Benchmarking community structure of estuarine-dependent nekton near the Aransas Pass inlet

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Abstract

The purpose of this study is to determine the baseline seasonal community structure of estuarine-dependent nekton (fish, shrimp, and crab) in the Aransas Pass inlet region to establish a pre-operational benchmark prior to newly proposed industrial development in this area. Many nekton occurring in coastal waters share a common estuarine-dependent life history strategy characterized by near-shore spawning in the Gulf of Mexico with larvae migrating through tidal inlets into shallow estuarine nursery habitats. Access to high-quality habitat and spawning grounds via tidal inlets is essential for the reproduction, growth, survival, and maintenance of these populations. Because 75% of commercially or recreationally important species in the Gulf are estuarine-dependent, evaluating how anthropogenic activities may impair this connection between Gulf and bay waters is critical to understanding the population dynamics in this system and how these factors may affect juvenile fish development and fishery productivity. The Aransas Pass inlet is the major tidal inlet for the region, and anthropogenic activities that may alter water chemistry, flow, and quality have the potential for significant negative impacts on the marine life using this migration corridor. The proposed industrial development of the Aransas Pass inlet region (e.g., Harbor Island) presents a critical opportunity to establish baseline community structure in the adjacent estuarine habitats. We found strong evidence that the Aransas Pass, where impacts from industrial development are likely to occur, and Packery Channel (located ~35 km south), where these impacts will likely be absent, inlets have wide-ranging differences in nekton recruitment and development at individual species and community levels. Based on the findings of this study, we recommend continued long-term monitoring in the Aransas Pass and Packery Channel inlet regions to establish baseline variability and appropriately capture planned and unplanned future natural and anthropogenic disturbances and scenarios of environmental change. Baseline studies such as this facilitate effective management plans to preserve the function of these inlet regions as nurseries and fulfill the CBBEP mission to protect and restore the health and productivity of Coastal Bend bays and estuaries while supporting continued economic growth and public use of the bays.

Introduction

The purpose of this study is to determine the baseline seasonal community structure of estuarine-dependent nekton (fish, shrimp, and crab) in the Aransas Pass inlet region to establish pre-operational benchmarks prior to newly proposed extensive industrial development in this area. Many nekton occurring in coastal waters share a common estuarine-dependent life history strategy characterized by near-shore spawning in the Gulf of Mexico (Gulf), with larvae migrating through tidal inlets into shallow estuarine nursery habitats (Weinstein 1979; Baltz et al. 1993; Minello 1999; Heck et al. 2003). Access to high-quality habitat and spawning grounds via tidal inlets is essential for the reproduction, growth, survival, and maintenance of these populations. Thus, tidal inlets play a direct role in nekton productivity, sustainability, and ecosystem health. Because 75% of commercially or recreationally important species in the Gulf are estuarine-dependent (Chambers 1992), evaluating how anthropogenic activities may impair this connection between Gulf and bay waters is critical to understanding the population dynamics in this system and how these factors may affect larval fish development.

The Aransas Pass inlet is the major tidal inlet for the region and is characterized by a channel confluence of several primary branches that has a notable bottlenecking effect resulting in an extraordinarily high abundance of economically and ecologically important estuarine-dependent species (e.g., Atlantic croaker, *Micropogonias undulates*; Gulf menhaden, *Brevoortia patronus*; pinfish, *Lagodon rhomboides*; red drum, *Sciaenops ocellatus*; southern flounder, *Paralichthys lethostigma*; blue crab, *Callinectes sapidus*, post-larval penaeid shrimps, *Farfantepenaeus aztecus*, *F. duorarum*, *Litopenaeus setiferus*) at several life stages (larval through adult). As a result, anthropogenic activities that may alter water chemistry, flow, and quality have the potential for significant negative impacts on the marine life using this migration corridor, which could lead to substantial changes in community structure, larval fish development, and fishery productivity. The development stage is one of the most sensitive times for juvenile fish populations and is associated with high mortality rates. Baseline data of a potential developmental gradient near the Aransas Pass inlet is needed to understand if the proposed industrial development will alter juvenile fish development.

The proposed industrial development of the Aransas Pass inlet region (e.g., Harbor Island) presented a critical opportunity to establish baseline community structure in the adjacent estuarine habitats (e.g., primarily seagrass meadows) and provide novel insights into developmental differences in juvenile fishes in response to different environmental conditions (present and future). This information will provide valuable baseline data against which future changes in this estuarine community can be measured. Furthermore, this baseline information will facilitate the development of Best Management Practices for the future preservation of estuarine habitats and aid in the protection of nursery areas for a diversity of economically and ecologically important estuarine-dependent species.

The overall goals of this project were to (1) determine seasonal abundance and densities of juvenile nekton species near the Aransas Pass inlet; (2) determine seasonal abundance and densities of juvenile nekton species near Packery Channel as a control area; and (3) evaluate developmental differences in juvenile fish in response to different environmental conditions near the Aransas Pass Inlet.

Methods

Study Site

The Aransas Pass inlet, between San José and Mustang barrier islands, is the primary connection from the Gulf to Aransas and Corpus Christi Bays, Texas. The inlet entrance is protected by jetties and connects to the Corpus Christi Ship Channel. The channel is currently 14.3-m mean lower low water (MLLW) deep and 183-m wide and is authorized and permitted for a depth of 16.5 m MLLW and width of 213 m to accommodate larger commercial vessels. Harbor Island is located opposite the inner end of the pass, separating Aransas Bay from Corpus Christi Bay, and has large oil-handling plants with berths on the southeast end of the island. From the inner basin off Harbor Island, a dredged channel leads northwest for about 8.4 km and intersects with the Gulf Intracoastal Waterway.

In an effort to restore flow between the Gulf and the upper Laguna Madre, the United States Army Corps of Engineers permanently reopened Packery Channel in 2006, a historic tidal inlet that was closed since the 1930s due to natural sedimentation (Reese et al. 2008). The Packery Channel inlet, between Mustang and North Padre barrier islands, is located between the northern tip of the upper Laguna Madre and the southeastern corner of Corpus Christi Bay, Texas, approximately 35 km south of Aransas Pass. The inlet is approximately 4-m deep and 37-m wide and extends 5.6 km from the seaward end of the jetties to the Gulf Intracoastal Waterway. Packery Channel is primarily used for recreational purposes, and no commercial shipping vessels enter Corpus Christi Bay via this channel.

Study Design and Sample Site Delineation

Eight sites near Aransas Pass inlet, where impacts from industrial development are likely to occur; and four sites near Packery Channel (located ~35 km south), where these impacts will likely be absent, were selected using a before-after control-impact (BACI) experimental design (Reese et al. 2008; Hall et al. 2016; **Figure 1**). The BACI concept seeks to determine if an event (e.g., industrial development) influences specified and predetermined ecological variables (Smith 2002). Seagrass meadows are the predominant habitat type used by recruiting nekton in this region (Stunz et al. 2002a, b); therefore, sites were established within shallow seagrass meadows (*Halodule wrightii*) near the Aransas Pass and Packery Channel inlets that estuarine-dependent nekton would encounter upon ingressing.

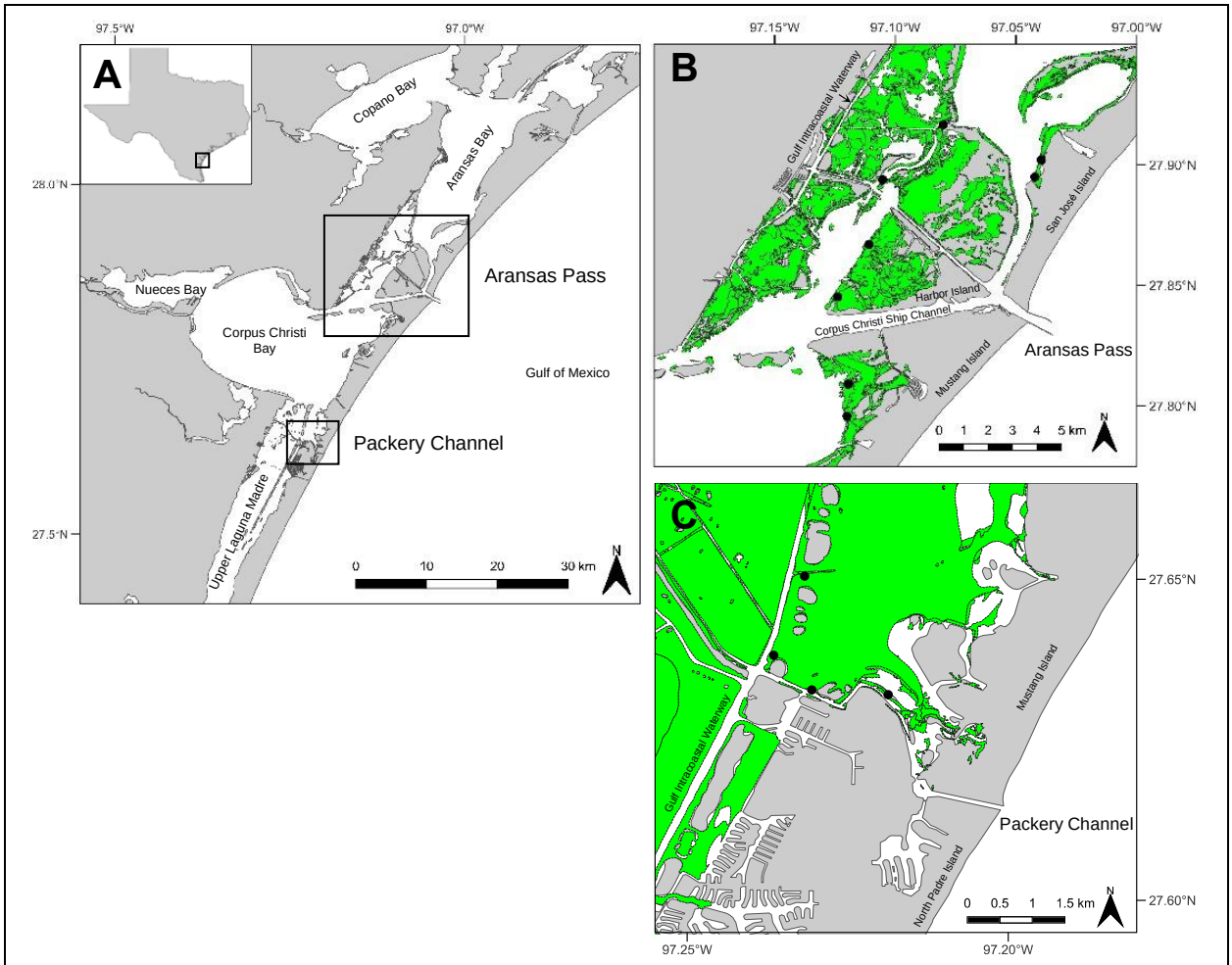

Figure 1. (A) Map of study sites along the Texas coast, including (B) the Aransas Pass inlet near Harbor Island and (C) Packery Channel inlet (control site; nearest tidal inlet). The map depicts a before-after-control-impact study design that includes sites (black circles) to be sampled before impacts at Aransas Pass. Additionally, locations at Packery Channel will serve as control sites. The distribution of seagrass habitat among sites is represented in green (Pulich et al. 1997; Onuf 2007).

