOSIM) to test for factors contributing in community structure where *F. jenkinsi* were captured and not captured. ANOSIM was 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 were pooled from both bay systems as well as from quarterly and monthly sampling events in order to run an ANOSIM to analyze differences in fish assemblages across gear types. Non-metric multidimensional scaling (NMDS) plots of assemblages were also constructed in PRIMER 6 to display assemblage similarities by season and gear type. Seasons were classified according to the astrological calendar with

switches between seasons being marked by the summer and winter solstices and the spring and fall equinoxes.

Standard length, weight, and GSI data were entered into Minitab 17 and Kruskal-Wallis H-tests were run to compare median standard lengths across gear types, genders, and seasons. A two-way ANOVA was also used to compare GSI values across months and seasons while a two-sample T-test was used to compare GSI values between genders. If significant differences in average values between categories (months, genders, and seasons) a Tukey's pairwise comparison was run to detect potential differences between pairs of target groups. Linear regression analyses were run to test the association between length and weight to GSI values of both male and females. Length and weight values from both male and female individuals were  $\text{Log}_{10}$  transformed and entered and plotted against each other in Excel. Solver was then used to find the best-fit relationship equation.

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.

The percent total occurrence and total percent composition of prey organisms in the gut was measured and used for two separate multivariate analyses. This information along with the abundance data of individuals from all other species of fish including

saltmarsh topminnow was input into the Primer software package. Sample transformations included a 4<sup>th</sup> root transformation and subsequent calculation of a Bray-Curtis similarity matrix based on the composition of prey or other fish species. One-way ANOSIMs were then run to compare the differences in gut content across sexes and seasons. Similarity percentages analysis (SIMPER) was run to compare the mean dissimilarity (%) of gut contents found in *F. jenkinsi* by season. The diversity ( $H'$ ) of the content of each gut was also calculated and values compared across genders and seasons. Regression analysis was also conducted to determine if abundance of dominant prey taxa varied with standard length. An  $\alpha$ -level of 0.05 was used to determine statistical significance in all tests.

## RESULTS

### **Distribution and Abundance**

A total of 134 sites were sampled along the upper Texas coast from February 17, 2014 to November 20, 2014 including 81 sampling sites within Galveston Bay and 53 within Sabine Lake. *Fundulus jenkinsi* were caught in the upper portion of Sabine Lake in both the Neches and Sabine River drainages (Figure 6). *Fundulus jenkinsi* were also caught within three areas of Galveston Bay in the East Bay, Trinity Bay, and Dickinson and Moses Bayou drainages (Figure 7). The distribution of historical occurrences along with the locations and relative abundance where *F. jenkinsi* were captured during the course of this study are depicted on the map of Sabine Lake (Figure 6) and Galveston Bay (Figure 7).

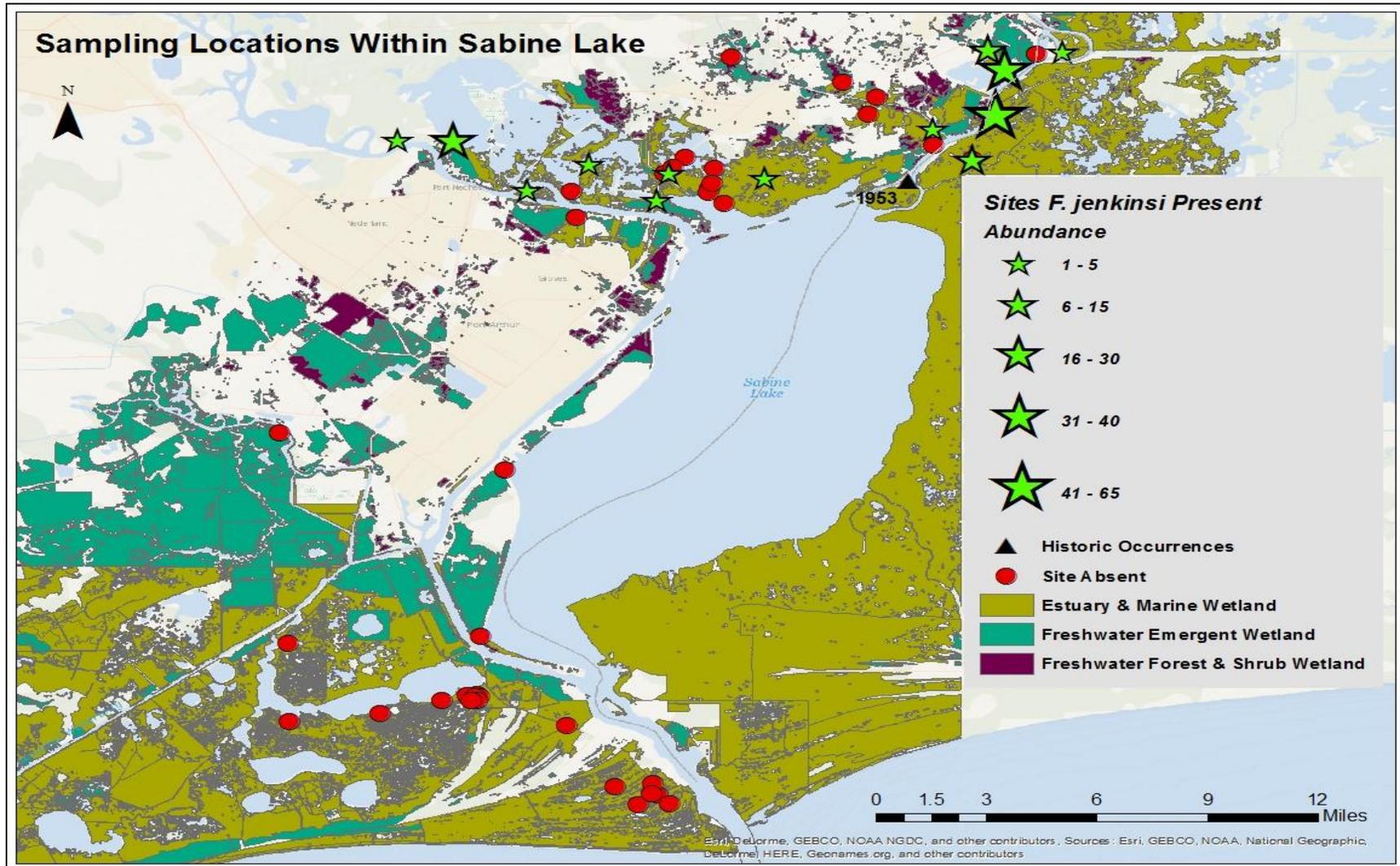


Figure 6. Map of sampling sites located around Sabine Lake, TX. Green stars and red circles represents sites where *F. jenkinsi* were found and not found respectively. Size of the star corresponds to the total number of *F. jenkinsi* collected at that site.

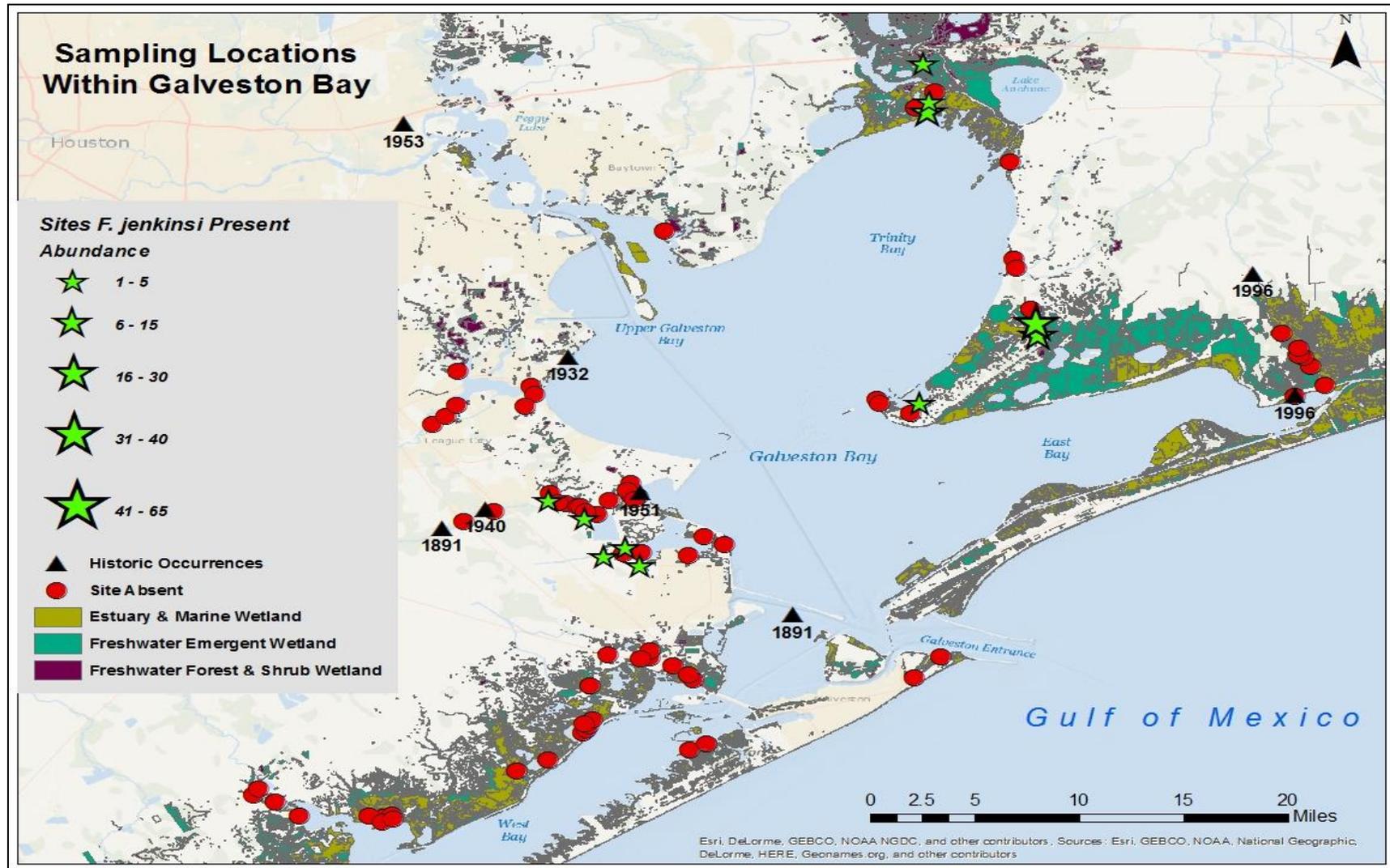


Figure 7. Map of sampling sites located around Galveston Bay, TX. Green stars and red circles represents sites where *F. jenkinsi* were found and not found respectively. Size of the star corresponds to the total number of *F. jenkinsi* collected at that site.

We collected *F. jenkinsi* at six sites within 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 where this species has not historically been reported before. We also failed to collect *F. jenkinsi* individuals from locations that they had previously been captured including the Clear Lake and Oyster Bayou regions of Galveston Bay. *Fundulus jenkinsi* were collected during every quarterly sampling event except for one in Galveston Bay (June 2014) and only during two quarterly sampling events (February 2014 and November 2014) for Sabine Lake.

The overall percentage of sites where *Fundulus jenkinsi* were captured was higher for the Sabine Lake system than that of Galveston Bay. The total number of *F. jenkinsi* collected was also three times greater in Sabine Lake than in Galveston Bay and the average number of *F. jenkinsi* collected per site in Sabine Lake was over twice the average number found per site in Galveston Bay (Table 2).

**Table 2 Summary of sites for quarterly sampling events from February 2014-November 2014 within each bay system where *F. jenkinsi* were collected and of the number of individuals captured within each estuary region.**

Sites				<i>F. jenkinsi</i> Abundance		
Site Regions	% Sites <i>F. jenkinsi</i> Present	% Sites <i>F. jenkinsi</i> Not Collected	Total Sites	Min.-Max.	Average per Site	Total <i>F. jenkinsi</i> Captured
<b>Sabine Lake</b>	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
<b>Galveston Bay</b>	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

Within the Sabine Lake system, specifically the Sabine and Neches River drainages, sites where *F. jenkinsi* were collected versus not collected occurred in relatively equal proportions. However, catch rates within these two drainages 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 2).

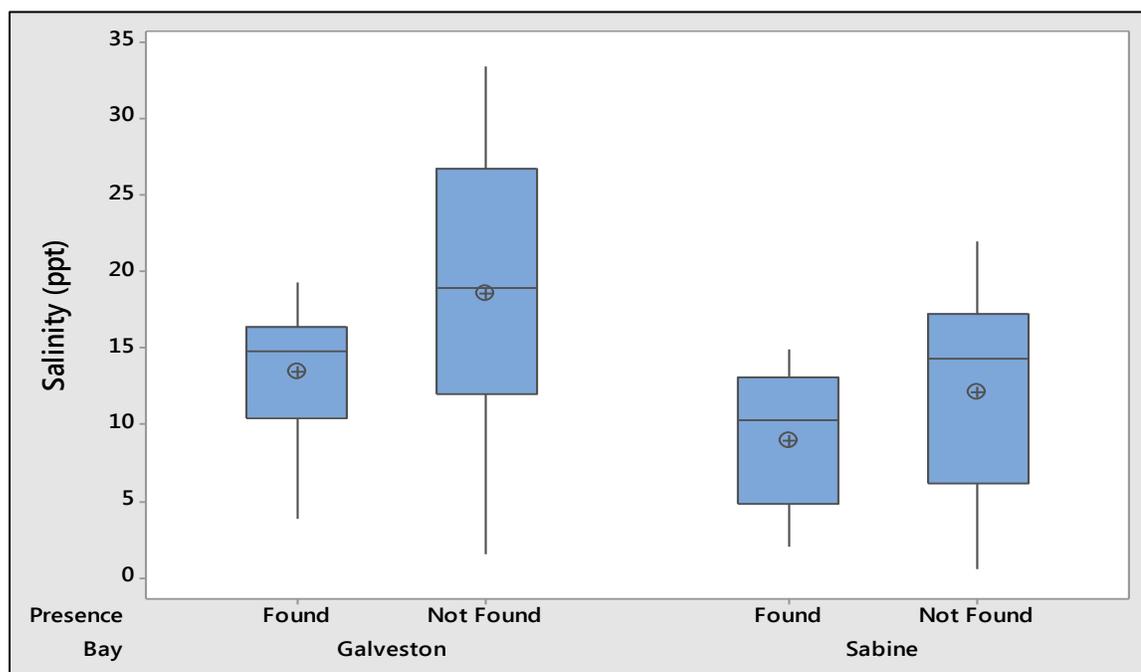
Within the Galveston Bay system, the Trinity Bay drainage contained the greatest proportion of sites where *F. jenkinsi* were collected. Sites sampled within the East Bay and Dickinson and Moses drainages contained similar, but smaller proportions of sites containing positive catches of *F. jenkinsi*. 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 in Dickinson and Moses Bayou (Table 2).