Sampling Procedure

Nekton samples were collected during daylight hours using an epibenthic sled, an efficient and standard device for sampling small nekton of the size we were targeting in seagrasses and other estuarine habitats (see Stunz et al. 2002b; Reese et al. 2008; Neahr et al. 2010). Briefly, it is composed of a metal frame 0.6 m wide by 0.75 m high, which supports a 1-mm-mesh conical plankton net mounted to skids. Each tow consisted of pulling the sled 16.6 m covering 10 m^2 of the seagrass bed. Samples from each tow were rough sorted in the field and preserved in 10% buffered formalin (Reese et al. 2008; Hall et al. 2016).

Two sampling events were conducted in each recruitment season (fall, winter, spring) for one year (November 2020 – May 2021). Three independent epibenthic sled tows were taken at each of the

twelve sites (eight near Aransas Pass and four near Packery Channel) during each sampling event totaling 216 samples over the entire study period. At each sample site, water temperature (°C), dissolved oxygen (mg L^{-1}), salinity (‰), and pH were recorded using a Hydrolab MS5 multiparameter water quality sonde for accounting for environmental variability and assessing potential changes following impact (i.e., industrial development). In the laboratory, fishes and crustaceans in each sample were sorted, counted, identified to the lowest possible taxon, and measured to the nearest 0.1 mm. Fishes were measured using standard length (SL), shrimps were measured using total length (TL) between the tip of the rostrum and the telson, and crab species were measured using carapace width (CW). If more than 22 individuals of the same species were collected in a single tow, the largest, smallest, and 20 randomly selected individuals were measured. We assumed that these measurements of randomly sampled individuals were representative of the entire size distribution in the two. Once a sample was processed, fish were preserved in fresh 10% buffered formalin for histological analyses (see below), and crustaceans were preserved in 70% ethanol for long-term storage. The 10% buffered formalin was refreshed for fish samples again after 30 days.

Statistical Analysis

Physical Variables

Water temperature, dissolved oxygen, salinity, and pH were compared seasonally and between inlets with two-way nested analysis of variance (ANOVA) constructed using linear mixed-effects models in the 'nlme' package (Pinheiro et al. 2021) in R 4.0.3. Linear mixed-effects models allow for random, nested factors and use restricted maximum likelihood (REML) to generate a set of contrasts calculated from original data. The REML technique can produce unbiased estimates of variance parameters while ensuring nuisance parameters have no effect. The main effects model was constructed for each physical variable and tested for a significant interaction between the inlet (Aransas Pass and Packery Channel) and season (fall, winter, and spring) main factors using type III sum of squares ($\alpha = 0.05$). Site was treated as a random factor nested in the inlet factor. A Tukey-Kramer test was used for post hoc pairwise comparisons of groups, accounting for unequal sample sizes. Results were tested for normality and homoscedasticity using Shapiro-Wilk's and Levene's test, respectively; though, physical variables were balanced as a result of experimental design and, therefore, robust to heterogeneity of variance. Water temperature data failed to produce normally-distributed residuals and therefore was aligned rank transformed (ART; Wobbrock et al. 2011) and fit with a parametric two-way ANOVA to the ranked data using the 'ARTool' package in R (Kay et al. 2021). The ART ANOVA is a nonparametric procedure that can detect main and interaction effects in multifactor analyses and aligned rank transform contrasts (ART-C) with Tukey-adjusted *p* values were used for post hoc pairwise comparisons of groups (Elkin et al. 2021).

Nekton General Comparisons

Mean density (m^{-2}), mean size (mm), and relative abundance (RA %) were calculated for each species during each recruitment season at the Aransas Pass and Packery Channel inlet sites. Mean density was calculated from a total of 48 and 24 samples collected each season at the Aransas Pass and Packery Channel inlet sites, respectively. Mean sizes were calculated from the number of individuals of a species measured during each season. The RA (%) was calculated by dividing the

number of individuals of a species collected by the total number of fishes or crustaceans within a particular season and multiplied by 100.

Primary Recruitment Seasons

Recruitment of nekton into estuaries is highly variable; thus, data used to test for significant differences were restricted to peak recruitment seasons for each species group or individual species (Reese et al. 2008; Hall et al. 2016). Total nekton, total fish, total crustaceans, and resident shrimp densities were tested over all sample seasons. Resident shrimp density was calculated by summing the densities of arrow shrimp (*Tozeuma carolinense*), cleaner shrimps (Hippolytidae spp.), and grass shrimps (*Palaemonetes* spp.), which comprised over 89% of the total crustaceans collected during this study. Mean densities and sizes of red drum were determined using fall samples only (Holt et al. 1983; Rooker and Holt 1997; Rooker et al. 1998a; Stunz et al. 2002b), while southern flounder and Atlantic croaker were determined using winter samples only (Haven 1957; Hansen 1969; Rooker et al. 1998b; Searcy et al. 2007; Nañez-James et al. 2009). Mean densities and sizes of Gulf menhaden were calculated by combining winter and spring samples (Brown-Peterson et al. 2017). Mean densities and sizes of pinfish, post-larval penaeid shrimps, and blue crabs were calculated by combining fall, winter, and spring samples, given that these taxa have complex life histories, disperse widely, and spawn year-around (Pile et al. 1996; Blackmon and Eggleston 2001; Reese et al. 2008; Hall et al. 2016).

Nekton Diversity, Density, and Size Comparisons

Nekton diversity was calculated using Hill's diversity number one (N1; Hill 1973), which measures the effective number of species in a sample and indicates the number of abundant species. It is calculated as the exponentiated form of the Shannon diversity index:

$$\mathrm{N1} = e^{H'} \tag{1}$$

As diversity decreases, N1 will tend toward 1. The Shannon diversity index is the average uncertainty per species in an infinite community made up of species with known proportional abundances. The Shannon diversity index is calculated by:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i \tag{2}$$

Where p_i is the proportion of individuals belonging to the *i*th species in the sample and *S* is the total number of species in the sample.

Nekton diversity and density were compared seasonally and between inlets using two-way nested ANOVAs constructed for each group (e.g., N1 diversity, total nekton) and tested for a significant interaction using type III sum of squares ($\alpha = 0.05$). Site was treated as a random factor nested in the inlet factor and a Tukey-Kramer test was used for post hoc pairwise comparisons of groups. N1 diversity was log-transformed, whereas total nekton, total fish, total crustaceans, and resident shrimp densities were log ($x + 1$) transformed to ensure homogeneity of variance and normality of residuals. Following transformation, data were tested for normality and homoscedasticity using Shapiro-Wilk's and Levene's test, respectively; though, nekton diversity and density data were balanced as a result of experimental design and, therefore, robust to heterogeneity.

To test for species-specific differences in nekton density and size between inlets, one-way nested ANOVAs were constructed for each estuarine-dependent species of interest (e.g., red drum, southern flounder) during peak recruitment seasons. Site remained treated as a random factor nested in the inlet factor. Since size data were unbalanced, given that the number of individuals of a species varied by tow, all one-way nested ANOVAs used to test these data utilized Helmert contrasts. Transformations were used to normalize each species-specific density and size dataset but failed to produce normally distributed residuals except for Atlantic croaker density (fourth root transformation). Therefore, the remaining species-specific density and size datasets were aligned rank transformed and fit with a parametric one-way ANOVA to the ranked data. For a single factor design, an ART *F*-test will produce the same result as the more conventional Kruskal-Wallis test for nonparametric factorial analysis (Neter et al. 1996).

Juvenile Fish Histo-morphometric Analysis

To assess potential differences in juvenile development between the Aransas Pass and Packery Channel inlets, histo-morphometric analyses were conducted on three economically and ecologically important estuarine-dependent fish species with high seasonal RA (%), which included pinfish, Atlantic croaker, and Gulf menhaden. Pinfish are a key forage fish (Chacin et al. 2016) and recreational baitfish (Green 2007; Ropicki and Fuiman 2020) in the Gulf and Texas Coastal Bend. Forage fish are critical for energy and nutrient transfer throughout the marine food web and serve as prey for spotted seatrout (*Cynoscion nebulosus*) and red drum which drive local recreational fisheries (Chacin et al. 2016). Atlantic croaker is considered an indicator species for Gulf fish communities (Monk et al. 2015) and is an important recreational baitfish (Green 2007; Ropicki and Fuiman 2020). Gulf menhaden support the largest commercial fishery (by weight) in the Gulf and the second largest in the United States (NMFS 2022). Beyond the economic value of the commercial fishery, Gulf menhaden are a key forage fish in estuarine and coastal ecosystems that provide a primary food base for marine mammals, seabirds, sharks, and recreationally and commercially targeted fish (Sagarese et al. 2016). As filter feeders, Gulf menhaden provide a key energy transfer link between primary producers and top-level consumers and are thus critically important to the structure and functioning of GOM marine ecosystems (Olsen et al. 2014).

Five to ten individuals of each species of interest were sectioned per inlet across their respective peak recruitment season and assessed for their gut integrity through microvilli length and goblet cell counts, immune system development through thymus and head kidney compartmentalization, and environmental stressor exposure through melanomacrophage centers in the liver and liver tissue integrity (**Figure 2**). These selected tissues are representative of nutrient uptake, immune competence, and nutrition status, respectively, and determinants of individual survival and fitness. Pinfish (n = 8-10) tissue morphology was compared seasonally and between inlets using a two-way ANOVA and tested for a significant interaction using type III sum of squares ($\alpha = 0.05$). Goblet cell count per microvilli was reciprocal transformed ($1/x$), the ratio between hematopoietic and glomerular tissue in the head kidney was aligned ranked transformed, and the mean number of melanomacrophage centers per liver tissue section was log ($x + 1$) transformed to ensure homogeneity of variance and normality of residuals. An ART-C was used for post hoc pairwise comparison of groups for ART ratio between hematopoietic and glomerular tissue data and a Tukey-Kramer test was used for the remaining selected tissues.

Atlantic croaker (n = 10) and Gulf menhaden (n = 5-9) tissue morphology were compared between inlets within their peak recruitment season using one-way ANOVAs. Atlantic croaker microvilli surface area (µm^2), the ratio between medulla and cortex in the thymus, and the ratio between hematopoietic and glomerular tissue were log-transformed. Gulf menhaden ratio between hematopoietic and glomerular tissue was also log-transformed, but failed to produce equality of variance and was therefore tested using a heteroscedasticity-corrected coefficient covariance matrix HC3 (Long and Ervin 2000) along with the ratio between medulla and cortex. Since pinfish and Gulf menhaden histo-morphometric data were unbalanced, given that the number of individuals of a species varied by inlet, all ANOVAs used to test these data utilized Helmert contrasts.

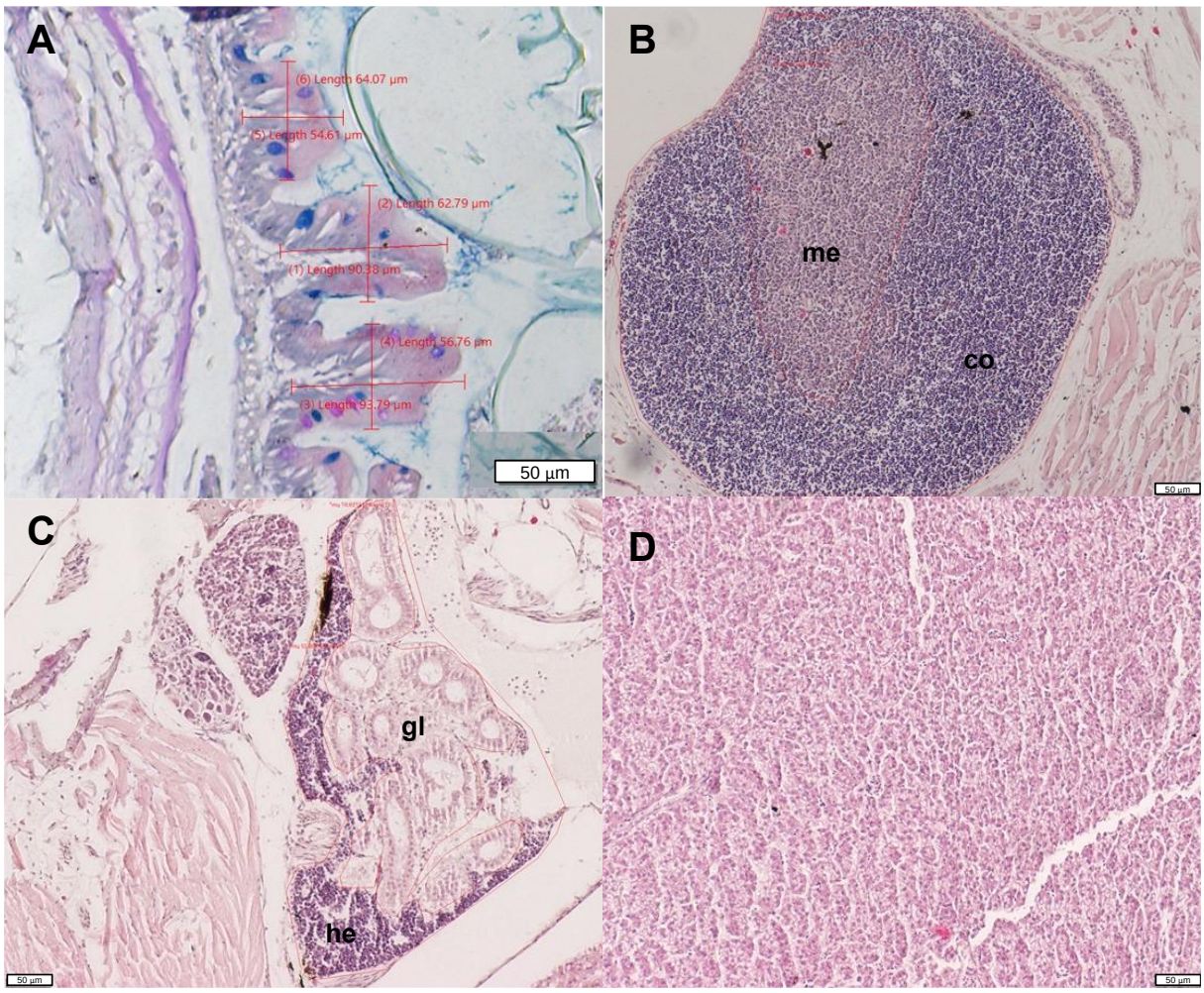

Figure 2. Representative images of histological longitudinal sections for each tissue from pinfish. (A) Gut epithelial layer with microvilli and goblet cells. (B) Thymus with cortical (co) and medullar (me) compartments. (C) Head kidney with glomerular (gl) and hematopoietic (he) tissue. (D) Liver tissue. Scale bars for each image represent 50 µm.

Results

Physical Variables

Sample site water temperatures (°C), dissolved oxygen levels (mg L^{-1}), salinities (‰), and pH changed seasonally (**Table 1**). Mean dissolved oxygen ranged from 6.8 to 10.4 mg L^{-1}, water temperatures from 17.8 to 26.8 °C, salinity from 28.3 to 34.8, and pH from 8.3 to 8.8 (**Table 2**). Differences between inlets within a season were only statistically significant for water temperature and salinity in the fall and dissolved oxygen in the spring and were likely not biologically significant. For example, mean dissolved oxygen concentrations (6.78 ± 0.17 mg L^{-1}) observed at sites near the Packery Channel inlet were significantly lower in the spring yet sustained supersaturated conditions (i.e., mean dissolved oxygen saturation 116.34 ± 3.18 %).

Table 1. Results of two-way nested analysis of variance (ANOVA) examining the main effects of inlet and season and their interaction on physical variables. See Table 2 for mean, standard error, and sample size. Values in bold indicate significance ($\alpha = 0.05$).

	df_{num}	df_{den}	*F*	*p*
Dissolved Oxygen (mg L^{-1})				
Inlet	1	10	11.0539	**0.0077**
Season	2	56	10.4173	**0.0001**
Inlet × Season	2	56	17.7828	**<0.0001**
Water Temperature (°C)				
Inlet	1	10	0.5532	0.4741
Season	2	56	83.2146	**<0.0001**
Inlet × Season	2	56	10.2828	**0.0002**
Salinity ‰				
Inlet	1	10	18.5560	**0.0015**
Season	2	56	43.0460	**<0.0001**
Inlet × Season	2	56	2.2920	0.1105
pH				
Inlet	1	10	2.7080	0.1309
Season	2	56	64.6560	**<0.0001**
Inlet × Season	2	56	12.6740	**<0.0001**

df_{num}, between-groups degrees of freedom; df_{den}, within-groups degrees of freedom

Table 2. Seasonal variation in mean and standard error (SE) of physical variables for Aransas Pass and Packery Channel inlet sites. Means and SE were calculated from measurements taken at each sample site twice per season (Aransas Pass: n = 16 and Packery Channel: n = 8 for each parameter seasonally). Mean values without a shared letter were significantly different (α = 0.05).

	Aransas Pass			Packery Channel		
	Mean	SE	*n*	Mean	SE	*n*
Dissolved Oxygen (mg L^{-1})						
Fall	7.39^{ab}	0.48	16	10.35^{bc}	0.78	8
Winter	8.61^{abc}	0.64	16	7.89^{abc}	0.21	8
Spring	10.22^{c}	0.45	16	6.78^{a}	0.17	8
Water Temperature (°C)						
Fall	21.01^{a}	0.66	16	22.55^{b}	0.16	8
Winter	18.60^{ab}	0.54	16	17.78^{ab}	1.46	8
Spring	26.75^{ab}	0.32	16	24.31^{a}	0.17	8
Salinity ‰						
Fall	31.92^{ce}	0.29	16	34.79^{f}	0.56	8
Winter	30.35^{bd}	0.37	16	31.85^{de}	0.36	8
Spring	28.34^{a}	0.23	16	30.16^{abc}	0.64	8
pH						
Fall	8.37^{ab}	0.04	16	8.53^{bc}	0.05	8
Winter	8.32^{ab}	0.05	16	8.30^{a}	0.03	8
Spring	8.82^{c}	0.06	16	8.55^{bc}	0.02	8

Overall Nekton Relative Abundance and Species Characterization

A total of 134,642 organisms (11,174 fishes and 123,468 crustaceans) were collected during this study, representing 30 fish and 9 crustacean species groups or individual species (**Table 3**). Any individuals that could not be identified to species were grouped into the lowest possible taxon (e.g., Clupeidae, Gobiidae, *Hippocampus*, Ophichthidae, Ophidiidae, *Symphurus*, and *Syngnathus* for fishes and Hippolytidae, *Palaemonetes*, Penaeidae, Porcellanidae, and Xanthidae for crustaceans). Estuarine resident Gobiidae (Aransas Pass RA 23.6 to 62.0%; Packery Channel RA 5.7 to 42.1%) and estuarine-dependent pinfish (Aransas Pass RA 5.1 to 59.6%; Packery Channel RA 1.0 to 30.5%) were among the most abundant fishes, and crustacean abundances were led by estuarine resident cleaner shrimps (Aransas Pass RA 45.6 to 86.3%; Packery Channel RA 14.0 to 65.3%) and estuarine-dependent post-larval penaeids (Aransas Pass RA 0.8 to 33.1%; Packery Channel RA 0.7 to 19.2%).

Table 3. The total seasonal catch and relative abundance (RA) of nekton for the Aransas Pass and Packery Channel inlets. Mean density and standard error (SE) were calculated from a total of 48 and 24 samples collected seasonally at Aransas Pass and Packery Channel inlets, respectively. Mean sizes and SE were calculated from the number of individuals of a species measured during each season.