### Habitat Characteristics

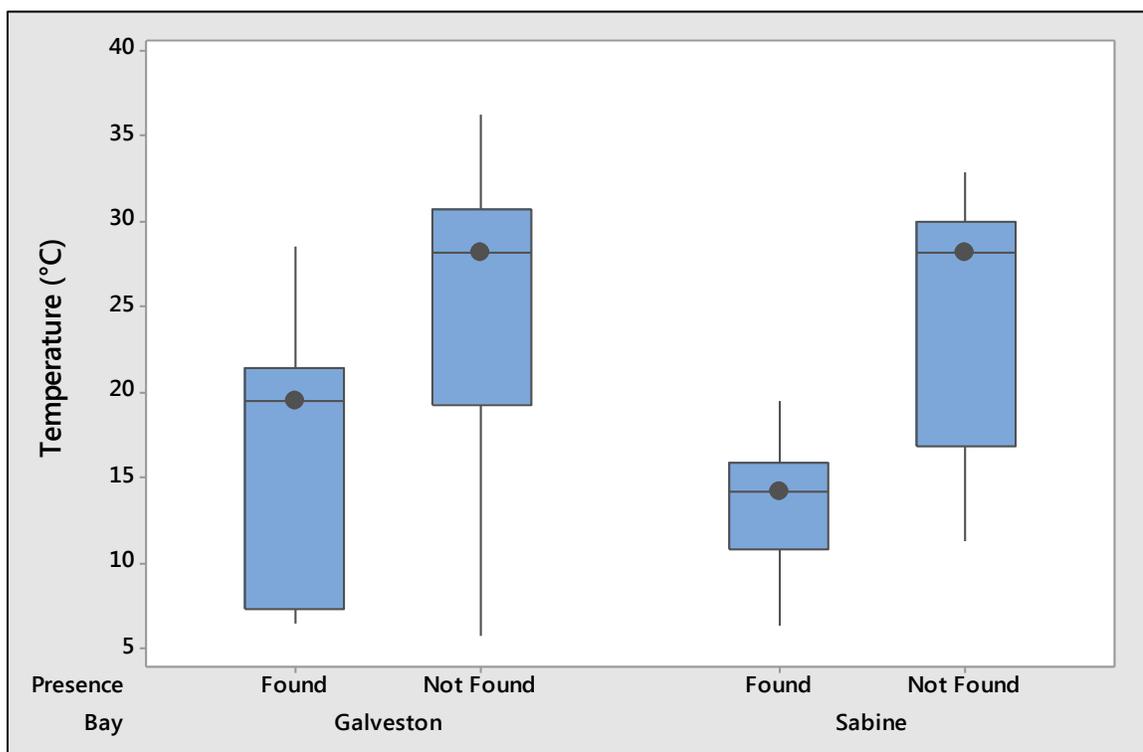
Salinity (ppt), temperature ( $^{\circ}\text{C}$ ), mean lower low water level (MLLW) values (m), and vegetation (% cover) were compared between sites where *F. jenkinsi* were collected and not collected within both bay systems.

Student T-tests comparing average salinities revealed that mean salinity values were significantly lower (13.5 ppt vs 18.5 ppt) in Galveston Bay sites where *F. jenkinsi* were captured ( $t_{21} = 3.07$ ;  $p = 0.006$ ) (Figure 8). However, average salinity (11.2 ppt) did not significantly vary between sites where *F. jenkinsi* were captured and not captured within Sabine Lake ( $t_{32} = 2.00$ ;  $p = 0.054$ ) (Figure 8).



**Figure 8** Boxplot comparing mean salinity (ppt) of sites where *F. jenkinsi* were found and not found for both Sabine Lake and Galveston Bay sites pooled across all seasons.

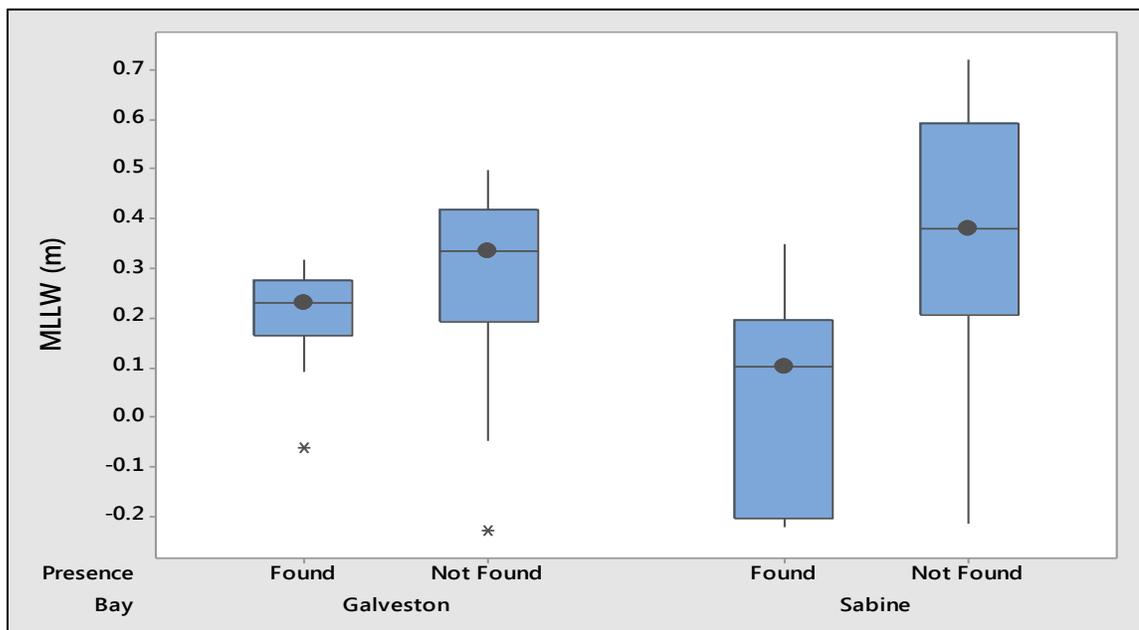
Mann-Whitney U tests of median temperature between Sabine Lake sites revealed that sites with *F. jenkinsi* present possessed significantly lower median temperatures (14.3°C vs 28.2°C) than sites where *F. jenkinsi* were not captured ( $U_{51} = 169.0; p < 0.001$ ) (Figure 9). Similar results were also found within Galveston Bay sites with median temperature being significantly lower at sites where *F. jenkinsi* were caught (19.5°C vs. 28.2°C) ( $U_{79} = 247; p = 0.005$ ) (Figure 9).



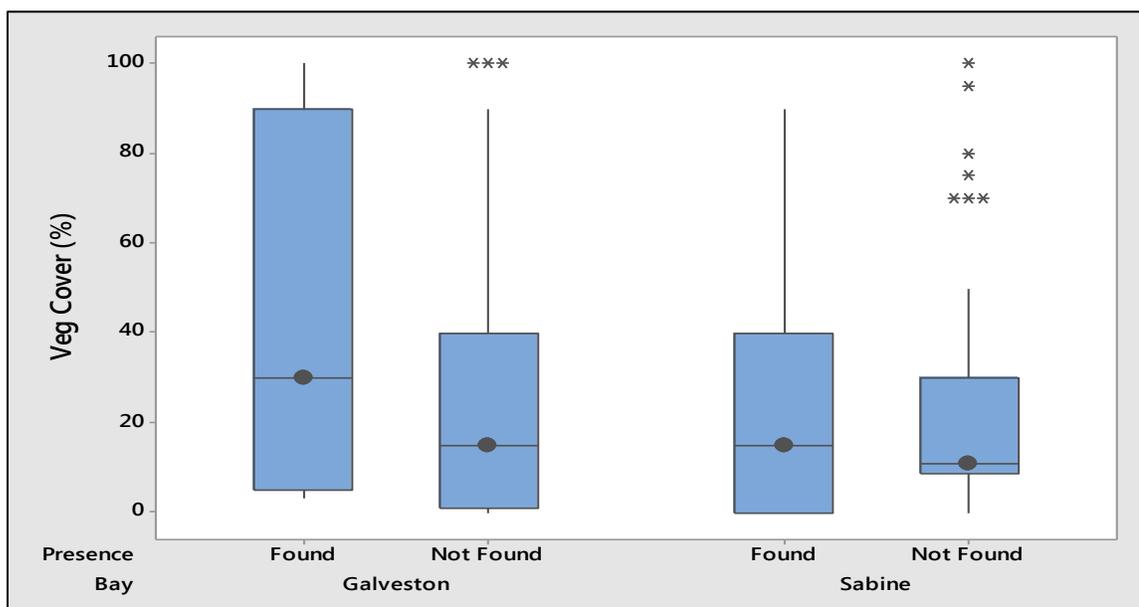
**Figure 9** Boxplot comparing median temperature (°C) of sites where *F. jenkinsi* were found and not found for both Sabine Lake and Galveston Bay sites pooled across all seasons.

Median water levels (MLLW) were significantly lower at sites where *F. jenkinsi* were found versus where they were not found within Sabine Lake (0.10 m vs. 0.38m) ( $U_{51} = 173.5; p < 0.001$ ) (Figure 10). However, median water levels (0.30 m) did not show a difference between sites where *F. jenkinsi* were captured within Galveston Bay ( $U_{79} = 315.0; p = 0.055$ ) (Figure 10). Mann-Whitney U-tests revealed that the median

percent vegetation cover did not significantly vary (Sabine  $U_{51} = 342.0$ ;  $p = 0.557$ ; Galveston  $U_{79} = 526.0$ ;  $p = 0.394$ ) between sites where *F. jenkinsi* were found and not found within either bay system (Figure 11).



**Figure 10** Boxplot comparing median MLLW (m) of sites where *F. jenkinsi* were found and not found for both Sabine Lake and Galveston Bay sites pooled across all seasons.



**Figure 11** Boxplot comparing median vegetative cover (%) of sites where *F. jenkinsi* were found and not found for both Sabine Lake and Galveston Bay sites pooled across all seasons.

*Spartina alterniflora* was the most frequently occurring plant species at all sites including locations with and without *F. jenkinsi* (Table 3). In general, *S. alterniflora*, *Phragmites australis* and *Typha latifolia* were prevalent at sites where *F. jenkinsi* were collected. In contrast, *Juncus 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 where *F. jenkinsi* were collected but was not found at any site where *F. jenkinsi* were not collected.

**Table 3 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 Sabine Lake and Galveston Bay.**

Primary Vegetation	Frequency of Occurrence (%)	
	Sites <i>F. jenkinsi</i> Collected	Sites <i>F. jenkinsi</i> Not Found
<i>Spartina alterniflora</i>	56%	82%
<i>Phragmites australis</i>	48%	10%
<i>Typha latifolia</i>	20%	3%
<i>Spartina patens</i>	8%	7%
<i>Iva frutescens</i>	4%	4%
<i>Ruppia maritima</i>	4%	3%
<i>Halodule wrightii</i>	4%	0%
<i>Juncus roemarianus</i>	0%	12%
<i>Batis maritima</i>	0%	5%
<i>Salicornia</i> spp.	0%	4%
<i>Taxodium distichum</i>	0%	1%
<i>Vallisneria americana</i>	0%	1%

## Fish Community Composition

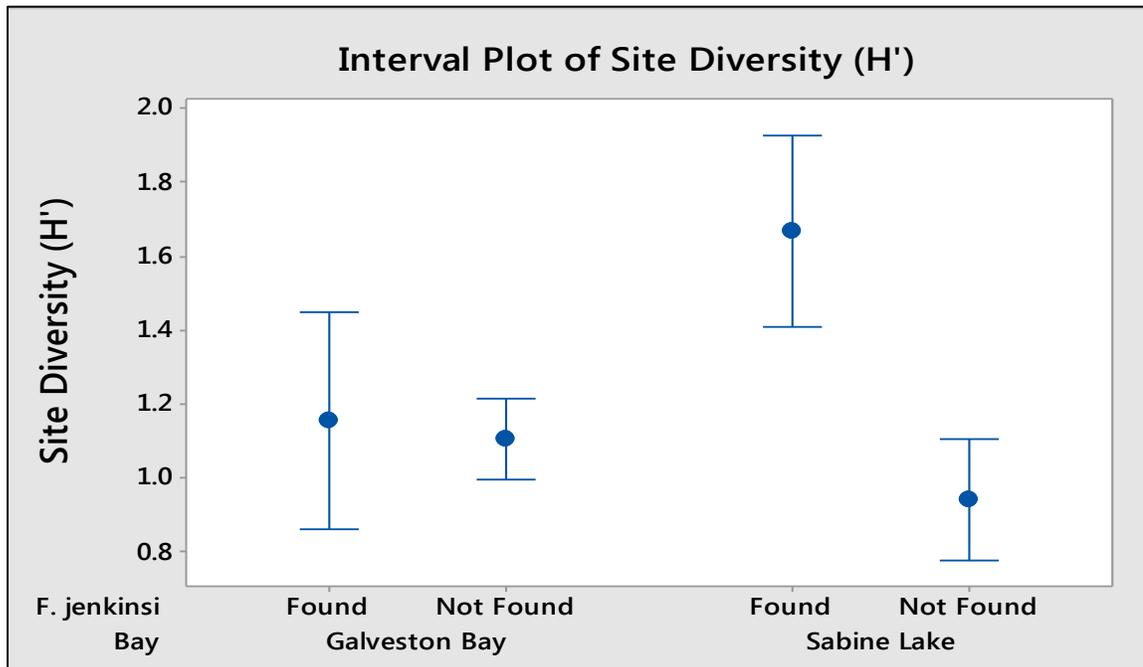
A total of 75,369 individual fish consisting of 27 families and 53 species were collected during all sampling events. The total abundance of all species captured during both quarterly and monthly sampling events across both gear types and bay systems is presented in Appendix A. The five most abundant families and species captured during all quarterly collections from both Sabine Lake and Galveston Bay are presented in Table 4. The families Clupeidae, Sciaenidae, and Engraulidae cumulatively represented 75% of the total species abundance. Similarly, the top 75% of all individuals collected consisted of four species including *Brevoortia patronus*, *Leiostomus xanthurus*, *Anchoa mitchilli*, and *Cyprinodon variegatus*.