		Aransas Inlet						Packery Channel					
		Total Catch	RA (%)	Mean Density (m^{-2})	SE	Mean Size (mm)	SE	Total Catch	RA (%)	Mean Density (m^{-2})	SE	Mean Size (mm)	SE
Fall													
Total Fish		972						535					
Atlantic Croaker	*Micropogonias undulatus*	98	10.1	0.204	0.079	9.90	0.217	27	2.8	0.113	0.067	9.44	0.322
Barbfish	*Scorpaena brasiliensis*	1	0.1	0.002	0.002	47.40	0.000	0	0.0	0.000	0.000	0.00	0.000
Bay Anchovy	*Anchoa mitcheli*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Bay Whiff	*Citharichthys spilopterus*	2	0.2	0.004	0.003	12.05	0.550	1	0.1	0.004	0.004	10.50	0.000
Clupeidae	Clupeidae spp.	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Cusk Eel	Ophidiidae spp.	2	0.2	0.004	0.004	24.10	2.100	0	0.0	0.000	0.000	0.00	0.000
Feather Blenny	*Hypsoblennius hentz*	1	0.1	0.002	0.002	31.50	0.000	0	0.0	0.000	0.000	0.00	0.000
Gobiidae	Gobiidae spp.	603	62.0	1.256	0.172	16.39	0.261	409	42.1	1.704	0.486	17.23	0.413
Gulf Menhaden	*Brevoortia patronus*	11	1.1	0.023	0.015	18.17	0.257	0	0.0	0.000	0.000	0.00	0.000
Inland Silverside	*Menidia beryllina*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Ladyfish	*Elops saurus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Ocellated Flounder	*Ancylopsetta ommata*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Pigfish	*Orthopristis chrysoptera*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Pinfish	*Lagodon rhomboides*	50	5.1	0.104	0.027	13.23	1.172	10	1.0	0.042	0.016	32.35	6.461
Pipefish	*Syngnathus* spp.	120	12.3	0.250	0.040	54.23	2.324	71	7.3	0.296	0.069	55.15	2.925
Rainwater Killifish	*Lucania parva*	10	1.0	0.021	0.017	22.72	0.869	0	0.0	0.000	0.000	0.00	0.000
Red Drum	*Sciaenops occelatus*	47	4.8	0.098	0.030	12.01	0.726	2	0.2	0.008	0.008	12.45	0.750
Seahorse	*Hippocampus* spp.	10	1.0	0.021	0.007	13.72	1.646	5	0.5	0.021	0.010	26.88	9.752
Sheepshead Minnow	*Cyprinodon variegatus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Shrimp Eel	*Ophichthus gomesii*	1	0.1	0.002	0.002	94.10	0.000	0	0.0	0.000	0.000	0.00	0.000
Silver Perch	*Bairdiella chrysoura*	0	0.0	0.000	0.000	0.00	0.000	1	0.1	0.004	0.004	58.00	0.000
Snake Eel	Ophichthidae spp.	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Southern Flounder	*Paralichthys lethostigma*	2	0.2	0.004	0.004	11.35	0.050	1	0.1	0.004	0.004	10.10	0.000
Southern Kingfish	*Menticirrhus americanus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Spot	*Leiostomus xanthurus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Spotfin Mojarra	*Eucinostomus argenteus*	1	0.1	0.002	0.002	30.80	0.000	6	0.6	0.025	0.011	25.00	1.147
Spotted Seatrout	*Cynoscion nebulosus*	2	0.2	0.004	0.004	5.00	0.200	0	0.0	0.000	0.000	0.00	0.000
Striped Blenny	*Chasmodes bosquianus*	1	0.1	0.002	0.002	20.40	0.000	0	0.0	0.000	0.000	0.00	0.000
Tonguefish	*Symphurus* spp.	10	1.0	0.021	0.008	21.94	1.354	2	0.2	0.008	0.006	17.85	1.750
White Mullet	*Mugil curema*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Total Crustaceans		34538						13043					
Arrow Shrimp	*Tozeuma carolinense*	2153	6.2	4.485	0.863	17.28	0.277	2403	7.0	10.013	2.187	19.19	0.396
Blue Crab	*Callinectus sapidus*	377	1.1	0.785	0.135	8.13	0.242	139	0.4	0.579	0.168	7.55	0.525
Cleaner Shrimp	Hippolytidae spp.	29813	86.3	62.110	6.273	9.33	0.112	9698	28.1	40.408	7.534	9.96	0.185
Grass Shrimp	*Palaemonetes* spp.	1783	5.2	3.715	1.285	14.85	0.259	453	1.3	1.888	0.591	15.50	0.377
Longnose Spider Crab	*Libinia dubia*	1	0.0	0.002	0.002	9.10	0.000	0	0.0	0.000	0.000	0.00	0.000
Mud Crabs	Xanthidae spp.	131	0.4	0.273	0.076	6.15	0.366	96	0.3	0.400	0.123	5.33	0.287
Post-larval Penaeid	Penaeidae spp.	275	0.8	0.573	0.064	21.75	0.866	254	0.7	1.058	0.182	22.78	0.959
Porcelain Crab	Porcellanidae spp.	1	0.0	0.002	0.002	3.70	0.000	0	0.0	0.000	0.000	0.00	0.000
Snapping Shrimp	*Alpheus heterochaelis*	4	0.0	0.008	0.006	13.95	1.962	0	0.0	0.000	0.000	0.00	0.000

Table 3 (continued)

		Aransas Pass						Packery Channel					
		Total Catch	RA (%)	Mean Density (m^{-2})	SE	Mean Size (mm)	SE	Total Catch	RA (%)	Mean Density (m^{-2})	SE	Mean Size (mm)	SE
Winter													
Total Fish		2191						1773					
Atlantic Croaker	*Micropogonias undulatus*	264	12.0	0.550	0.157	10.94	0.166	499	22.8	2.079	0.472	11.75	0.149
Barbfish	*Scorpaena brasiliensis*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Bay Anchovy	*Anchoa mitcheli*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Bay Whiff	*Citharichthys spilopterus*	16	0.7	0.033	0.019	11.08	0.329	35	1.6	0.146	0.043	10.04	0.237
Clupeidae	Clupeidae spp.	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Cusk Eel	Ophidiidae spp.	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Feather Blenny	*Hypsoblennius hentz*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Gobiidae	Gobiidae spp.	517	23.6	1.077	0.239	12.57	0.324	197	9.0	0.821	0.129	13.22	0.499
Gulf Menhaden	*Brevoortia patronus*	23	1.0	0.048	0.036	17.31	0.496	313	14.3	1.304	0.656	16.67	0.290
Inland Silverside	*Menidia beryllina*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Ladyfish	*Elops saurus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Ocellated Flounder	*Ancylopsetta ommata*	0	0.0	0.000	0.000	0.00	0.000	1	0.0	0.004	0.004	31.80	0.000
Pigfish	*Orthopristis chrysoptera*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Pinfish	*Lagodon rhomboides*	1305	59.6	2.719	0.425	14.24	0.130	669	30.5	2.788	0.502	12.98	0.134
Pipefish	*Syngnathus* spp.	42	1.9	0.088	0.024	68.62	3.079	22	1.0	0.092	0.051	72.05	2.516
Rainwater Killifish	*Lucania parva*	8	0.4	0.017	0.012	22.39	1.478	0	0.0	0.000	0.000	0.00	0.000
Red Drum	*Sciaenops occelatus*	1	0.0	0.002	0.002	56.90	0.000	0	0.0	0.000	0.000	0.00	0.000
Seahorse	*Hippocampus* spp.	10	0.5	0.021	0.007	16.04	0.864	10	0.5	0.042	0.024	16.04	1.448
Sheepshead Minnow	*Cyprinodon variegatus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Shrimp Eel	*Ophichthus gomesii*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Silver Perch	*Bairdiella chrysoura*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Snake Eel	Ophichthidae spp.	0	0.0	0.000	0.000	0.00	0.000	3	0.1	0.013	0.009	73.10	2.571
Southern Flounder	*Paralichthys lethostigma*	2	0.1	0.004	0.003	10.10	1.300	20	0.9	0.083	0.037	13.67	1.114
Southern Kingfish	*Menticirrhus americanus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Spot	*Leiostomus xanthurus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Spotfin Mojarra	*Eucinostomus argenteus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Spotted Seatrout	*Cynoscion nebulosus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Striped Blenny	*Chasmodes bosquianus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Tonguefish	*Symphurus* spp.	3	0.1	0.006	0.005	29.47	1.362	2	0.1	0.008	0.006	27.10	3.200
White Mullet	*Mugil curema*	0	0.0	0.000	0.000	0.00	0.000	2	0.1	0.008	0.006	23.00	2.400
Total Crustaceans		45639						13359					
Arrow Shrimp	*Tozeuma carolinense*	1611	3.5	3.356	0.695	22.92	0.253	1621	3.6	6.754	3.159	20.30	0.408
Blue Crab	*Callinectus sapidus*	487	1.1	1.015	0.246	8.08	0.325	700	1.5	2.917	0.420	5.17	0.223
Cleaner Shrimp	Hippolytidae spp.	35816	78.5	74.617	11.260	10.49	0.101	6410	14.0	26.708	10.434	8.69	0.104
Grass Shrimp	*Palaemonetes* spp.	6816	14.9	14.200	2.898	18.56	0.177	322	0.7	1.342	0.654	17.30	0.329
Longnose Spider Crab	*Libinia dubia*	1	0.0	0.002	0.002	15.10	0.000	0	0.0	0.000	0.000	0.00	0.000
Mud Crabs	Xanthidae spp.	406	0.9	0.846	0.223	5.14	0.168	123	0.3	0.513	0.151	4.74	0.298
Post-larval Penaeid	Penaeidae spp.	494	1.1	1.029	0.167	10.27	0.307	4181	9.2	17.421	2.874	12.21	0.348
Porcelain Crab	Porcellanidae spp.	1	0.0	0.002	0.002	14.80	0.000	1	0.0	0.004	0.004	7.00	0.000
Snapping Shrimp	*Alpheus heterochaelis*	7	0.0	0.015	0.006	19.27	1.851	1	0.0	0.004	0.004	10.20	0.000

Table 3 (continued)

		Aransas Pass						Packery Channel					
		Total Catch	RA (%)	Mean Density (m^{-2})	SE	Mean Size (mm)	SE	Total Catch	RA (%)	Mean Density (m^{-2})	SE	Mean Size (mm)	SE
Spring													
Total Fish		4730						973					
Atlantic Croaker	*Micropogonias undulatus*	0	0.0	0.000	0.000	0.00	0.000	1	0.0	0.004	0.004	53.00	0.000
Barbfish	*Scorpaena brasiliensis*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Bay Anchovy	*Anchoa mitcheli*	73	1.5	0.152	0.109	14.99	0.256	3	0.1	0.013	0.009	9.37	2.042
Bay Whiff	*Citharichthys spilopterus*	1	0.0	0.002	0.002	27.00	0.000	2	0.0	0.008	0.006	22.95	4.850
Clupeidae	Clupeidae spp.	0	0.0	0.000	0.000	0.00	0.000	3	0.1	0.013	0.013	18.33	0.176
Cusk Eel	Ophidiidae spp.	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Feather Blenny	*Hypsoblennius hentz*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Gobiidae	Gobiidae spp.	1286	27.2	2.679	0.414	20.95	0.247	268	5.7	1.117	0.116	22.26	0.392
Gulf Menhaden	*Brevoortia patronus*	462	9.8	0.963	0.547	18.26	0.351	15	0.3	0.063	0.058	13.59	0.593
Inland Silverside	*Menidia beryllina*	5	0.1	0.010	0.005	23.14	3.205	1	0.0	0.004	0.004	22.50	0.000
Ladyfish	*Elops saurus*	2	0.0	0.004	0.004	31.95	2.050	0	0.0	0.000	0.000	0.00	0.000
Ocellated Flounder	*Ancylopsetta ommata*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Pigfish	*Orthopristis chrysoptera*	1	0.0	0.002	0.002	13.70	0.000	0	0.0	0.000	0.000	0.00	0.000
Pinfish	*Lagodon rhomboides*	2796	59.1	5.825	0.760	19.83	0.198	583	12.3	2.429	0.460	19.86	0.283
Pipefish	*Syngnathus* spp.	77	1.6	0.160	0.024	55.03	1.911	43	0.9	0.179	0.032	64.18	4.278
Rainwater Killifish	*Lucania parva*	2	0.0	0.004	0.003	18.50	6.500	0	0.0	0.000	0.000	0.00	0.000
Red Drum	*Sciaenops occelatus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Seahorse	*Hippocampus* spp.	6	0.1	0.013	0.006	11.32	0.384	4	0.1	0.017	0.008	11.50	0.803
Sheepshead Minnow	*Cyprinodon variegatus*	3	0.1	0.006	0.004	25.23	1.068	3	0.1	0.013	0.007	19.70	1.815
Shrimp Eel	*Ophichthus gomesii*	1	0.0	0.002	0.002	81.20	0.000	0	0.0	0.000	0.000	0.00	0.000
Silver Perch	*Bairdiella chrysoura*	8	0.2	0.017	0.007	15.85	1.766	31	0.7	0.129	0.062	6.96	0.363
Snake Eel	Ophichthidae spp.	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Southern Flounder	*Paralichthys lethostigma*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Southern Kingfish	*Menticirrhus americanus*	0	0.0	0.000	0.000	0.00	0.000	1	0.0	0.004	0.004	22.10	0.000
Spot	*Leiostomus xanthurus*	7	0.1	0.015	0.007	39.87	1.551	15	0.3	0.063	0.025	42.63	1.430
Spotfin Mojarra	*Eucinostomus argenteus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Spotted Seatrout	*Cynoscion nebulosus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Striped Blenny	*Chasmodes bosquianus*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Tonguefish	*Symphurus* spp.	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
White Mullet	*Mugil curema*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Total Crustaceans		8673						8216					
Arrow Shrimp	*Tozeuma carolinense*	157	1.8	0.327	0.103	18.99	0.737	169	1.9	0.704	0.125	14.26	0.484
Blue Crab	*Callinectus sapidus*	107	1.2	0.223	0.056	16.37	0.475	24	0.3	0.100	0.033	13.90	1.163
Cleaner Shrimp	Hippolytidae spp.	3951	45.6	8.231	0.979	10.00	0.132	5664	65.3	23.600	4.558	9.74	0.225
Grass Shrimp	*Palaemonetes* spp.	1370	15.8	2.854	0.692	18.56	0.326	485	5.6	2.021	0.424	19.61	0.273
Longnose Spider Crab	*Libinia dubia*	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Mud Crabs	Xanthidae spp.	219	2.5	0.456	0.157	4.76	0.224	225	2.6	0.938	0.270	5.30	0.203
Post-larval Penaeid	Penaeidae spp.	2867	33.1	5.973	0.571	23.24	0.383	1649	19.0	6.871	0.665	25.03	0.517
Porcelain Crab	Porcellanidae spp.	0	0.0	0.000	0.000	0.00	0.000	0	0.0	0.000	0.000	0.00	0.000
Snapping Shrimp	*Alpheus heterochaelis*	2	0.0	0.004	0.003	29.35	3.950	0	0.0	0.000	0.000	0.00	0.000