**Table 4 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%	<i>Brevoortia patronus</i>	39.3%
Sciaenidae	19.3%	<i>Leiostomus xanthurus</i>	15.8%
Engraulidae	13.9%	<i>Anchoa mitchilli</i>	13.9%
Cyprinodontidae	5.9%	<i>Cyprinodon variegatus</i>	5.9%
Fundulidae	5.2%	<i>Menidia beryllina</i>	4.9%
All Other Families	14.2%	All Other Species	20.2%

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 = 0.064,  $p=0.005$ ). Due to this difference, data collected from Sabine Lake and Galveston Bay was separated and analyzed independently of each other. A *T*-test revealed that the mean species diversity ( $H'$ ) at sites where *F. jenkinsi* were collected

did not differ significantly from sites where *F. jenkinsi* were absent within Galveston Bay ( $t_{13} = 0.37; p = 0.720$ ). However, the mean diversity of site containing *F. jenkinsi* did significantly differ ( $t_{25} = 5.03; p < 0.001$ ) from sites lacking *F. jenkinsi* within Sabine Lake (Figure 12).



**Figure 12** Interval plot of mean fish species diversity ( $\text{Log}_e$ ) and 95% confidence interval between sites where *F. jenkinsi* were found and not found in both Galveston Bay and Sabine Lake.

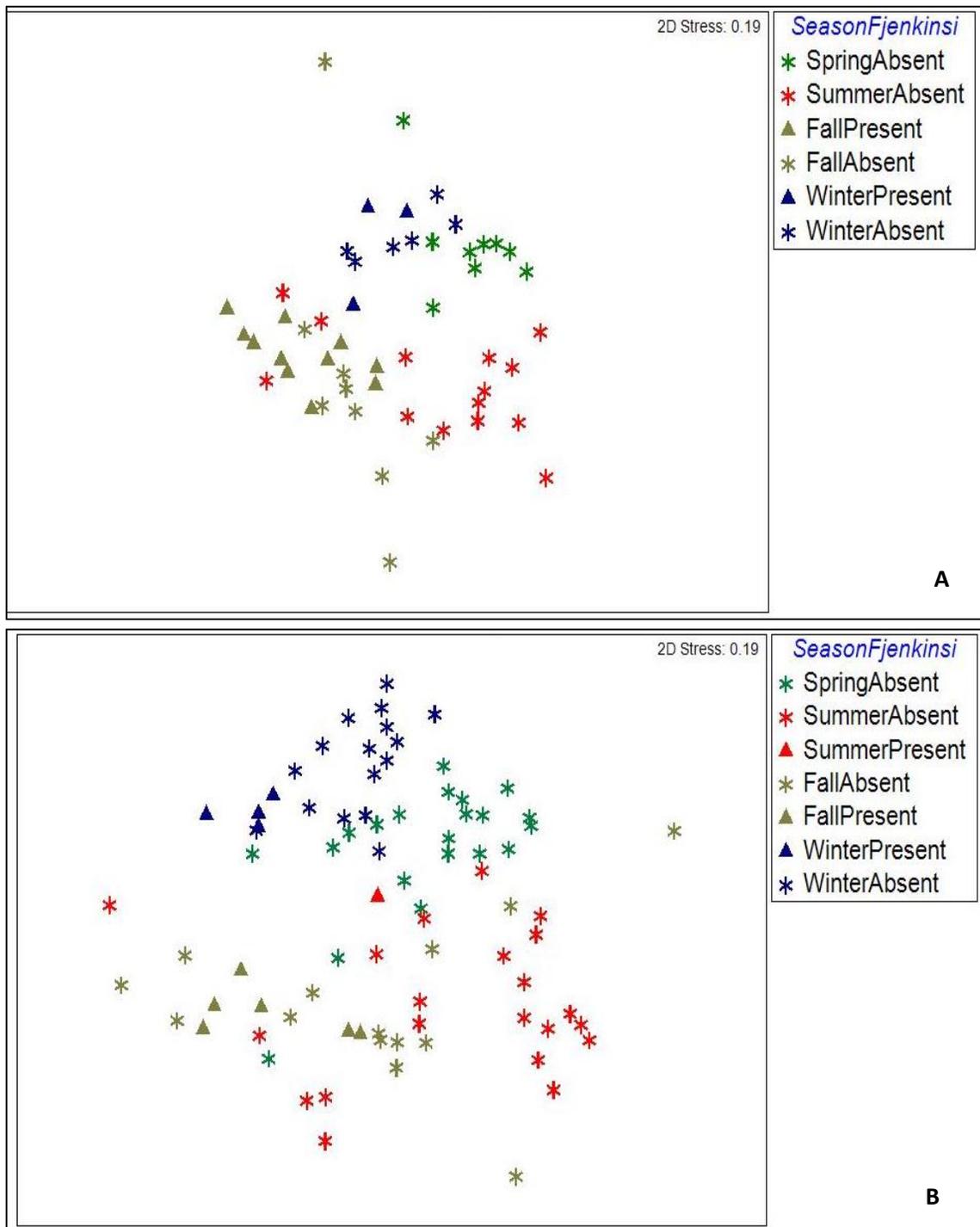
A one-way ANOSIM documented significant differences in fish assemblages between sites where *F. jenkinsi* were present versus assemblages where *F. jenkinsi* were not present for both Sabine Lake (Global  $R=0.110, p=0.037$ ) and Galveston Bay sites (Global  $R=0.117, p=0.023$ ). Additional similarity percentage analysis (SIMPER) revealed that within the Sabine Lake sites assemblages containing *F. jenkinsi* had an average similarity of 48.4% while assemblages that did not contain *F. jenkinsi* only had an average similarity of only 32.1%. Galveston Bay assemblages containing *F. jenkinsi* had an average similarity of 43.4% with assemblages lacking *F. jenkinsi* possessing a

similarity of 31.7%. Similar species differences were found between Galveston Bay and Sabine Lake groups. Species within the family Fundulidae as well as other livebearers contributed to the top 70% of similarity between assemblages where *F. jenkinsi* occurred while more pelagic species from the families Engraulidae, Clupeidae, and Sciaenidae compromised the top 70% of similarity between assemblages where *F. jenkinsi* did not occur (Table 5).

**Table 5. Species contributing to the majority of similarity in assemblages containing *F. jenkinsi* and assemblages not containing *F. jenkinsi* for Sabine Lake and Galveston Bay systems based on similarity percentages analysis (SIMPER). Species are listed in order of their contribution to the average similarity with a cutoff when cumulative percent contribution approaches about 70%.**

	Sabine Lake Sites		Galveston Bay Sites	
	Species	Percent Contribution (%)	Species	Percent Contribution (%)
<b>Sites <i>F. jenkinsi</i> Collected</b>	<i>Fundulus grandis</i>	15.9%	<i>Cyprinodon variegatus</i>	29.9%
	<i>Poecilia latipinna</i>	15.5%	<i>Fundulus grandis</i>	21.3%
	<i>Fundulus pulvereus</i>	12.4%	<i>Poecilia latipinna</i>	12.4%
	<i>Cyprinodon variegatus</i>	11.3%	<i>Adinia xenica</i>	12.3%
	<i>Gambusia affinis</i>	11.2%		
	<i>Menidia beryllina</i>	8.2%		
		<b>Total 74.5 %</b>		<b>Total 75.9 %</b>
<b>Sites <i>F. jenkinsi</i> Not Collected</b>				
	<i>Menidia beryllina</i>	28.8%	<i>Menidia beryllina</i>	28.4%
	<i>Anchoa mitchilli</i>	27.4%	<i>Anchoa mitchilli</i>	11.9%
	<i>Brevoortia patronus</i>	7.9%	<i>Leiostomus xanthurus</i>	11.5%
	<i>Leiostomus xanthurus</i>	6.7%	<i>Mugil cephalus</i>	10.7%
	<i>Micropogonias undulatus</i>	5.9%	<i>Cyprinodon variegatus</i>	8.1%
		<b>Total 76.7 %</b>	<i>Brevoortia patronus</i>	7.2%
			<b>Total 77.8 %</b>	

A two-way crossed ANOSIM on site assemblages from Sabine Lake showed that assemblages differed significantly between all seasonal groups (Global R= 0.468;  $p=0.001$ ) and that assemblages where *F. jenkinsi* were present differed significantly from assemblages where *F. jenkinsi* were not found to be present across all seasons (Global R= 0.262;  $p=0.001$ ). 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= 0.227;  $p=0.021$ ). Non-metric multidimensional scaling (NMDS) 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 almost exclusively during the winter and fall within both bay systems (Figure 13).

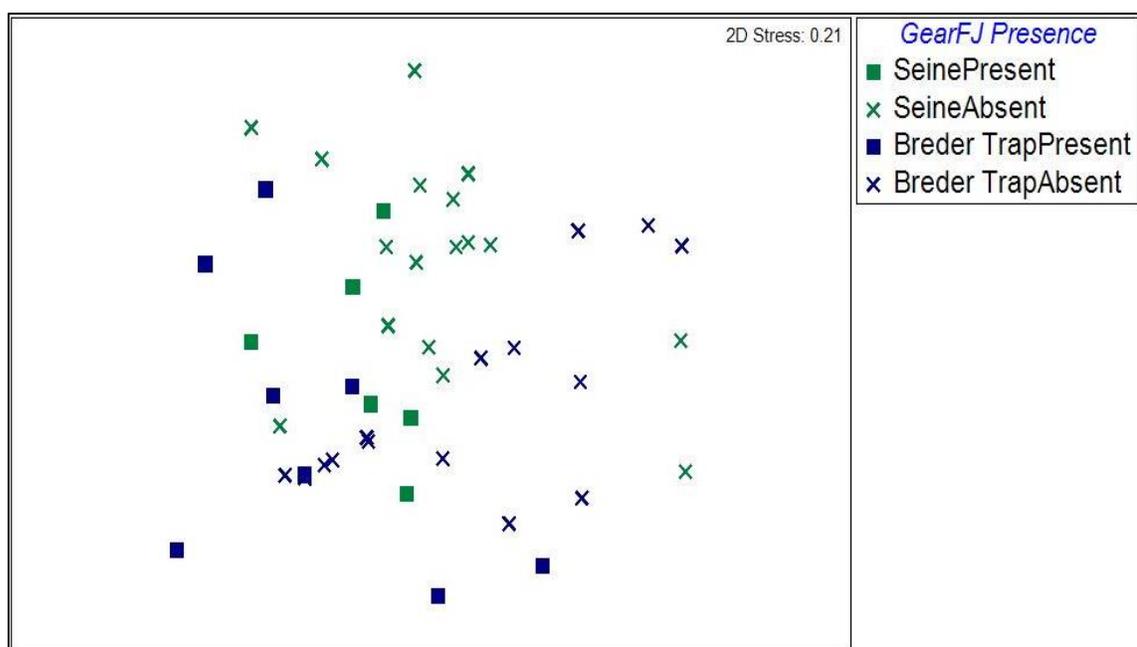


**Figure 13** NMDS plot illustrating the similarity of fish assemblages at sites where *F. jenkinsi* were collected versus not collected by season sampled for (A) Sabine Lake and (B) Galveston Bay. Distance between points represents similarity of site assemblages. Assemblages are labeled by season (color) and *F. jenkinsi* occurrence (shape). Filled triangles represent assemblages containing *F. jenkinsi* while crosses represent assemblages they are lacking from.

The results of a two-way ANOSIM on fish assemblages at sites within Sabine Lake failed to detect any differences in fish assemblages between tidal stages (Global R= 0.052;  $p= 0.209$ ) and that assemblages where *F. jenkinsi* were present did not differ significantly from assemblages where *F. jenkinsi* were not found across all tidal stage groups (Global R= 0.045;  $p= 0.265$ ). Galveston Bay fish assemblages also did not show a significant difference in composition across tidal stages (Global R= 0.040;  $p= 0.158$ ) or a significant difference when *F. jenkinsi* was present or absent across tidal stage groups (Global R= 0.099;  $p= 0.173$ ).