Nekton Diversity, Density, and Size Comparisons

A two-way nested ANOVA revealed that there was a significant interaction between the effects of inlet and season on N1 diversity ($F(2, 200) = 21.015$, $p = <0.0001$). Post hoc pairwise comparisons revealed no significant difference in N1 diversity between inlets within a season; however, we observed significant differences within and between inlets across seasons (**Figure 3**; **Appendix Table A1**). For example, N1 diversity significantly increased at Aransas Pass sites from Fall to Spring, whereas N1 diversity peaked at Packery Channel sites during the winter.

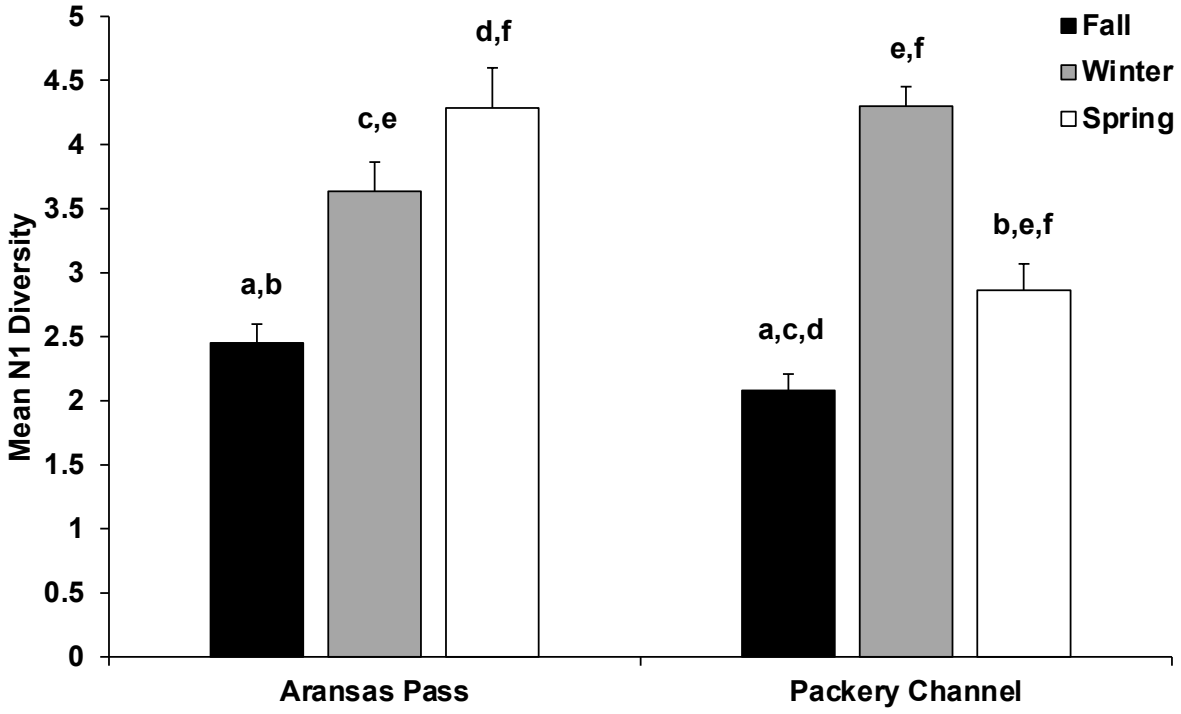

Figure 3. Mean Hill's N1 diversity compared seasonally and between inlets. Error bars represent the standard error among epibenthic sled tows. Bars without a shared letter were significantly different ($\alpha = 0.05$).

Two-way nested ANOVAs revealed significant interactions between the effects of inlet and season on total nekton density, total fish density, total crustacean density, and resident shrimp density (**Table 4**). Post hoc pairwise comparisons revealed no significant difference in nekton density between inlets within a season; however, we observed significant differences within and between inlets across seasons (**Figure 4**; **Table 5**). Mean densities for total nekton and total crustaceans were significantly lower during the spring at Aransas Pass sites, whereas there was no significant difference between seasons among Packery Channel sites. Similarly, resident shrimp mean density was significantly lower during the spring at Aransas Pass sites. In contrast, there was only a significant difference between fall (mean 52.308 m^{-2}) and winter (mean 34.804 m^{-2}) among Packery Channel sites. Total fish mean density significantly increased at Aransas Pass sites from Fall to Spring, whereas total fish mean density peaked at Packery Channel sites during the winter.

Table 4. Results of two-way nested analysis of variance (ANOVA) examining the main effects of inlet and season and their interaction on nekton density (m^{-2}). Resident shrimp density was calculated by summing the total of arrow shrimp, cleaner shrimp, and grass shrimp species. See Table 4 for mean, standard error, and sample size. Values in bold indicate significance ($\alpha = 0.05$).

	Density (m^{-2})			
	df_{num}	df_{den}	*F*	*p*
Total Nekton				
Inlet	1	10	1.6426	0.2289
Season	2	200	22.7005	**<0.0001**
Inlet × Season	2	200	5.1086	**0.0069**
Total Fish				
Inlet	1	10	0.1357	0.7202
Season	2	200	46.0716	**<0.0001**
Inlet × Season	2	200	11.0313	**<0.0001**
Total Crustaceans				
Inlet	1	10	1.4484	0.2565
Season	2	200	37.4028	**<0.0001**
Inlet × Season	2	200	8.9451	**0.0002**
Resident Shrimps				
Inlet	1	10	0.91765	0.3607
Season	2	200	38.6027	**<0.0001**
Inlet × Season	2	200	15.4398	**<0.0001**

df_{num}, between-groups degrees of freedom; df_{den}, within-groups degrees of freedom

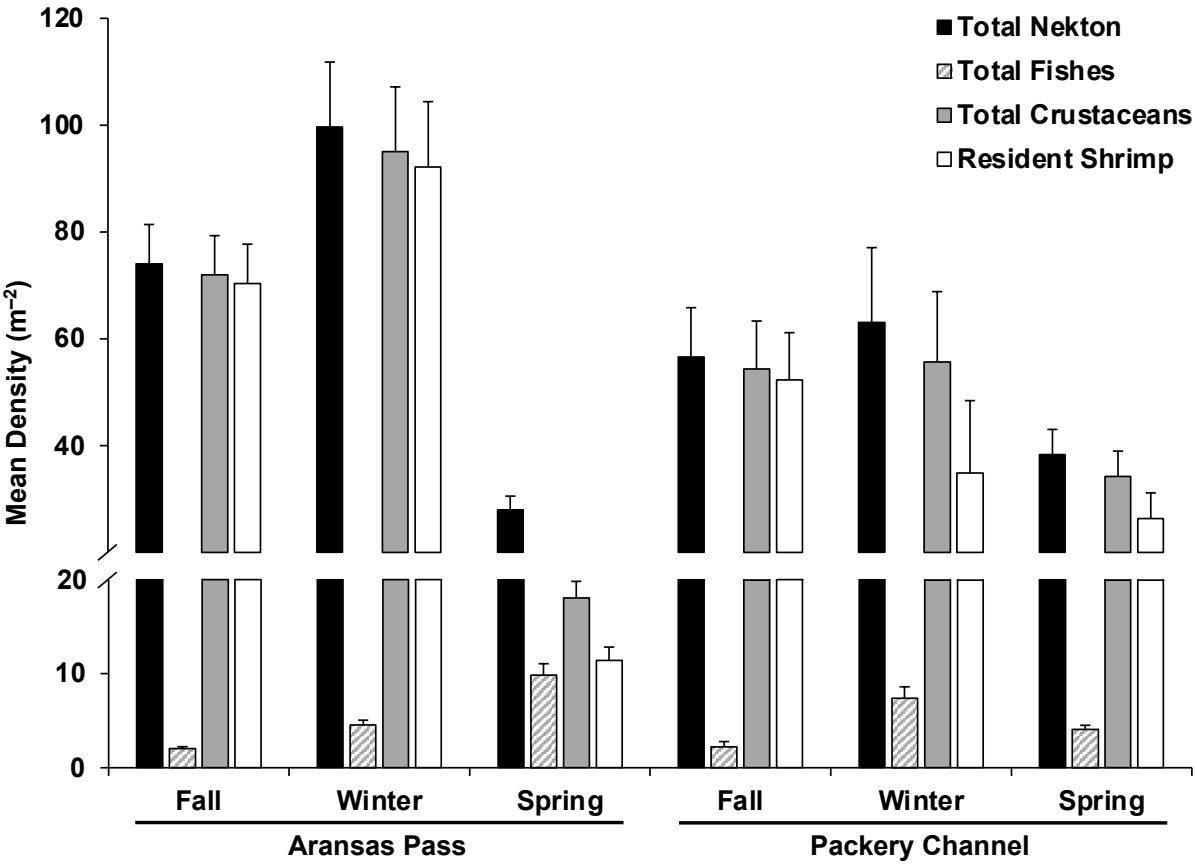

Figure 4. Mean nekton density compared seasonally and between inlets. Resident shrimp density was calculated by summing the total of arrow shrimp, cleaner shrimp, and grass shrimp species. Error bars represent the standard error among epibenthic sled tows. See Table 4 for mean, standard error, and sample size.