### **Gear Collection**

*Fundulus jenkinsi* were found in fish assemblages sampled by both seines and Breder traps (Figure 14). A one-way ANOSIM showed that fish assemblages evaluated for the influence of capture methods did not significantly differ from each other by bay system (Global R= 0.030;  $p= 0.373$ ). For this reason, data from both bay systems was pooled together for subsequent analyses.



**Figure 14** NMDS plot illustrating the similarity of fish assemblages at sites where *F. jenkinsi* were collected versus not collected pooled from both gear types and bay systems. Distance between points represents similarity of site assemblages. Assemblages are labeled by capture method (color) and *F. jenkinsi* occurrence (shape). Filled squares represent assemblages containing *F. jenkinsi* while stars represent collection where they were not collected.

One-way ANOSIM showed that fish assemblages did differ by collection method (Global R= 0.195;  $p=0.001$ ). A subsequent two-way crossed ANOSIM showed a significant difference in fish assemblages across both gear types, regardless of *F. jenkinsi* presence, (Global R= 0.238;  $p= 0.001$ ) but not a significant difference in *F. jenkinsi* presence across both gear types (Global R= 0.061;  $p= 0.184$ ). Additional similarity percentage analysis (SIMPER) was done and revealed that Breder trap and seine assemblages had an average dissimilarity of 80.8%. Assemblages within the Breder trap grouping had an average similarity of 22.8% and that approximately the top 70% of this similarity was contributed by mostly edge species like *P. latipinna*, *A. xenica*, *F. grandis*, *C. variegatus*, as well as *M. cephalus*. Meanwhile, assemblages within the seine grouping possessed an average similarity of 29.3% with more pelagic species like *M. beryllina*, *A.*

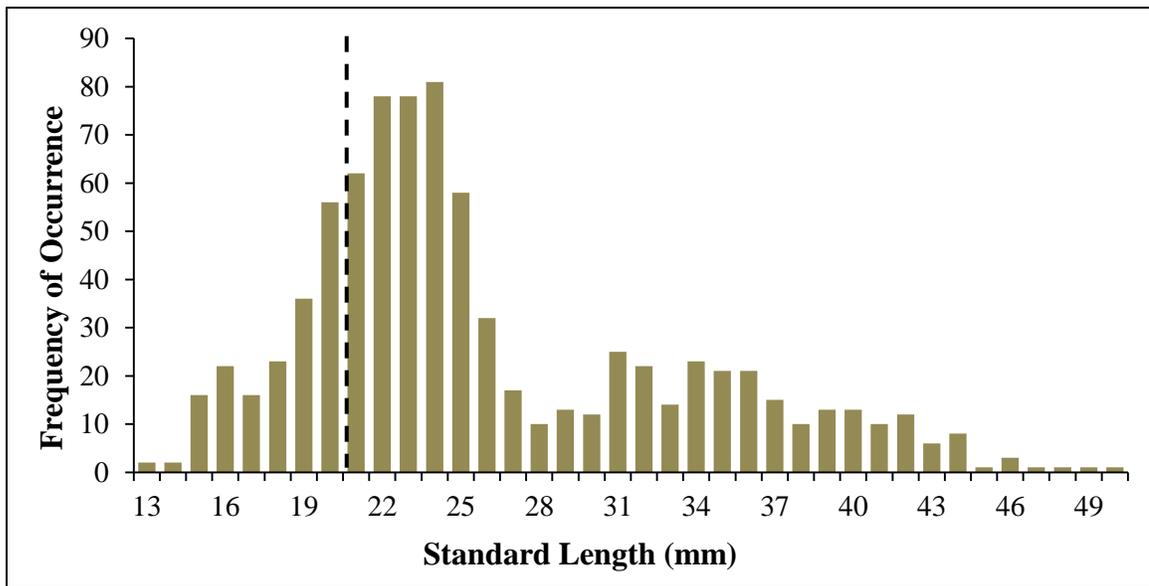
*mitchilli*, *M. cephalus*, and *L. xanthurus* as well as edge species like *P. latipinna* and *C. variegatus* contributing to about the top 70% of this similarity.

The range of *F. jenkinsi* standard lengths collected with seines was 13-50 mm in contrast a range of 18-42 mm captured by Breder traps. A Mann-Whitney U-test revealed that the median standard length of *F. jenkinsi* did not vary significantly by gear type (median = 24 mm;  $U = 3316$ ;  $p = 0.968$ ).

## **Life History Characteristics**

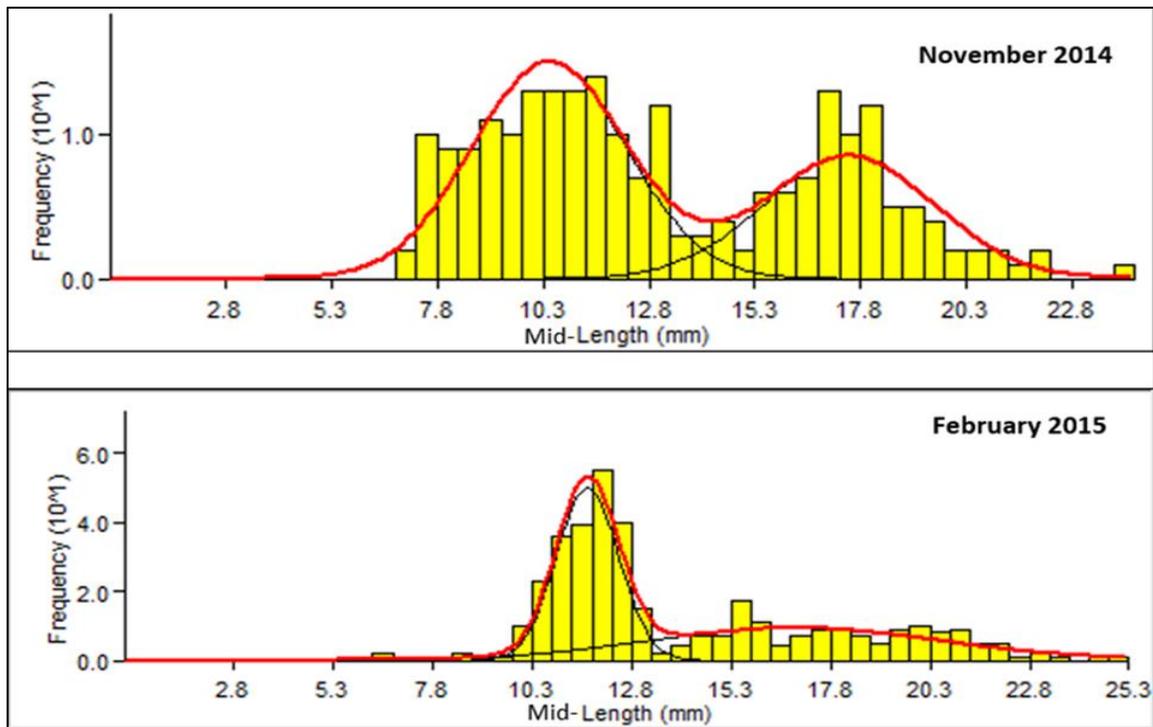
### *Size Distribution*

Standard lengths frequencies of *F. jenkinsi* individuals were pooled from both collection methods and shown to not be normality distributed within either bay system ( $p < 0.01$ ). Kruskal Wallis H-tests revealed that the median length frequencies did not significantly differ by bay system ( $H = 0.37$ ;  $p = 0.545$ ), season ( $H = 6.36$ ;  $p = 0.095$ ), or sex ( $H = 1.069$ ,  $p = 0.194$ ). For this reason, standard length measurements from all *F. jenkinsi* caught were pooled by bay system and collection methods to graph the overall distribution of length frequencies (Figure 15).



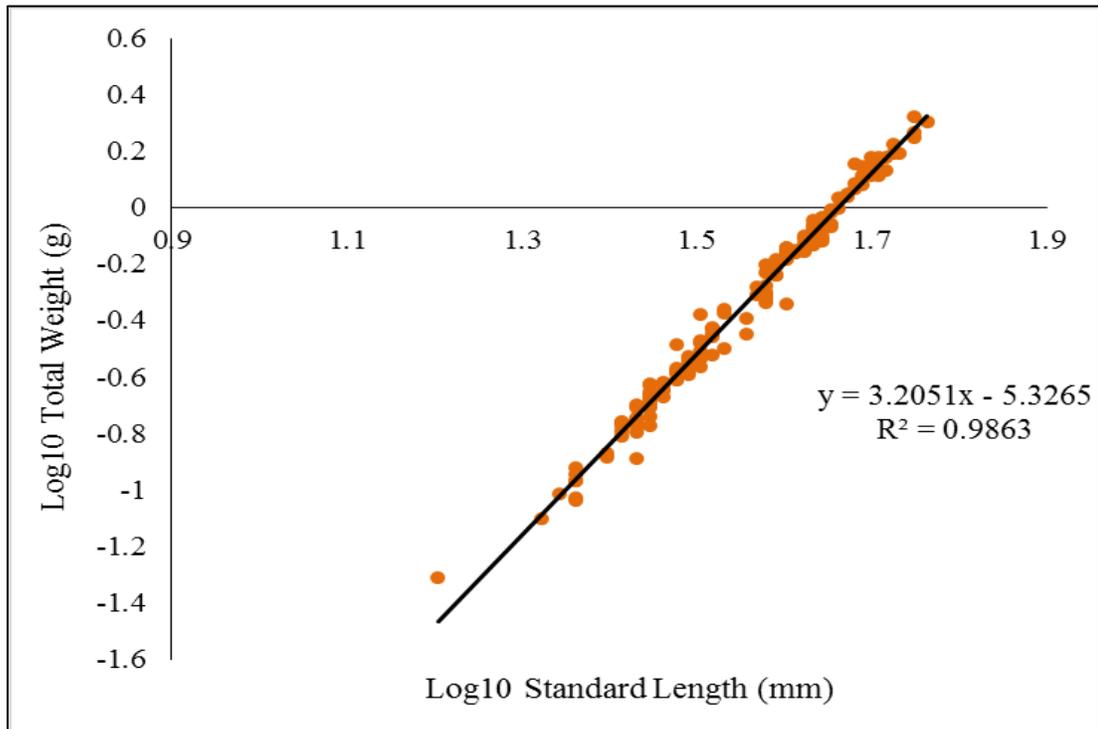
**Figure 15 Standard length (mm) frequency of all *F. jenkinsi* individuals collected from all sampling events across all bays, seasons, and sexes. Dotted vertical line represents the size break between juveniles and adults as defined by (Ross 2001).**

A Kruskal-Wallis H-test revealed that standard length frequencies did significantly differ between months ( $H= 144.8, p<0.001$ ) and monthly length frequencies were run through NORMSEP's method of modal progression analysis. Due to a low sample sizes within most months, effective analysis utilizing length frequency data to estimate relative age classes was unable to be conducted for most months. 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 evidence of at least two distinct age classes (Figure 16). Modal class sizes for November occurred at 20.8 mm ( $\pm 3.5$  SD) and 35.0 mm ( $\pm 4.0$  SD). February's modal classes followed a very similar pattern with size classes centered at 23.3 mm ( $\pm 1.6$  SD) and 33.7 mm ( $\pm 7.3$  SD).



**Figure 16** Length frequency histograms for the month of November 2014 and February 2015 with approximate age classes outlined (red line).

A Kruskal-Wallis H-test revealed that median total body weight of *F. jenkinsi* did not significantly differ between genders ( $H= 1.84$ ;  $p= 0.175$ ). Data was therefore pooled from both sexes to plot the association of *F. jenkinsi* length and total weight (Figure 17). The best-fit model ( $r^2 = 0.9863$ ) for the relationship between length and weight from the data collected was the linear equation of:  $\text{Log}_{10} W = 3.20511 \text{ Log}_{10} \text{SL} + \text{Log}_{10} (-5.3265)$ .



**Figure 17** Scatter plot of  $\text{Log}_{10}$  *F. jenkinsi* standard length (mm) versus  $\text{Log}_{10}$  total body weight (grams). Each point represents data from one individual.

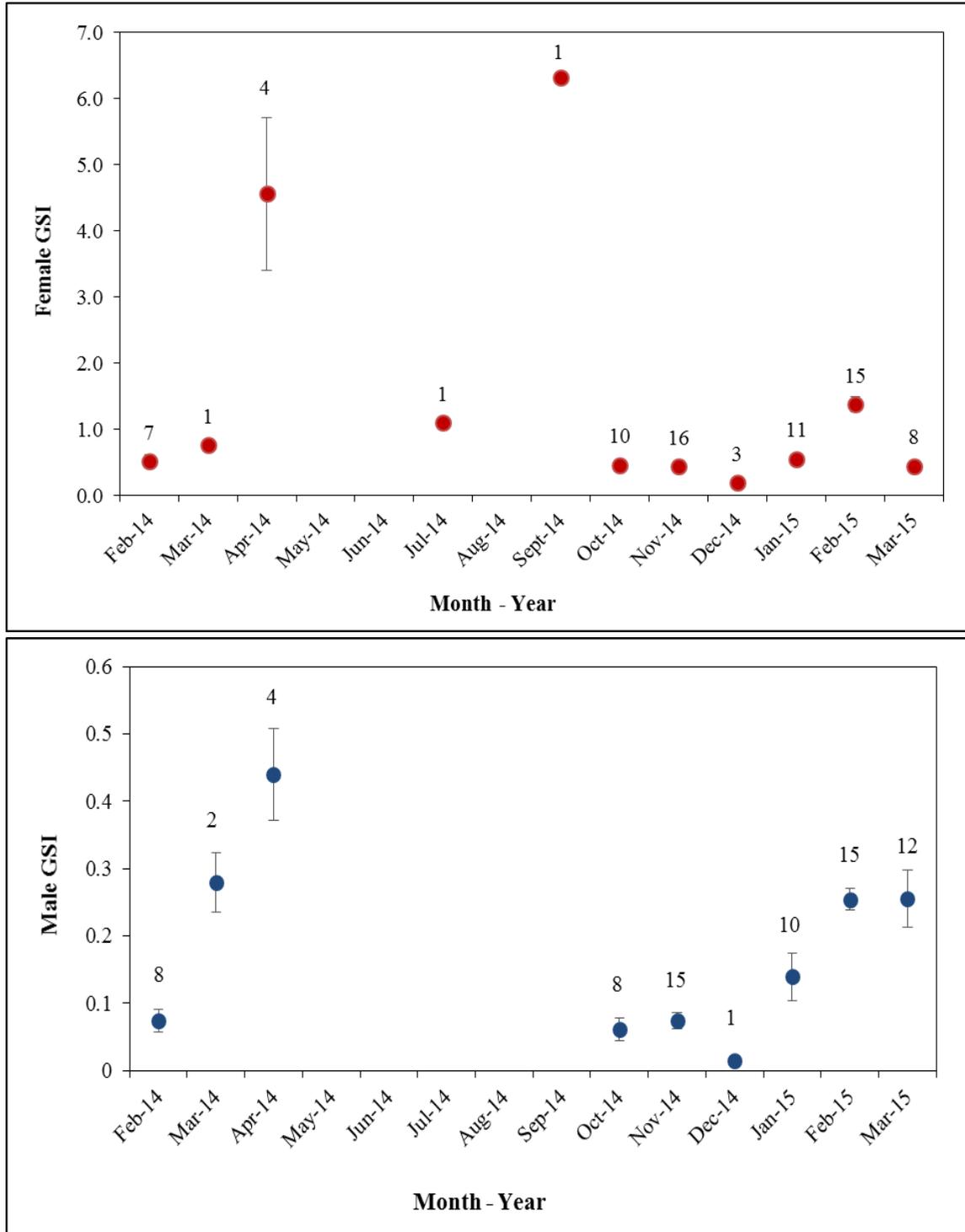
### *Reproduction*

During monthly sampling, *Fundulus jenkinsi* were collected every month except for the months of May, June, and August. 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 sample size varied greatly between months.

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

Year	Month	Total (N)	Mean SL (mm) $\pm$ 1 SD	Min – Max SL (mm)	Mean TW (g) $\pm$ 1 SD	Min. – Max TW (g)
2014	February	15	23.5 $\pm$ 1.0	20 - 32	0.2 $\pm$ 0.1	0.1 – 0.5
	March	3	29.3 $\pm$ 4.2	26 - 34	0.4 $\pm$ 0.2	0.3 – 0.7
	April	8	32.4 $\pm$ 5.8	26 - 44	0.6 $\pm$ 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 $\pm$ 7.3	17 - 37	0.4 $\pm$ 0.3	0.1 – 1.4
	November	31	35.5 $\pm$ 6.7	25 - 47	0.8 $\pm$ 0.4	0.1 – 1.5
	December	4	18.3 $\pm$ 4.2	14 - 22	0.1 $\pm$ 0.03	0.05 – 0.1
2015	January	21	29.4 $\pm$ 5.1	23 - 42	0.5 $\pm$ 0.3	0.2 – 0.9
	February	30	40.6 $\pm$ 4.7	31 - 50	1.2 $\pm$ 0.4	0.7 – 2.0
	March	20	25.1 $\pm$ 5.2	23 - 46	0.3 $\pm$ 0.3	0.2 – 1.7

Elevated GSI values were observed during April and September for females and February-April for males (Figure 18). 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 (Figure 18). One-way ANOVA revealed that mean GSI values significantly differed across months for both females ( $F_{76} = 31.58$ ;  $p < 0.001$ ) and males ( $F_{74} = 13.11$ ;  $p < 0.001$ ).

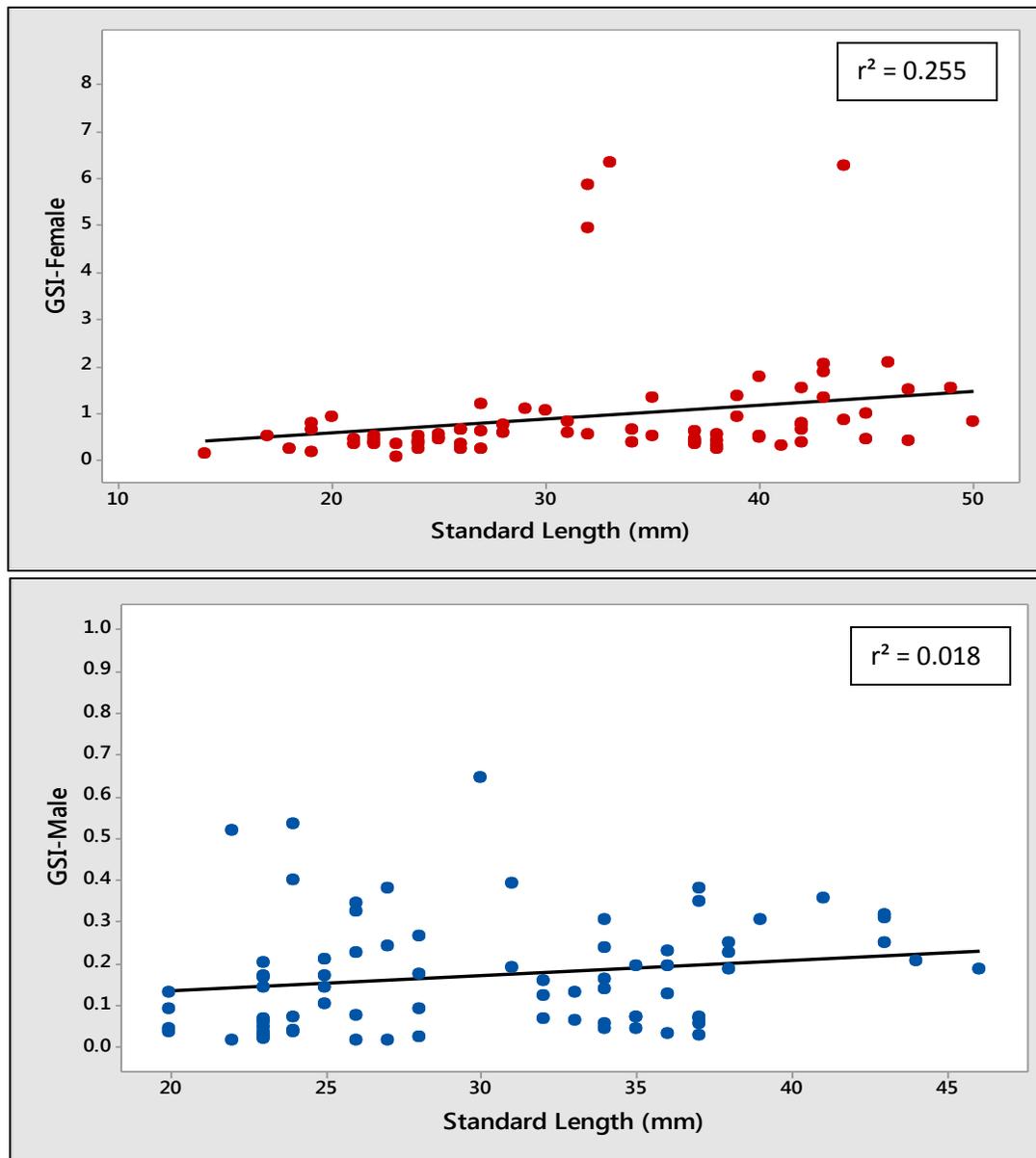


**Figure 18** Plot of female (above) and male (below) mean gonadosomatic index (GSI) by month for *F. jenkinsi*. Bars represent  $\pm 1$  SE. Numbers above data points represent that month's sample size (n). In some months SE bar values are very small and consequently hidden by the data point.

Subsequent post hoc Fisher LSD analysis revealed several significant groupings of monthly mean GSI values for both females and males that are listed fully in Appendix B along with each pairwise comparisons. September 2014 female GSI value was significantly higher than all other months (all  $p$ -values < 0.001). April 2014 female GSI value was the next highest value and statistically significant from all other months (all  $p$ -values < 0.005). April 2014 male mean GSI value was significantly greater than all other months (all  $p$ -values < 0.05). Male mean GSI values for the months of March 2014, February 2015, and March 2015 contained the next significantly highest grouping (all  $p$ -values < 0.05). The third grouping of significantly different monthly mean GSI values consisted of the months of February 2014, October 2014, November 2014, December 2014, and January 2015 (all  $p$ -values < 0.05).

GSI values were pooled by season for each gender and mean GSI values significantly differed by season for both females ( $F_{76} = 8.36; p < 0.001$ ) and males ( $F_{74} = 22.4; p < 0.001$ ). Fisher's LSD post hoc revealed that females had the significantly highest ranked mean GSI values in summer ( $\bar{x} = 3.71$ ; all  $p$ -values < 0.05) followed by spring ( $\bar{x} = 1.73$ ; all  $p$ -values < 0.05). Female fall and winter GSI means (fall = 0.45; winter = 0.85) did not significantly differ from each other ( $p$ -value = 0.153) but were both significantly lower than the spring or summer seasons (all  $p$ -values < 0.05). Fisher pairwise comparisons for male seasonal GSI values showed that all male mean seasonal GSI values significantly differed from each other (all  $p$ -values < 0.001) with the spring season containing the highest GSI values for males ( $\bar{x} = 0.30$ ) followed by winter ( $\bar{x} = 0.17$ ) and then fall ( $\bar{x} = 0.07$ ). Detailed results of Fisher LSD pairwise comparison data for seasonal GSI values for both genders are presented in Appendix B.

Regression analysis revealed that body weight of both males and females was not significantly correlated to GSI value (Females  $R^2=0.026$ ,  $p= 0.085$ ; Males  $R^2=0.022$ ,  $p= 0.108$ ). However, while male standard length did not correlate significantly to GSI value ( $R^2=0.180$ ,  $p= 0.122$ ) female standard length did show a significant positive correlation to GSI value ( $R^2=0.255$ ,  $p= 0.025$ ) (Figure 19).



**Figure 19** Regression plot of female (above) and male (below) standard lengths against GSI value for *F. jenkinsi*.

The resulting sample sizes classified into each ovarian phase were latent (n= 37), early maturing (n= 24), late maturing (n= 12), mature (n= 2), and ripe (n= 2). 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 positive relationship to ovary phase. Ovary maturation phase increased with larger standard length values as well as increased with greater GSI values. Ovarian phases were pooled by season (Figure 20) in order to assess temporal shifts in the reproductive activity of *F. jenkinsi*. Ovarian development showed signs of seasonal progression. Ripe and mature ovaries were found exclusively in the spring and summer seasons with fall and winter seasons being composed mostly of early maturing or latent ovaries. Also, the percentage of early maturing ovaries steadily decreased from fall to summer.

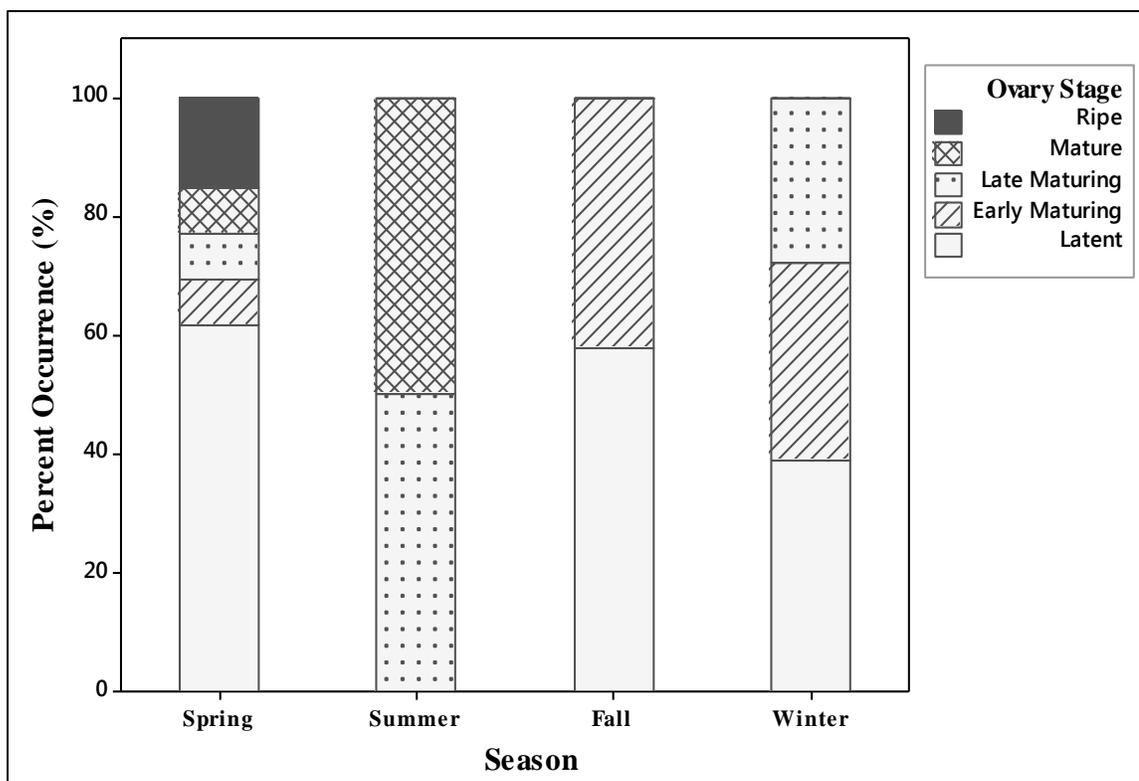


Figure 20 Seasonal (spring n= 13, summer n= 2, fall n= 26, and winter n= 36) percentage of occurrence for ovarian phases of female *F. jenkinsi*.