Table 5. Mean nekton density, standard error (SE), and sample size (*n*) was compared seasonally and between inlets. Mean density values without a shared letter were significantly different ($\alpha = 0.05$). Resident shrimp density was calculated by summing the total of arrow shrimp, cleaner shrimp, and grass shrimp species.

	Aransas Pass			Packery Channel		
	Mean Density (m^{-2})	SE	*n*	Mean Density (m^{-2})	SE	*n*
Total Nekton						
Fall	73.979^{b}	7.404	48	56.575^{ab}	9.225	24
Winter	99.646^{b}	12.156	48	63.050^{ab}	13.994	24
Spring	27.923^{a}	2.597	48	38.288^{ab}	4.736	24
Total Fish						
Fall	2.025^{ac}	0.202	48	2.229^{ab}	0.555	24
Winter	4.565^{bd}	0.497	48	7.388^{de}	1.218	24
Spring	9.854^{e}	1.219	48	4.054^{cde}	0.455	24
Total Crustaceans						
Fall	71.954^{b}	7.354	48	54.346^{ab}	8.958	24
Winter	95.081^{b}	12.049	48	55.663^{ab}	13.171	24
Spring	18.069^{a}	1.764	48	34.233^{ab}	4.715	24
Resident Shrimps						
Fall	70.310^{cd}	7.384	48	52.308^{bd}	8.813	24
Winter	92.173^{cd}	12.213	48	34.804^{ac}	13.607	24
Spring	11.413^{ab}	1.419	48	26.325^{abcd}	4.813	24

Changes in mean density and size were assessed for estuarine-dependent species of interest during their peak recruitment season(s) to examine species-specific differences between Aransas Pass and Packery Channel inlets (**Figure 5**; **Table 6**). One-way nested ANOVAs revealed that the mean density of Atlantic croaker, Gulf menhaden, southern flounder, and post-larval penaeid shrimps was significantly higher at Packery Channel than at Aransas Pass sites during peak recruitment. Similarly, Atlantic croaker mean size was significantly larger at Packery Channel compared to Aransas Pass sites. In contrast, blue crab mean size was significantly smaller at Packery Channel compared to Aransas Pass sites. There were no significant differences in mean density or size among the remaining estuarine-dependent species of interest between Aransas Pass and Packery Channel. We could not perform statistical analyses for the mean size of red drum or southern flounder because only two individuals were measured at Packery Channel and Aransas Pass sites, respectively, during peak recruitment seasons.

Table 6. Results of one-way nested analysis of variance (ANOVA) examining the main effect of the inlet on species-specific mean density (m^{-2}) and mean sizes (mm) with corresponding standard errors (SE) and sample sizes (*n*). Each mean density and SE are calculated from samples collected during peak recruitment seasons. Mean sizes and SE were calculated from the number of individuals of a species measured during peak recruitment seasons. No analyses were performed for the mean size of red drum or southern flounder due to relatively few measurements (n = 2) at Packery Channel and Aransas Pass sites, respectively. Values in bold indicate significance ($\alpha = 0.05$).

	Aransas Pass			Packery Channel						
	Mean	SE	*n*	Mean	SE	*n*	df_{num}	df_{den}	*F*	*p*
Density (m^{-2})										
Atlantic Croaker	0.550	0.157	48	2.079	0.472	24	1	10	7.5310	**0.0207**
Gulf Menhaden	0.505	0.277	96	0.683	0.338	48	1	10	6.4992	**0.0289**
Pinfish	2.883	0.348	144	1.753	0.266	72	1	10	0.9145	0.3615
Red Drum	0.098	0.030	48	0.008	0.008	24	1	10	2.4908	0.1456
Southern Flounder	0.004	0.003	48	0.083	0.037	24	1	10	10.6250	**0.0086**
Blue Crab	0.674	0.099	144	1.199	0.209	72	1	10	0.3571	0.5634
Post-larval Penaeid	2.525	0.285	144	8.450	1.261	72	1	10	15.2530	**0.0029**
Size (mm)										
Atlantic Croaker	10.939	0.166	208	11.748	0.149	286	1	10	11.5290	**0.0136**
Gulf Menhaden	18.055	0.297	107	16.408	0.278	177	1	10	2.2256	0.2155
Pinfish	17.310	0.144	1677	16.574	0.226	728	1	10	0.9521	0.3518
Red Drum	12.015	0.726	47	12.450	0.750	2				
Southern Flounder	10.100	1.300	2	13.670	1.114	20				
Blue Crab	9.221	0.212	790	6.055	0.221	590	1	10	11.1740	**0.0084**
Post-larval Penaeid	19.788	0.309	1641	19.278	0.351	1269	1	10	0.2930	0.6005

df_{num}, between-groups degrees of freedom; df_{den}, within-groups degrees of freed

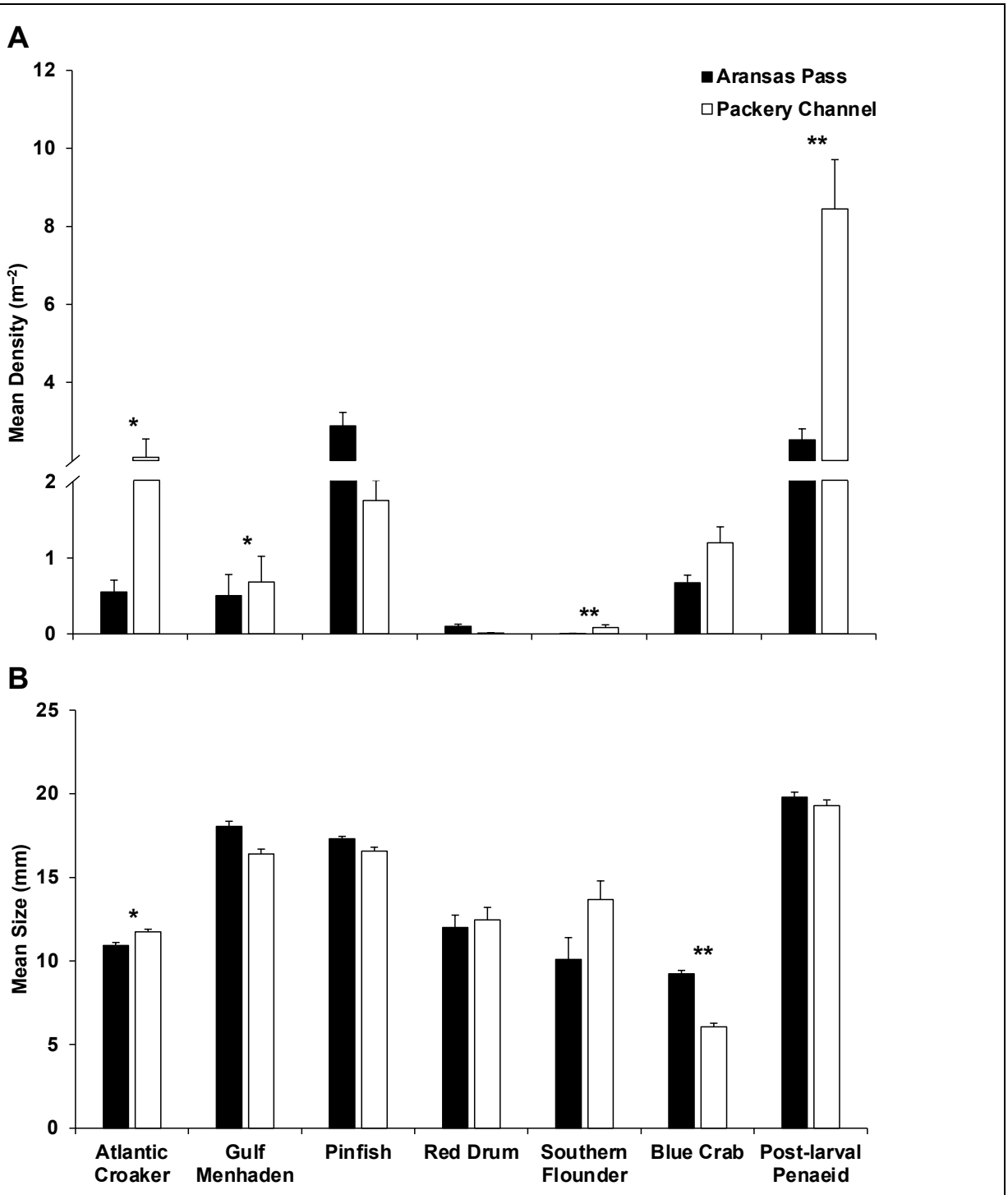

Figure 5. (A) Mean density and (B) mean size of estuarine-dependent species of interest during their peak recruitment season(s) at each inlet. Error bars represent the standard error among (A) epibenthic sled tows and (B) individuals for species-specific density and size comparisons, respectively. Asterisks represent significant differences between inlets (*$p<0.05$, **$p<0.01$). No analyses were performed for the mean size of red drum or southern flounder due to relatively few measurements (n = 2) at Packery Channel and Aransas Pass sites, respectively.

Juvenile Fish Histo-morphometric Analysis

Pinfish

Pinfish were the most relatively abundant fish species (identified to species level) across all three recruitment seasons. No significant differences were observed among pinfish (1.63 ± 0.25 cm TL) between inlets for microvilli surface area and goblet cell numbers, thus indicating no differences in gut integrity (**Figure 6**; **Table 7; Appendix Table A2**). Seasonal changes in microvilli surface area were observed for Packery Channel samples with significantly increased microvilli surface in winter compared to fall (**Figure 6A**), possibly indicating a higher food abundance in winter (Day et al. 2014), while the goblet cell numbers remained similar (**Figure 6B**). Pinfish collected in spring had a significantly increased medullary compartment of the thymus (**Figure 6C**) and a higher amount of hematopoietic tissue in the head kidney (**Figure 6D**), indicating an advanced immune system development in the spring cohort, notably for Aransas Pass (Seemann et al. 2015a, b). A significant increase in melanomacrophage centers in the spring cohort from Packery Channel demonstrates a possible increased exposure to environmental stressors (**Figure 6E**). Together these data may indicate reduced health of juvenile pinfish originating from Packery Channel.