## *Diet*

The content of *F. jenkinsi* intestinal tracts was found to consist of an array of material spanning both the biotic and abiotic spectra. Gut contents were composed of four different phyla, at least five different classes, and over nine orders (Table 7). These taxa involved mainly members from Phylum Arthropoda and consisted of both aquatic and terrestrial organisms. There was also a variety of unconventional items including vegetative debris, sand or grit, and microplastics. The total percent occurrence and abundance of each of these contents were found (Table 7) and are further outlined below. The most frequently seen items, as well as the one that contributed the most to the total percent composition were from suborder Nematocera (mosquitoes and midges), order Collembola (springtails), and order Amphipoda (amphipods) (Table 7).

**Table 7. Percent total occurrence and percent total composition of gut content pooled from all individuals (n= 107).**

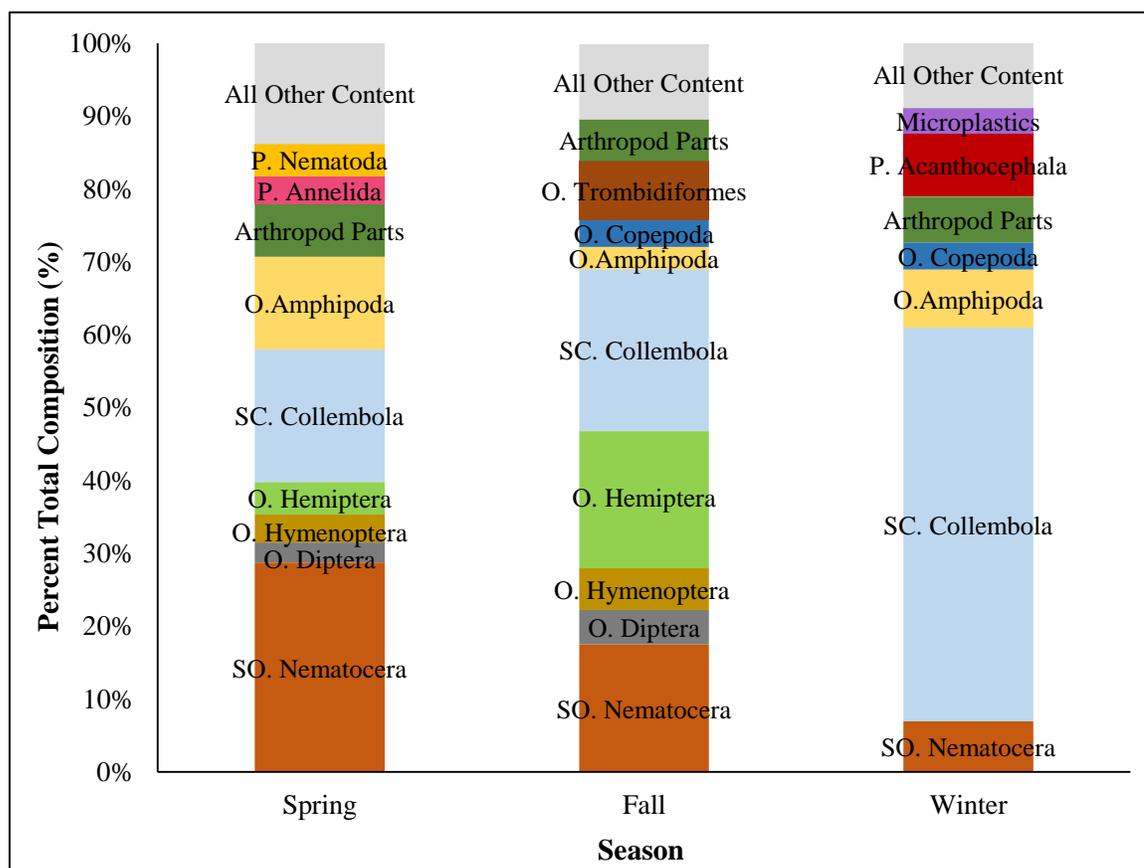
<b>Gut Content</b>	<b>Percent Total Occurrence (%)</b>	<b>Percent Total Composition (%)</b>
Phylum Nematoda	7.5%	1.3%
Phylum Acanthocephala	6.5%	4.5%
Phylum Annelida	2.8%	0.7%
Phylum Arthropoda		
Subphylum Chelicerata		
Class Arachnida		
Order Araneae	9.3%	1.1%
Order Trombidiformes	9.3%	3.1%
Subphylum Crustacea		
Class Maxillopoda		
Order Copepoda	9.3%	3.3%
Class Malacostraca		
Order Amphipoda	33.6%	7.1%
Order Isopoda	6.5%	1.6%
Subphylum Hexapoda		
Class Entognatha		
Subclass Collembola	36.4%	36.0%
Class Insecta		
Order Hemiptera	17.8%	6.8%
Order Orthoptera	0.9%	0.1%
Order Hymenoptera	16.8%	2.5%
Order Diptera	18.7%	4.6%
Suborder Nematocera	43.9%	14.0%
Arthropod Parts	42.1%	6.4%
Unidentified Plankton	5.6%	0.9%
Detritus & Vegetation Debris	21.5%	3.5%
Sand / Grit	2.8%	0.3%
Microplastics	15.0%	2.2%

Regression analysis failed to detect a significant relationship between total number of gut items and standard length ( $R^2= 0.0$ ;  $p$ -value= 0.535) or weight ( $R^2= 0.0$ ;  $p$ -values= 0.593). A two sample T-test also revealed that there was no significant difference in mean total gut items between females ( $10.3\pm 1.6$  SE) and males ( $7.3\pm 1.4$  SE) ( $t_{103}= 1.39$ ;  $p$ -value= 0.166). The mean species diversity ( $H'$ ) of gut content for females ( $\bar{x} = 0.921$ ) did not significantly differ from the mean species diversity of gut content for males ( $\bar{x} = 0.815$ ) ( $t_{101} = 1.05$ ;  $p = 0.299$ ).

Only two individuals were available for analysis for the summer season and were therefore omitted from seasonal analysis. A one-way ANOVA did not show a significant difference in total gut items across seasons ( $F_{1,107} = 1.22$ ;  $p$ -value= 0.301). A One-way ANOVA did show that mean diversity ( $H'$ ) of gut contents did significantly differ across seasons ( $F_{1,103} = 6.79$ ;  $p = 0.002$ ). A post hoc Fisher LSD showed that spring ( $\bar{x} = 1.03$ ) and fall ( $\bar{x} = 1.01$ ) mean diversity values did not significantly differ from each other ( $p$ -value = 0.871) but were both significantly higher than winter ( $\bar{x} = 0.66$ ; all  $p$ -values < 0.005). The seasonal composition and variation of gut contents across seasons are depicted in Figure 21 from which several seasonal variations can be seen. Only unidentified Arthropod parts occurred in relatively equal percentages across all seasons. All other content classes were either not found across all seasons or occurred in highly variable different proportions across seasons (Figure 21).

A greater variety of contents are found in the spring and fall season than in the winter, reflects the higher diversity ( $H'$ ) of species found. The percentage of gut items represented by the suborder Nematocera (mosquitoes and midges) steadily decreased from spring to winter while an inverse trend was observed for the subclass Collembola

(springtails). The percentage composition of order Hemiptera (true bugs), Hymenoptera (wasps, bees, ants), and Diptera (flies) also appear to increase from spring to fall but are found in extremely low numbers during the winter. Worms from the phyla Nematoda, Annelida, and Acanthocephala only occurred in moderate abundance during the spring and winter but declined to very low numbers during the fall, while an inverse trend was observed for organisms of order Trombidiformes (mites). Also, while microplastics were found to occur across all seasons they were found in relatively greater abundance during the winter.



**Figure 21** Percent total composition (%) of major gut content groups for each season (spring n= 29; fall n= 31; and winter n= 45).

A one-way ANOSIM on individual gut contents showed that content did not vary significantly between genders (Global  $R = -0.014$ ;  $p = 0.894$ ) but did significantly differ across seasonal groups (Global  $R = 0.087$ ;  $p = 0.001$ ). Based on results of similarity percentage analysis (SIMPER) it was found that Saltmarsh Topminnow collected in the spring possessed an average similarity of 27.78% with prey items consisting of suborder Nematocera, order Amphipoda, Arthropod parts, and subclass Collembola contributing to the top 80% of this similarity. Gut content of individual fish examined from fall had an average similarity of 21.76% with arthropod parts, subclass Collembola, order Diptera, order Hymenoptera, suborder Nematocera, and order Hemiptera cumulatively representing the top 80% of this similarity. Finally, winter fish gut contents exhibited an average similarity of 25.66% with subclass Collembola, Arthropod parts, suborder Nematocera, and order Amphipoda cumulatively contributing to the top 80% of this similarity.

Similarity percentage analysis (SIMPER) also estimated the average dissimilarity of gut contents across seasonal groups. Based on this analysis spring and fall groups exhibited an average dissimilarity of 78.48%, while fall and winter had an average dissimilarity of 80.83%, and spring and winter had an average dissimilarity of 76.95%. A complete description of the contribution of each gut content category to the dissimilarity between seasons is provided in Appendix C.

## DISCUSSION

### **Distribution and Abundance**

Based on the results of this study the existence of a decreasing east to west gradient of *F. jenkinsi* density based on geographic location was observed within the study area. Not only did a greater percentage of sites contain *F. jenkinsi* in Sabine Lake compared to Galveston Bay (26% vs. 14%) but *F. jenkinsi* were found, on average, in greater numbers as well (12 vs. 5) (Table 2). This decline in *F. jenkinsi* frequency and abundance from east to west was seen within each bay system as well. Sites sampled along the Sabine River had a higher frequency of *F. jenkinsi* presence (50% vs. 42%) as well as a higher number of average individuals found per site (20 vs. 5) when compared to the more western sites sampled along the Neches River (Table 2). Likewise, within Galveston Bay the frequency of sites where *F. jenkinsi* were collected decreased from 50% (Trinity Bay) to 21% (Dickinson and Moses Bayous) with the average number of individual *F. jenkinsi* caught per site decreasing as well (Table 2).

Past historical records (Akin et al. 2003; Nicolau 2001; Martin et al. 2012) indicate that *Fundulus jenkinsi* may continue to be found sporadically in extremely small numbers west of the Galveston Bay estuary but these are more than likely small fragmented local populations. Galveston Bay represents the reported western most extent of this species' known range (Thompson 1991; Hoese & Moore 1998) and the data from this study confirms that *Fundulus jenkinsi* most likely decreases in both occurrence and abundance to spatially sporadic and small isolated groups as one travels further west. However, further surveys are needed in these locations and adjacent estuarine wetlands

where *Fundulus jenkinsi* have been collected including Cedar Lakes Creek and portions of Matagorda Bay and San Antonio Bay, the western most cited occurrence of *F. jenkinsi* (Akin et al. 2003; Nicolau 2001), to confirm this hypothesis.

Another potential explanation for the observed east to west gradient in *Fundulus jenkinsi* density may be due to the fact that Galveston Bay is significantly more developed and contains less undisturbed habitat than in Sabine Lake. As a consequence of habitat fragmentation and loss of wetlands in the western portion of Galveston Bay local populations of *Fundulus jenkinsi* have become disconnected and more isolated from each other and the lack of habitat corridors needed to expand and colonize suitable wetland habitat. This is in contrast to the Sabine Lake area which contains large extensive and continuous wetlands extending along the eastern shoreline and to a lesser extent the western portion of the watershed. These extensive wetlands sustain suitable saltmarsh habitat and tidal creek corridors for expansion and migration of saltmarsh topminnow within the system.

It is possible that the occurrences and numbers of this species were under represented in this study due to the difficulty in accurately sampling this type of habitat. For example, 293 specimens were captured during one seine haul in February 2015 during monthly sampling. The high catch rate is attributed to the extremely low water levels at the time of sampling which had forced fish and other marsh nekton into a highly concentrated mass within a single disconnected pool. In contrast, most of the locations lacking *Fundulus jenkinsi* occurred at sites sampled during the summer when water levels were highest, allowing small nekton to seek refuge in higher elevation inundated marsh vegetation therefore evading our standard sampling gear. This would effectively produce

a high false negative (zero catch; absence) rate. Evidence for this is further supported by the fact that *F. jenkinsi* were only captured in the fall and winter months during quarterly sampling (Figure 13) despite them being considered year-long estuarine residents (Neill & Turner 1987). 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 do in fact lack *F. jenkinsi*. In addition, the use of throw traps or high marsh net pens might aid in capturing organisms during high water events.

### **Habitat Characteristics**

This study supports previous literature which states that *F. jenkinsi* prefer lower to mid salinity ranges (4-20ppt) (Peterson & Ross 1991; Lopez et al. 2011; and Griffith 1974). During the course of this study *F. jenkinsi* were collected within similar salinity ranges for Sabine Lake and Galveston Bay (Figure 8). The mean catch of *F. jenkinsi* did differ between systems with Sabine Lake having a higher number of *F. jenkinsi* in lower salinities than Galveston Bay (Figure 8). This difference in part is most likely due to the differing degree of freshwater inflow and resulting salinity for each bay 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* are able to inhabit a wide salinity range (2-20 ppt) it is equally important to have appropriate marsh habitat and migration corridors available along the entirety of the existing salinity gradients.

Median water temperatures were significantly lower at sites where *F. jenkinsi* were collected versus not collected. In addition higher numbers of *F. jenkinsi* were

collected in lower temperatures. It can be assumed that the significantly lower temperatures at sites where *F. jenkinsi* were collected were primarily due to the seasonality (cooler months) of when *F. jenkinsi* were captured which in turn was correlated with lower water levels and less saltmarsh inundation. As noted earlier, low water levels resulted in easier and more efficient seine collections. This is supported by the fact that there was a significant decline in MLLW where *F. jenkinsi* were found vs. not found ( $p < 0.001$ ) within Sabine Lake. Also, all *F. jenkinsi* individuals were captured when MLLW levels were below 0.4m and were absent at sites when water levels exceeded  $> 0.4$  to 0.7m MLLW (Figure 11).

The association of occurrences and higher catches with lower temperatures may also be due to the interaction and influence of water temperature on *F. jenkinsi* physiology and gear avoidance. Cold weather typically slows down the metabolism of poikilothermic temperate estuarine species (Clarke & Johnson 1999) which causes a decline in swimming activity and gear avoidance. During this study *Fundulus jenkinsi* were found in coastal wetlands composed of various dominant plant species. While previous studies have stressed the strong 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*. This difference in presence across vegetation types might also be a secondary observance of salinity. 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/  $0.25\text{m}^{-2}$ ) (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 density vegetation facilitated more efficient sampling equipment (seining).

### **Fish Community Composition**

Analysis of the fish assemblages caught over the course of this study shows that *F. jenkinsi* closely associate with certain other 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 documented 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 that *F. jenkinsi* did not occur in were dominated by more open water, pelagic species (Table 5) suggesting that the habitat utilized by those species is not the type favored by *F. jenkinsi*.

A higher average number of fish species and diversity ( $H'$ ) 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*. The overall species diversity was relatively low across many sites, attributed to the relatively lower total number of species (richness) across sites. Lower species diversity found among Galveston Bay sites could be a reflection of the greater habitat fragmentation and saltmarsh degradation that has occurred within that system compared to Sabine Lake. It appears that, when available,

*Fundulus jenkinsi* may prefer saltmarsh habitat that is also able to support a higher array of fish species. Low fish diversity may therefore be associated with a lower likelihood of finding *F. jenkinsi* in coastal saltmarshes.

Based on the results of ANOSIM analysis (Figure 13) it is clear that seasonality is a major factor structuring 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 all tide levels influence fish assemblage composition or *F. jenkinsi* presence there is evidence that minimum water levels (MLLW) does have an influence on *F. jenkinsi* capture (Figure 11). This is also a reasonable conclusion since lower water levels enhance the effectiveness of sampling gear. Also, 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 experiences weak lunar induced tidal fluctuation compared to other coastlines it can exhibit large meteorological induced fluctuations (Ward 1980) which in turn influences the amount of saltmarsh inundation, and thus ability to accurately capture *F. jenkinsi*. Along the Texas coast extreme high water levels usually occur during warmer months when prevailing winds are from the southeast, facilitating the movement of water into the estuary. 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 the Gulf coast. During these periods water levels recede to deeper tidal creeks and expose most of the marsh surface causing fish to be much more vulnerable to capture.

### **Gear Collection**

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 (Figure 14). I found that Breder traps 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 (i.e. summer months) seining failed to capture species that are closely associated with marsh edge habitat and tended to capture more open water species (Table 5). 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 this study I 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. I also observed that during times of low water levels and/or little to no inundation or when tidal channel bank slope was steep seining was very effective. During these low water periods we were able to seine the entire creek from bank to bank at a relatively rapid rate at most sites. Conversely Breder traps were less effective as a sampling tool during extended periods of low water 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 since the traps would frequently fall over or were oriented in less than ideal sampling orientation. 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.

### **Life History Characteristics**

The overall length distribution for *F. jenkinsi* consisted of two modal peaks including one at the 20-22 mm (SL) and the second at the 32-34 mm (Figure 15). These peaks correspond with the values estimated by the limited analysis conducted with FISAT II (Figure 16). This data provides supportive preliminary evidence that these two modes of standard length most likely represents at least two separate age classes. Unfortunately, due to low catch rates I lacked the data needed to make inferences about the preliminary growth rate of this species. In addition to length frequency analysis this study provides estimates of size distribution based on total weight and standard length. However, these estimates are biased by the lack of younger (i.e. larval) stage individuals. Smaller younger stages of saltmarsh topminnow were not collected by my gear.

Due to very little data existing on the growth rates of this species makes estimating an individual specimen's age difficult to accomplish. This is due in part to the difficulty of aging individuals and defining class groups. Since *F. jenkinsi* are batch spawners (Lopez et al. 2010) and spawn over a relatively long season (Lang et al. 2012) the size distribution of age classes have the potential to vary greatly as individuals in the same class may have hatched months apart. 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 along the Mississippi and Alabama coast have documented that *F. jenkinsi*'s spawning season extends from March through August and that individuals are capable of spawning multiple times throughout a single season (Lopez et al. 2010; Lang et al. 2012). My study confirms these estimates of reproductive activity for the western edge of *F. jenkinsi*'s range. I found that there is a significant rise in the GSI values for both male and females during the spring and early 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. Despite this limit in data there is an obvious 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. It is important to note that only two females were caught during the summer which due to low sample size, may not be representative of the larger population. Despite the lack of a complete annual data set, our data agrees with and supports previously documented patterns and assumptions regarding *F. jenkinsi* reproduction.

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) individuals. This is not surprising as these older and larger individuals are most often associated with greater reproductive potential in marine bony fishes. We would also expect that ovary phase and GSI values to be

directly related as the size of the ovary and its maturation stage are mutually 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; Lang et al. 2012) that there is a seasonal trend in *F. jenkinsi* reproductive 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.

Analysis of gut content consisted of a wide array of both terrestrial and aquatic invertebrates (Table 7), reflecting Saltmarsh Topminnow's utilization of the edge habitat existing within the inundated saltmarsh vegetation. No significant difference was found in gut content across genders or sizes. A significant difference in gut content composition was found between seasons and several seasonal trends in diet composition can be easily seen (Figure 21). The diversity of gut content items was higher in the spring and fall months in comparison to the winter season. Also, terrestrial arthropods (suborder Nematocera, order Hemiptera, order Hymenoptera, order Diptera) composed a higher proportion of total gut content in the spring and fall while aquatic arthropods (subclass Collembola) existed in higher proportion in the winter. This difference could be a reflection of the difference in productivity between seasons. It could also be influenced by the higher water levels during the warmer months allowing Saltmarsh Topminnow further up into the saltmarsh vegetation and therefore having greater access to the terrestrial invertebrates inhabiting the area there.

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APPENDIXES

**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 February 2014 – March 2015.**

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

Family	Scientific Name	Common Name	Sabine Lake			Galveston Bay			Total N
			S	BT	Sabine Total	S	BT	Galveston Total	
Fundulidae	<i>Adinia xenica</i>	Diamond Killifish	52	5	57	2170	34	2204	<b>2261</b>
	<i>Fundulus chrysotus</i>	Golden Topminnow	0	0	0	2	0	2	<b>2</b>
	<i>Fundulus grandis</i>	Gulf Killifish	480	53	533	1032	37	1069	<b>1602</b>
	<i>Fundulus jenkinsi</i>	Saltmarsh Topminnow	161	0	161	622	9	631	<b>792</b>
	<i>Fundulus pulvereus</i>	Bayou Killifish	117	18	135	429	13	442	<b>577</b>
	<i>Fundulus similis</i>	Longnose Killifish	0	0	0	43	0	43	<b>43</b>
	<i>Lucania parva</i>	Rainwater Killifish	429	3	432	155	0	155	<b>587</b>
Cyprinodontidae	<i>Cyprinodon variegatus</i>	Sheepshead Minnow	1059	86	1145	3657	12	3669	<b>4814</b>
Poeciliidae	<i>Gambusia affinis</i>	Mosquito Fish	892	13	905	655	6	661	<b>1566</b>
	<i>Heterandria formosa</i>	Least Killifish	1	0	1	0	0	0	<b>1</b>
	<i>Poecilia latipinna</i>	Sailfin Molly	1155	113	1268	1699	18	1717	<b>2985</b>
Syngnathidae	<i>Syngnathus louisianae</i>	Chain Pipefish	1	0	1	24	0	24	<b>25</b>
	<i>Syngnathus scovelli</i>	Gulf Pipefish	1	0	1	1	0	1	<b>2</b>
Carangidae	<i>Oligoplites saurus</i>	Leatherjack	1	0	1	21	0	21	<b>22</b>
Lutjanidae	<i>Lutjanus griseus</i>	Grey Snapper	7	0	7	8	0	8	<b>15</b>

Family	Scientific Name	Common Name	Sabine Lake			Galveston Bay			Total N
			S	BT	Sabine Total	S	BT	Galveston Total	
Gerreidae	<i>Eucinostomus argenteus</i>	Spotfin Mojarra	2	0	2	96	0	96	<b>98</b>
	<i>Eucinostomus melanopterus</i>	Flagfin Mojarra	3	0	3	142	2	144	<b>147</b>
Sparidae	<i>Lagodon rhomboides</i>	Pinfish	20	0	20	491	1	492	<b>512</b>
Sciaenidae	<i>Bairdiella chrysoura</i>	Silver Perch	0	0	0	2	0	2	<b>2</b>
	<i>Cynoscion arenarius</i>	Sand Seatrout	22	0	22	38	0	38	<b>60</b>
	<i>Cynoscion nebulosus</i>	Spotted Seatrout	42	0	42	48	3	50	<b>93</b>
	<i>Cynoscion nothus</i>	Silver Seatrout	0	0	0	1	0	1	<b>1</b>
	<i>Larimus fasciatus</i>	Banded Drum	0	0	0	1	0	1	<b>1</b>
	<i>Leiostomus xanthurus</i>	Spot	836	0	836	10765	22	10787	<b>11623</b>
	<i>Micropogonias undulatus</i>	Atlantic Croaker	778	3	781	1244	13	1257	<b>2038</b>
Sciaenidae	<i>Pogonias cromis</i>	Black Drum	1	1	2	0	0	0	<b>2</b>
	<i>Stellifer lanceolatus</i>	Star Drum	3	0	3	5	0	5	<b>8</b>
	<i>Sciaenops ocellatus</i>	Red Drum	29	0	29	192	7	199	<b>228</b>
Centrarchidae	<i>Lepomis miniatus</i>	Redspotted Sunfish	2	0	2	0	0	0	<b>2</b>
	<i>Micropterus salmoides</i>	Largemouth Bass	1	0	1	0	0	0	<b>1</b>
	<i>Lepomis gulosus</i>	Warmouth	1	0	1	0	0	0	<b>1</b>
	<i>Lepomis macrochirus</i>	Bluegill	7	0	7	1	0	1	<b>8</b>

Family	Scientific Name	Common Name	Sabine Lake			Galveston Bay			Total N
			S	BT	Sabine Total	S	BT	Galveston Total	
Eleotridae	<i>Dormitator maculatus</i>	Fathead Sleeper	1	1	2	1	0	1	3
Gobiidae	<i>Ctenogobius boleosoma</i>	Darter Goby	32	0	32	9	0	9	41
	<i>Ctenogobius shufeldti</i>	Freshwater Goby	2	0	2	0	0	0	2
	<i>Gobiosoma bosc</i>	Naked Goby	96	0	96	20	0	20	116
	<i>Gobiosoma robustum</i>	Code Goby	5	0	5	1	0	1	6
	<i>Microgobius gulosus</i>	Clown Goby	13	0	13	7	0	7	20
Ephippidae	<i>Chaetodipterus faber</i>	Atlantic Spadefish	0	0	0	2	0	2	2
Achiridae	<i>Achirus lineatus</i>	Lined Sole	0	0	0	1	0	1	1
Cynoglossidae	<i>Symphurus plagiusa</i>	Blackcheek Tonguefish	1	0	1	8	1	9	10
Tetraodontidae	<i>Sphoeroides parvus</i>	Least Puffer	0	0	0	2	0	2	2

## Appendix B1. Fisher Pairwise Comparisons for Female Monthly GSI Values

Grouping Information Using the Fisher LSD Method and 95% Confidence

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

Means that do not share a letter are significantly different.