Table 7. Results of two-way analysis of variance (ANOVA) examining the main effects of inlet and season and their interaction on selected tissues from pinfish. See Appendix Table A2 for mean, standard error, and sample size. Values in bold indicate significance ($\alpha = 0.05$).

	df_{num}	df_{den}	*F*	*p*
Microvilli Surface Area (μm^2)				
Inlet	1	47	0.7529	0.3900
Season	2	47	9.8894	**0.0003**
Inlet × Season	2	47	2.0526	0.1398
Goblet Cell Count (per microvilli)				
Inlet	1	47	0.0059	0.9388
Season	2	47	0.9371	0.3990
Inlet × Season	2	47	0.5404	0.5861
Ratio Medulla:Cortex				
Inlet	1	47	2.2255	0.1424
Season	2	47	4.4164	**0.0175**
Inlet × Season	2	47	2.3231	0.1091
Ratio Hematopoietic:Glomerular				
Inlet	1	47	1.9690	0.1671
Season	2	47	29.1889	**<0.0001**
Inlet × Season	2	47	1.8776	0.1643
Mean Melanomacrophage Centers				
Inlet	1	47	13.8389	**0.0005**
Season	2	47	0.9282	0.4024
Inlet × Season	2	47	1.0596	0.3547

df_{num}, between-groups degrees of freedom; df_{den}, within-groups degrees of freedom

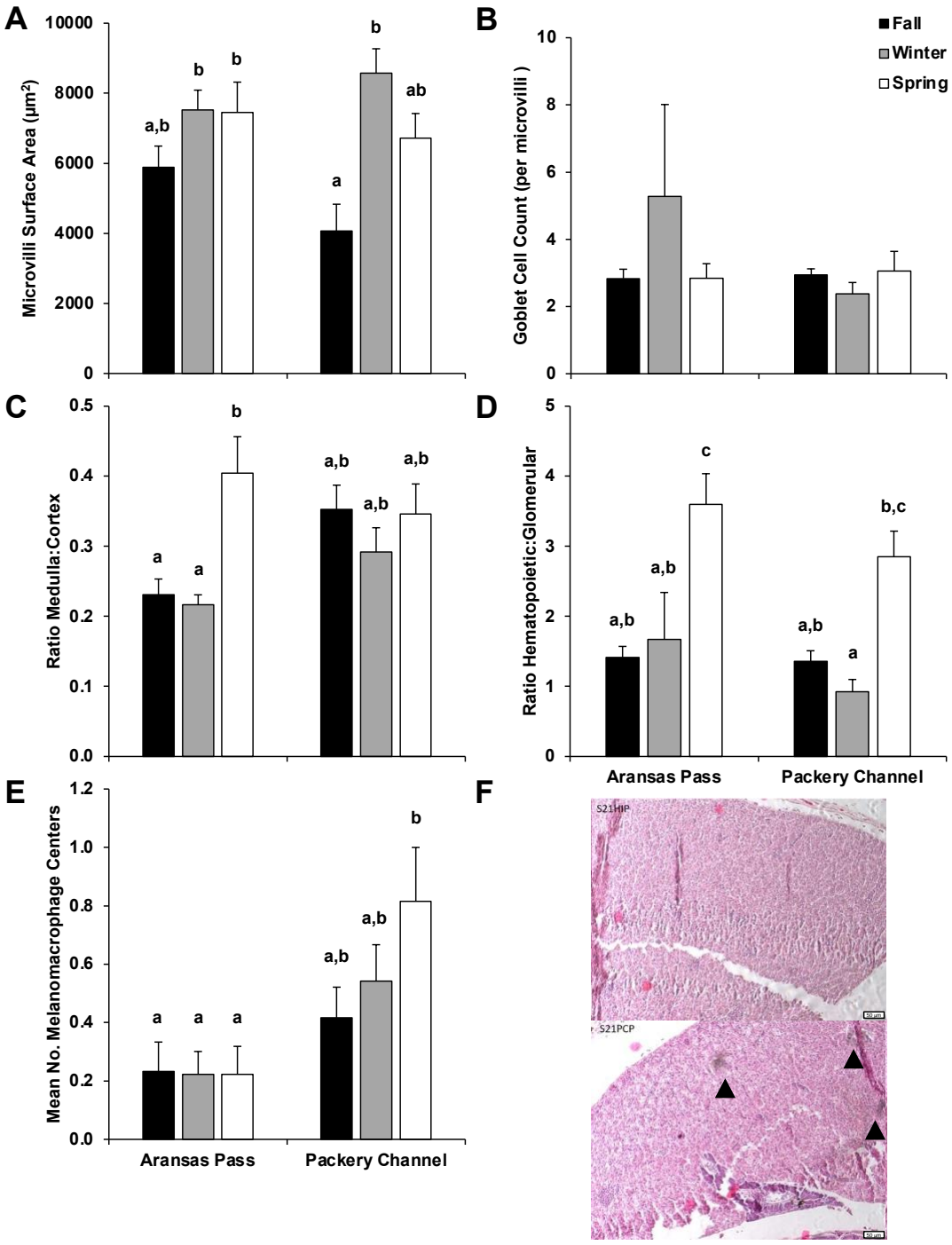

Figure 6. Pinfish tissue morphology compared seasonally and between inlets. A) Microvilli surface area (µm^2). B) Goblet cell count per microvilli. C) The ratio between medulla and cortex in the thymus. D) The ratio between hematopoietic and glomerular tissue in the head kidney. E) The mean number of melanomacrophage centers per liver tissue section. Error bars represent the standard error among individuals. Error bars without a shared letter indicate a significant difference between seasons for each inlet. No significant differences were observed for goblet cell count. F) Images of liver sections with melanomacrophage centers (arrowheads) from Aransas Pass (top) and Packery Channel (bottom). Scale bars indicate 50 µm.

Atlantic Croaker

Atlantic croaker was the second most relatively abundant fish species collected during the winter. In this study, juvenile Atlantic croaker (1.28 ± 0.15 cm TL) was the species showing the least variation between the Aransas Pass and Packery Channel inlets, as no significant differences were observed for all selected tissues (**Figure 7**; **Table 8**).

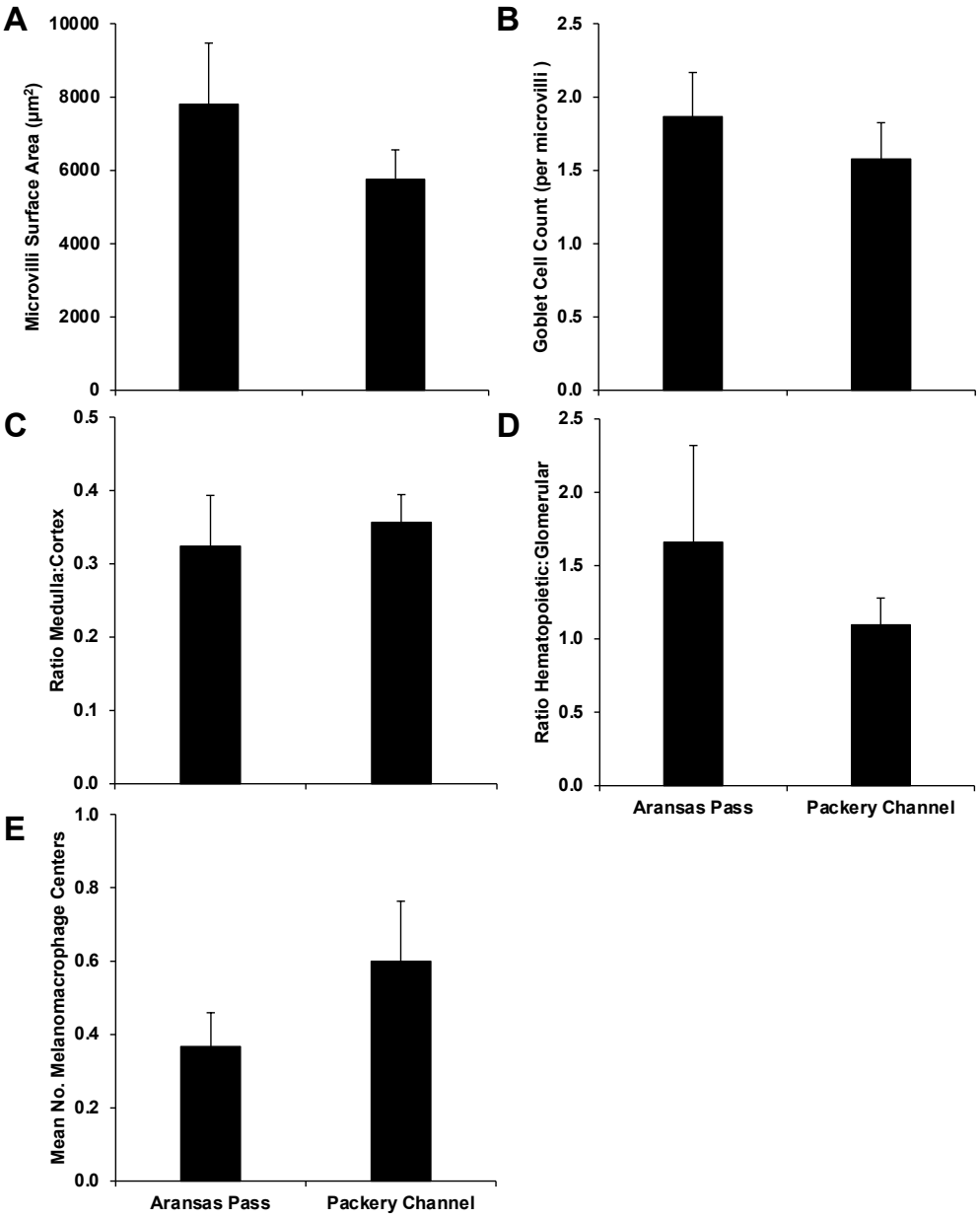

Figure 7. Atlantic croaker tissue morphology collected during winter from Aransas Pass and Packery Channel inlets. A) Microvilli surface area (µm2). B) Goblet cell count per microvilli. C) The ratio between medulla and cortex in the thymus. D) The ratio between hematopoietic and glomerular tissue in the head kidney. E) The average number of melanomacrophage centers per liver tissue section. Error bars represent the standard error among individuals.

Table 8. Results of one-way analysis of variance (ANOVA) examining the main effect of the inlet on selected tissues from Atlantic croaker and Gulf menhaden for histo-morphometric analysis with corresponding standard errors (SE) and sample sizes (n). Mean values and SE were calculated from the number of individuals of a species sectioned per inlet during peak recruitment seasons. Values in bold indicate significance ($\alpha = 0.05$).