Fisher Individual Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Dec-14 - April-14	-4.373	0.424	(-5.219, -3.526)	-10.31	0.000
Feb-14 - April-14	-4.051	0.348	(-4.746, -3.356)	-11.64	0.000
Feb-15 - April-14	-3.189	0.312	(-3.813, -2.566)	-10.21	0.000
Jan-15 - April-14	-4.016	0.324	(-4.663, -3.368)	-12.39	0.000
July-14 - April-14	-3.468	0.621	(-4.707, -2.228)	-5.59	0.000
March-14 - April-14	-3.801	0.621	(-5.040, -2.562)	-6.12	0.000
March-15 - April-14	-4.122	0.340	(-4.801, -3.444)	-12.13	0.000
Nov-14 - April-14	-4.124	0.310	(-4.744, -3.505)	-13.29	0.000
Oct-14 - April-14	-4.100	0.328	(-4.756, -3.444)	-12.48	0.000
Sept-14 - April-14	1.763	0.621	( 0.524, 3.002)	2.84	0.006
Feb-14 - Dec-14	0.322	0.383	(-0.443, 1.087)	0.84	0.404
Feb-15 - Dec-14	1.183	0.351	( 0.482, 1.884)	3.37	0.001
Jan-15 - Dec-14	0.357	0.362	(-0.365, 1.079)	0.99	0.327
July-14 - Dec-14	0.905	0.641	(-0.375, 2.185)	1.41	0.163
March-14 - Dec-14	0.571	0.641	(-0.709, 1.851)	0.89	0.376
March-15 - Dec-14	0.250	0.376	(-0.500, 1.001)	0.67	0.508
Nov-14 - Dec-14	0.248	0.349	(-0.449, 0.946)	0.71	0.480
Oct-14 - Dec-14	0.273	0.365	(-0.457, 1.002)	0.75	0.458
Sept-14 - Dec-14	6.136	0.641	( 4.856, 7.416)	9.57	0.000
Feb-15 - Feb-14	0.862	0.254	( 0.354, 1.369)	3.39	0.001
Jan-15 - Feb-14	0.035	0.268	(-0.501, 0.571)	0.13	0.895
July-14 - Feb-14	0.583	0.594	(-0.602, 1.768)	0.98	0.329
March-14 - Feb-14	0.250	0.594	(-0.935, 1.435)	0.42	0.675
March-15 - Feb-14	-0.071	0.287	(-0.645, 0.502)	-0.25	0.804
Nov-14 - Feb-14	-0.073	0.252	(-0.576, 0.429)	-0.29	0.771
Oct-14 - Feb-14	-0.049	0.274	(-0.595, 0.497)	-0.18	0.859
Sept-14 - Feb-14	5.814	0.594	( 4.629, 6.999)	9.80	0.000
Jan-15 - Feb-15	-0.826	0.220	(-1.266, -0.386)	-3.75	0.000
July-14 - Feb-15	-0.278	0.573	(-1.423, 0.867)	-0.49	0.629
March-14 - Feb-15	-0.612	0.573	(-1.757, 0.533)	-1.07	0.290
March-15 - Feb-15	-0.933	0.243	(-1.418, -0.448)	-3.84	0.000
Nov-14 - Feb-15	-0.935	0.200	(-1.333, -0.537)	-4.69	0.000
Oct-14 - Feb-15	-0.910	0.227	(-1.363, -0.458)	-4.02	0.000
Sept-14 - Feb-15	4.953	0.573	( 3.808, 6.097)	8.64	0.000
July-14 - Jan-15	0.548	0.580	(-0.610, 1.706)	0.94	0.348
March-14 - Jan-15	0.214	0.580	(-0.943, 1.372)	0.37	0.713
March-15 - Jan-15	-0.107	0.258	(-0.622, 0.408)	-0.41	0.680
Nov-14 - Jan-15	-0.109	0.217	(-0.543, 0.325)	-0.50	0.619
Oct-14 - Jan-15	-0.084	0.243	(-0.569, 0.400)	-0.35	0.729
Sept-14 - Jan-15	5.779	0.580	( 4.621, 6.936)	9.97	0.000

March-14 - July-14	-0.334	0.785	(-1.901, 1.234)	-0.42	0.672
March-15 - July-14	-0.655	0.589	(-1.831, 0.521)	-1.11	0.270
Nov-14 - July-14	-0.657	0.572	(-1.799, 0.486)	-1.15	0.255
Oct-14 - July-14	-0.632	0.582	(-1.795, 0.530)	-1.09	0.281
Sept-14 - July-14	5.231	0.785	( 3.663, 6.798)	6.66	0.000
March-15 - March-14	-0.321	0.589	(-1.497, 0.854)	-0.55	0.587
Nov-14 - March-14	-0.323	0.572	(-1.466, 0.819)	-0.56	0.574
Oct-14 - March-14	-0.299	0.582	(-1.461, 0.864)	-0.51	0.610
Sept-14 - March-14	5.564	0.785	( 3.997, 7.132)	7.09	0.000
Nov-14 - March-15	-0.002	0.240	(-0.482, 0.478)	-0.01	0.994
Oct-14 - March-15	0.023	0.263	(-0.503, 0.548)	0.09	0.932
Sept-14 - March-15	5.886	0.589	( 4.710, 7.061)	9.99	0.000
Oct-14 - Nov-14	0.024	0.224	(-0.422, 0.471)	0.11	0.913
Sept-14 - Nov-14	5.887	0.572	( 4.745, 7.030)	10.29	0.000
Sept-14 - Oct-14	5.863	0.582	( 4.700, 7.026)	10.07	0.000

Simultaneous confidence level = 34.82%

## Appendix B2. Fisher Pairwise Comparisons for Male Monthly GSI Values

Grouping Information Using the Fisher LSD Method and 95% Confidence

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

Means that do not share a letter are significantly different.

Fisher Individual Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Dec-14 - April-14	-0.425	0.100	( -0.625, -0.224)	-4.23	0.000
Feb-14 - April-14	-0.3649	0.0550	(-0.4747, -0.2551)	-6.64	0.000
Feb-15 - April-14	-0.1851	0.0505	(-0.2859, -0.0842)	-3.66	0.000
Jan-15 - April-14	-0.3002	0.0531	(-0.4063, -0.1941)	-5.65	0.000
March-14 - April-14	-0.1599	0.0778	(-0.3151, -0.0046)	-2.06	0.044
March-15 - April-14	-0.1847	0.0518	(-0.2882, -0.0812)	-3.56	0.001
Nov-14 - April-14	-0.3650	0.0505	(-0.4659, -0.2642)	-7.22	0.000
Oct-14 - April-14	-0.3783	0.0550	(-0.4881, -0.2685)	-6.88	0.000
Feb-14 - Dec-14	0.0598	0.0952	( -0.1303, 0.2500)	0.63	0.532
Feb-15 - Dec-14	0.2397	0.0927	( 0.0545, 0.4248)	2.58	0.012
Jan-15 - Dec-14	0.1245	0.0942	(-0.0635, 0.3125)	1.32	0.191
March-14 - Dec-14	0.265	0.110	( 0.045, 0.484)	2.41	0.019
March-15 - Dec-14	0.2400	0.0935	( 0.0534, 0.4266)	2.57	0.012
Nov-14 - Dec-14	0.0597	0.0927	(-0.1255, 0.2448)	0.64	0.522
Oct-14 - Dec-14	0.0464	0.0952	(-0.1438, 0.2365)	0.49	0.628
Feb-15 - Feb-14	0.1798	0.0393	( 0.1013, 0.2583)	4.57	0.000
Jan-15 - Feb-14	0.0647	0.0426	(-0.0203, 0.1497)	1.52	0.134
March-14 - Feb-14	0.2050	0.0710	( 0.0633, 0.3467)	2.89	0.005
March-15 - Feb-14	0.1802	0.0410	( 0.0984, 0.2620)	4.40	0.000
Nov-14 - Feb-14	-0.0002	0.0393	(-0.0786, 0.0783)	-0.00	0.997
Oct-14 - Feb-14	-0.0134	0.0449	(-0.1031, 0.0762)	-0.30	0.766
Jan-15 - Feb-15	-0.1151	0.0367	(-0.1883, -0.0419)	-3.14	0.003
March-14 - Feb-15	0.0252	0.0676	(-0.1098, 0.1601)	0.37	0.711
March-15 - Feb-15	0.0004	0.0348	(-0.0691, 0.0698)	0.01	0.991

Nov-14 - Feb-15	-0.1800	0.0328	(-0.2454, -0.1145)	-5.49	0.000
Oct-14 - Feb-15	-0.1933	0.0393	(-0.2718, -0.1148)	-4.92	0.000
March-14 - Jan-15	0.1403	0.0696	( 0.0015, 0.2792)	2.02	0.048
March-15 - Jan-15	0.1155	0.0384	( 0.0388, 0.1923)	3.00	0.004
Nov-14 - Jan-15	-0.0648	0.0367	(-0.1380, 0.0083)	-1.77	0.082
Oct-14 - Jan-15	-0.0781	0.0426	(-0.1632, 0.0069)	-1.83	0.071
March-15 - March-14	-0.0248	0.0686	(-0.1617, 0.1121)	-0.36	0.719
Nov-14 - March-14	-0.2052	0.0676	(-0.3401, -0.0702)	-3.04	0.003
Oct-14 - March-14	-0.2185	0.0710	(-0.3602, -0.0767)	-3.08	0.003
Nov-14 - March-15	-0.1804	0.0348	(-0.2498, -0.1109)	-5.19	0.000
Oct-14 - March-15	-0.1936	0.0410	(-0.2755, -0.1118)	-4.72	0.000
Oct-14 - Nov-14	-0.0133	0.0393	(-0.0918, 0.0652)	-0.34	0.737

Simultaneous confidence level = 44.87%

### Appendix B3. Fisher LSD Pairwise Comparison for Female Seasonal GSI values.

Grouping Information Using the Fisher LSD Method and 95% Confidence

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

Means that do not share a letter are significantly different.

Fisher Individual Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Spring - Fall	1.286	0.372	( 0.544, 2.027)	3.46	0.001
Summer - Fall	3.263	0.804	( 1.661, 4.865)	4.06	0.000
Winter - Fall	0.407	0.282	(-0.155, 0.969)	1.44	0.153
Summer - Spring	1.977	0.832	( 0.319, 3.635)	2.38	0.020
Winter - Spring	-0.879	0.354	(-1.585, -0.172)	-2.48	0.015
Winter - Summer	-2.856	0.796	(-4.442, -1.270)	-3.59	0.001

Simultaneous confidence level = 79.99%

### Appendix B4. Fisher LSD Pairwise Comparison for Male Seasonal GSI values.

Grouping Information Using the Fisher LSD 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.

Fisher Individual Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Spring - Fall	0.2288	0.0342	( 0.1606, 0.2969)	6.69	0.000
Winter - Fall	0.1014	0.0293	( 0.0429, 0.1598)	3.46	0.001
Winter - Spring	-0.1274	0.0317	(-0.1905, -0.0643)	-4.02	0.000

Simultaneous confidence level = 87.89%

**Appendix C. Mean dissimilarity (%) of gut contents found in *Fundulus jenkinsi* by season based on similarity percentages analysis (SIMPER). Contents are listed in order of their contribution to the average dissimilarity between pairs of seasons (column 5), with a cutoff when cumulative percent contribution to mean dissimilarity approaches about 90%. Sp: spring, F: fall, W: winter.**

Gut Content	Pair	Average Abundance		Average Dissimilarity (%)	Mean Diss./SD	Contribution (%)
		F vs Sp	Fall	Spring		
SO. Nematocera		0.46	0.81	10.56	1.10	13.46
SC. Collembola		0.50	0.37	8.43	0.80	10.74
O. Amphipoda		0.16	0.57	7.65	0.95	9.75
Arthropod Parts		0.44	0.34	7.11	0.89	9.05
O. Hemiptera		0.43	0.25	6.72	0.79	8.56
O. Hymenoptera		0.42	0.21	5.60	0.84	7.13
O. Diptera		0.40	0.17	5.46	0.81	6.96
Dentritus/Veg. Debris		0.23	0.18	5.18	0.65	6.60
O. Copepoda		0.22	0.08	3.52	0.52	4.48
P. Nematoda		0.07	0.19	3.13	0.46	3.99
O. Araneae		0.17	0.14	3.12	0.55	3.97
O. Trombidiformes		0.21	0.03	2.64	0.47	3.36
Microplastic		0.06	0.10	2.08	0.41	2.65
<b>Total</b>						<b>90.71</b>
	<b>F vs. W</b>	<b>Fall</b>	<b>Winter</b>	<b>80.83%</b>		
SC. Collembola		0.50	0.70	12.46	0.91	15.42
Arthropod Parts		0.44	0.50	8.62	0.88	10.66
SO. Nematocera		0.46	0.43	8.29	0.90	10.26
O. Amphipoda		0.16	0.44	7.09	0.75	8.77
Dentritus/ Veg. Debris		0.23	0.27	6.29	0.68	7.78
O. Hemiptera		0.43	0.04	6.03	0.64	7.47
O. Diptera		0.40	0.02	5.15	0.75	6.37
O. Hymenoptera		0.42	0.00	5.09	0.74	6.30
Microplastic		0.06	0.26	4.49	0.57	5.55
O. Copepoda		0.22	0.07	3.77	0.48	4.66
O. Trombidiformes		0.21	0.07	3.17	0.50	3.92
Unidentified Plankton		0.11	0.04	2.32	0.37	2.87
<b>Total</b>						<b>90.03</b>
	<b>Sp vs. W</b>	<b>Spring</b>	<b>Winter</b>	<b>76.95%</b>		
SC. Collembola		0.37	0.70	11.95	0.88	15.53
SO. Nematocera		0.81	0.43	11.26	1.05	14.63
O. Amphipoda		0.57	0.44	8.89	1.01	11.55
Arthropod Parts		0.34	0.50	8.54	0.89	11.10
Dentritus/ Veg. Debris		0.18	0.27	5.26	0.68	6.83
Microplastic		0.10	0.26	4.56	0.61	5.92
O. Hemiptera		0.25	0.04	4.13	0.57	5.37
P. Nematoda		0.19	0.02	3.25	0.42	4.22
O. Diptera		0.17	0.02	2.75	0.46	3.58
O. Hymenoptera		0.21	0.00	2.73	0.50	3.55
P. Acanthocephala		0.08	0.13	2.58	0.39	3.35
O. Araneae		0.14	0.02	2.28	0.41	2.96
O. Isopoda		0.08	0.08	2.09	0.37	2.71
<b>Total</b>						<b>91.32</b>