	Aransas Pass			Packery Channel						
	Mean	SE	*n*	Mean	SE	*n*	df_{num}	df_{den}	*F*	*p*
Atlantic Croaker										
Microvilli Surface Area (μm^2)	7800.1	1667.9	10	5761.3	795.1	10	1	18	0.9594	0.3403
Goblet Cell Count (per microvilli)	1.867	0.300	10	1.578	0.247	10	1	18	0.5535	0.4665
Ratio Medulla:Cortex	0.324	0.070	10	0.357	0.038	10	1	18	0.9342	0.3466
Ratio Hematopoietic:Glomerular	1.659	0.661	10	1.098	0.181	10	1	18	0.2825	0.6016
Mean Melanomacrophage Centers	0.367	0.092	10	0.600	0.163	10	1	18	1.5474	0.2295
Gulf Menhaden										
Microvilli Surface Area (μm^2)	8922.1	1777.7	9	4312.7	1310.7	5	1	12	3.1292	0.1023
Goblet Cell Count (per microvilli)	1.457	0.137	9	0.756	0.074	5	1	12	13.0540	**0.0036**
Ratio Medulla:Cortex	0.384	0.059	9	0.177	0.021	5	1	12	9.6111	**0.0092**
Ratio Hematopoietic:Glomerular	1.899	0.360	9	1.547	0.716	5	1	12	0.9708	0.3439
Mean Melanomacrophage Centers	0.148	0.059	9	0.533	0.200	5	1	12	5.4663	**0.0375**

df_{num}, between-groups degrees of freedom; df_{den}, within-groups degrees of freedom

Gulf Menhaden

Gulf menhaden was the second most relatively abundant fish species collected during the spring. No significant differences were observed in microvilli surface area (**Figure 8A**); however, Gulf menhaden collected near Aransas Pass had a significantly increased number of goblet cells (**Figure 8B**), possibly indicative of an intact intestinal barrier and improved fish health (Knoop and Newberry 2018; Dawood 2021). Similarly, Gulf menhaden from Aransas Pass had a significantly increased medullary compartment of the thymus (**Figure 8C**), indicating advanced development of the immune competence (Van Loon et al. 1982; Nakanishi et al. 2015); however, no significant differences were observed for head kidney development (**Figure 8D**). The increased number of melanomacrophage centers may indicate an increased exposure to environmental stressors in juveniles near the Packery Channel inlet (**Figure 8E**; Ali et al. 2014; Steinel and Bolnick 2017). Overall, these data reveal possibly reduced health in Gulf menhaden from near the Packery Channel inlet compared to juveniles from Aransas Pass.

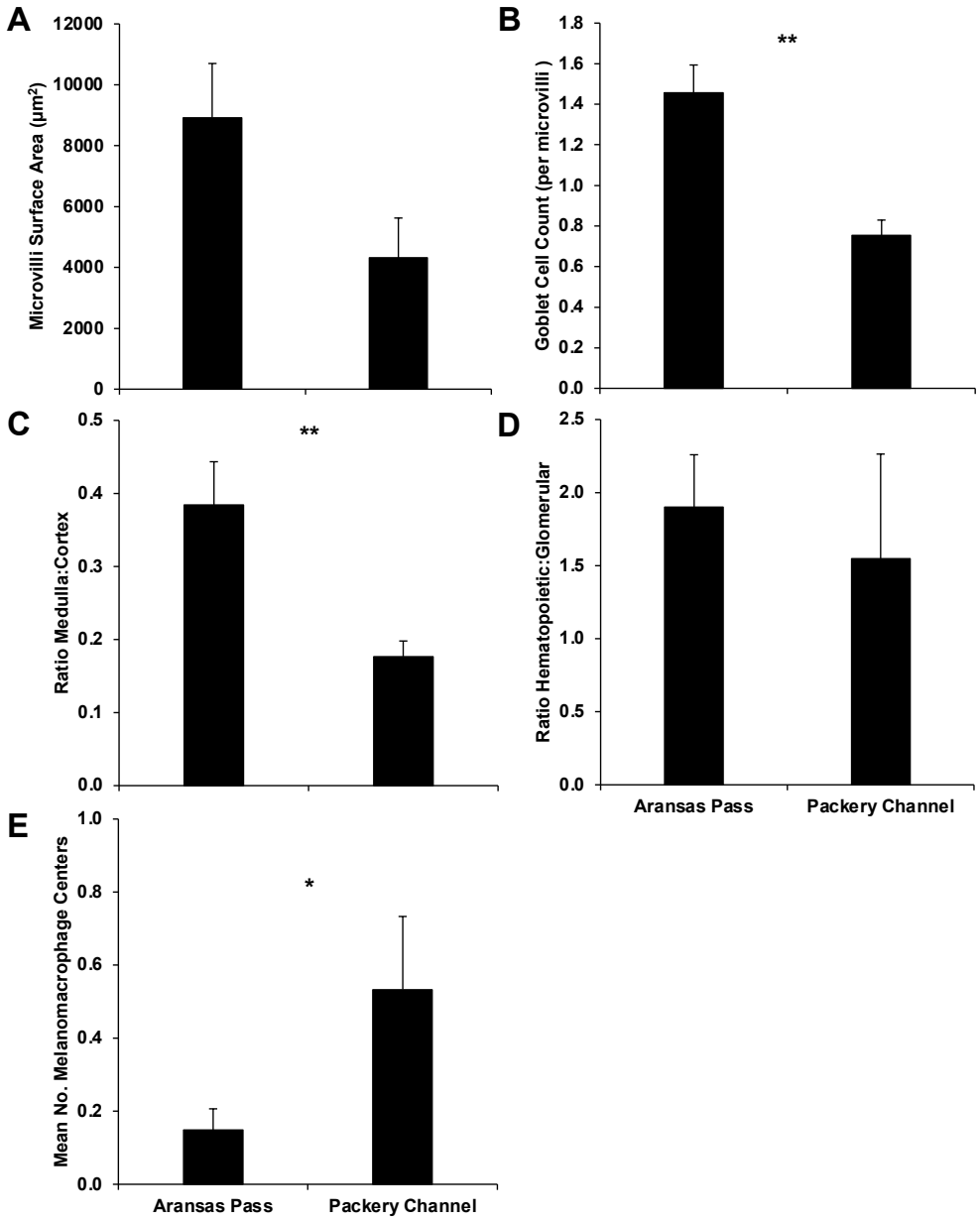

Figure 8. Gulf menhaden tissue morphology collected during spring from Aransas Pass and Packery Channel inlets. A) Microvilli surface area (µm^2). B) Goblet cell count per microvilli. C) The ratio between medulla and cortex in the thymus. D) The ratio between hematopoietic and glomerular tissue in the head kidney. E) The average number of melanomacrophage centers per liver tissue section. Error bars represent the standard error among individuals. Asterisks represent significant differences between inlets (*p<0.05, **p<0.01).

Discussion

The window of opportunity for obtaining quantitative baseline data is narrowing while estuarine habitats are being altered at a rapid rate (Peterson 2003). The proposed industrial development of the Aransas Pass inlet region presented a critical opportunity to establish baseline community structure in the adjacent estuarine nursery seagrass habitats and provide novel insights into developmental differences in juvenile fishes in response to different environmental conditions (present and future). We found strong evidence that the Aransas Pass, where impacts from industrial development are likely to occur, and Packery Channel, where these impacts will likely be absent, inlets have wide-ranging differences in nekton recruitment and development at individual species and community levels. Overall, these data provide the first assessment of seasonal and site-specific differences in juvenile fish development in Texas Coastal Bend estuaries, which warrant further investigation of potentially impaired fish health at the Packery Channel inlet. In addition, Gulf menhaden was revealed to be more sensitive to environmental conditions at Packery Channel than pinfish and Atlantic croaker, which were the least sensitive of these three species of interest. Further analyses are needed to reveal the underlying causes for possible increased fish health for the pinfish spring cohort in comparison to individuals developing in fall and winter. This study should be a valuable baseline and priority defining tool in developing management plans for these inlets, contributing to the safeguard of seagrass nursery habitats and estuarine-resident and estuarine-dependent species.

Environmental impact assessments of potential natural and anthropogenic change and coastal planning processes require careful selection of sampling techniques and experimental designs to tease apart the relative impacts of a stressor of interest from other, often co-occurring, natural and anthropogenic disturbances (Osenberg et al. 1994; Lotze et al. 2011). Given the unpredictable nature of many natural (e.g., hurricanes, severe winter storms) and anthropogenic (e.g., oil spills) disturbances, establishing relevant baseline conditions is of paramount importance in environmental assessment. Precise assessments of the impacts of disturbances require sufficient baseline data collected during the pre-disturbance period to quantify both natural variability and historical disturbance impacts, especially for designs such as before-after-control-impact (Stewart-Oaten et al. 1992; Osenberg et al. 1994; Reese et al. 2008; Hall et al. 2016). When appropriate pre-disturbance data are unavailable, assessments of impacted and unimpacted areas may be confounded by local and episodic large-scale weather events, seasonal and spatial variability, pulsed ecological events (e.g., recruitment), replication problems, and other unmeasured occurrences that may affect both impact and control areas.

In conclusion, our study of the Aransas Pass and Packery Channel inlets provides resource managers with important baseline information regarding community-level patterns for estuarine-dependent and estuarine-resident species, particularly concerning the relative abundance and size of nekton, their spatial and temporal distribution, species composition of the community, and developmental differences. Baseline studies such as this facilitate effective management plans to preserve the function of these inlet regions as nurseries and fulfill the CBBEP mission to protect and restore the health and productivity of Coastal Bend bays and estuaries while supporting continued economic growth and public use of the bays. Based on the findings of this study, we recommend continued long-term monitoring in the Aransas Pass and Packery Channel inlet

regions to establish baseline variability and appropriately capture planned and unplanned future natural and anthropogenic disturbances and scenarios of environmental change.

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Appendix

Table A1. Mean Hill's N1 diversity, standard error (SE), and sample size (*n*) were compared seasonally and between inlets. Mean N1 diversity values without a shared letter were significantly different ($\alpha = 0.05$).

	Aransas Pass			Packery Channel		
	Mean N1 Diversity	SE	*n*	Mean N1 Diversity	SE	*n*
Fall	2.450^{ab}	0.148	24	2.082^{acd}	0.126	48
Winter	3.636^{ce}	0.227	24	4.300^{ef}	0.152	48
Spring	4.285^{df}	0.313	24	2.861^{bef}	0.207	48

Table A2. Mean values of selected tissues from pinfish, standard errors (SE), and sample sizes (*n*) were compared seasonally and between inlets. Mean values and SE were calculated from the number of individuals sectioned per inlet each season. Mean values without a shared letter were significantly different ($\alpha = 0.05$). No significant differences were observed among pinfish for goblet cell count.

	Aransas Pass			Packery Channel		
	Mean	SE	*n*	Mean	SE	*n*
Microvilli Surface Area (μm^2)						
Fall	5880.5^{ab}	660.3	10	4072.2^{a}	738.3	8
Winter	7526.2^{b}	696.0	9	8567.5^{b}	738.3	9
Spring	7452.8^{b}	696.0	9	6722.0^{ab}	696.0	8
Goblet Cell Count (per microvilli)						
Fall	2.835	0.276	10	2.944	0.181	8
Winter	5.284	2.728	9	2.375	0.342	9
Spring	2.840	0.437	9	3.062	0.581	8
Ratio Medulla:Cortex						
Fall	0.231^{a}	0.022	10	0.353^{ab}	0.034	8
Winter	0.216^{a}	0.014	9	0.292^{ab}	0.035	9
Spring	0.405^{b}	0.052	9	0.346^{ab}	0.043	8
Ratio Hematopoietic:Glomerular						
Fall	1.413^{ab}	0.157	10	1.357^{ab}	0.151	8
Winter	1.669^{ab}	0.672	9	0.924^{a}	0.175	9
Spring	3.597^{c}	0.438	9	2.851^{bc}	0.364	8
Mean Melanomacrophage Centers						
Fall	0.233^{a}	0.100	10	0.417^{ab}	0.104	8
Winter	0.222^{a}	0.079	9	0.542^{ab}	0.125	9
Spring	0.222^{a}	0.096	9	0.815^{b}	0.185	8