Final Report

As Required By

The State Wildlife Grants Program

Texas

F19AP00294

Contract: 526332

Distribution, abundance, and habitat use of the Dwarf Seahorse, *Hippocampus zosterae*, along the Texas Coast.

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Report # EIH21-001

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December 1, 2021

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Recommended Citation:
Oakley, J.W., S. Lesher, G. Dennis, R. Ulibarri, and G. Guillen. 2022. Distribution, abundance, and habitat use of the Dwarf Seahorse, *Hippocampus zosterae,* along the Texas Coast. Final Report (EIH 21-001) submitted to the Texas Parks and Wildlife Department, Contract 526332. pp 78.

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Locations

This study was conducted along the Texas Coast from Galveston Bay to the Lower Laguna Madre. The following bay systems and sub-bays were surveyed: Galveston Bay including Christmas and West Bays, Matagorda Bay and East Matagorda Bay, San Antonio Bay including Espiritu Santo Bay, Aransas and Copano Bays, Corpus Christi Bay, and the Upper and Lower Laguna Madre systems. These waterbodies are located in the following coastal counties: Aransas, Brazoria, Calhoun, Cameron, Galveston, Kenedy, Matagorda, Nueces, Willacy. Only public waters were surveyed.

Objectives

In order to evaluate the status of Dwarf Seahorse (*Hippocampus zosterae*) populations along the Texas Coast, we addressed the following objectives:

1) Describe the distribution and abundance of Dwarf Seahorse along the Texas Coast
2) Describe the habitat associations of Dwarf Seahorse along the Texas Coast
3) Describe the sex and morphometrics of Dwarf Seahorse along the Texas Coast
4) Compare catch per unit effort of Dwarf Seahorse using different types of sampling gear

Acknowledgments

We would like to thank the team of EIH staff and graduate students that assisted with the field sampling for this project including: Jake Doyal, Natasha Zarnstorff, Matthew VanBemmel, and Kaylei Chau; as well as the field assistance from our United States Fish and Wildlife Department partner and support staff: Roy Ulibarri, Jennifer Whitt, Adriana Leiva, and volunteer Fernando Carvallo. Coordination and completion of the genetic component of the project was facilitated by Steven Mussmann from the Southwestern Native Aquatic Resources and Recovery Center (Unites States Fish and Wildlife Services, Dexter, NM), and Dr. Brian Stephens (University of Houston Clear Lake). Genetic samples from Florida were provided by Nate Fedrizzi (Pews Charitable Trust). Genetic sequencing was conducted with help from Zeb Woiak with the Midwest Fisheries Center (United States Fish and Wildlife Service, Onalaska, WI). Internal support for the project was provided by numerous UHCL departments and especially EIH's Department Assistant III, Patti Koch. All sampling was conducted under Texas Parks and Wildlife Department Scientific Collection Permit SPR-0504-383.

Abstract

Dwarf Seahorses (*Hippocampus zosterae*) are found in shallow waters along the Atlantic Ocean ranging from Bermuda to the Bahamas and within the Gulf of Mexico. The Dwarf Seahorse is the smallest species of seahorse in the Western Hemisphere, averaging about two centimeters in height. The preferred habitat of Dwarf Seahorses is seagrass beds, which provide anchors for their prehensile tails as well as places to hide from predators. There are five species of seagrass in Texas. Species composition and percent cover varies by bay system, but in general, have declined over time due to a combination of natural and anthropogenic stressors. Like other seahorse species, male Dwarf Seahorses gestate and birth the young. They exhibit annual protracted iteroparity with a life span of approximately 2 years. The Dwarf Seahorse is currently a candidate species for federal listing, and data are particularly lacking for this species in Texas. This study represents the first coast-wide survey of Dwarf Seahorse in Texas and provides valuable baseline data for future assessments. Previous studies and Texas Parks and Wildlife Department routine monitoring had documented the presence of Dwarf Seahorse as occasional or incidental catch. Field sampling was divided into two years. Year-1 (2020) was a coast-wide distribution and abundance assessment maximizing spatial coverage using push nets, and year-2 (2021) was a gear comparison study focused on a sub-set of sites with the highest Dwarf Seahorse catch from year-1. Seagrass species, percent cover, canopy height and biomass were monitored at each site, and water quality and other physical habitat characteristics were recorded. Morphometrics, maturity and sex were determined and genetic samples were collected from Dwarf Seahorses.

A total of 79 Dwarf Seahorses were captured at 30 of the 80 sites that were visited in year-1 with an overall catch per unit effort (CPUE) of 0.017 individuals per meter^2. They were caught in all bay systems except for Galveston Bay with the highest CPUE in Aransas Bay. Dwarf Seahorse CPUE was positively correlated with an increase in seagrass diversity. The presence and percent cover of Turtle Grass (*Thalassia testudinum*) was significantly correlated with the CPUE and presence of Dwarf Seahorses. In year-2, the most effective gear type for capturing Dwarf Seahorses was the Throw Trap (CPUE = 0.222) followed by the push net (CPUE = 0.019). The Beam Trawl captured a single individual (CPUE = 0.003). We failed to capture any Dwarf Seahorses using a 15’ straight seine or the 60’ bag seine, which was based on the same design as the standard bag seine used by by TPWD coastal fisheries monitoring program. With year-1 and year-2 Dwarf Seahorses combined, a total of 90 individuals (33 juveniles, 40 females and, 17 males) were included in demographic analysis. Tail to height ratio was helpful in distinguishing both maturity and sex; while the snout to height ratio was helpful for determining maturity. Initial genetic assessment confirmed that Texas Dwarf Seahorses have different genetic lineages compared to samples examined from Florida. Preliminary analyses suggest that, within the Texas population, there are at least two distinct lineages which appear to follow a latitudinal gradient along the coast. Dwarf Seahorse catch was highest at sites which had a high diversity of seagrass and contained Turtle Grass, which is a climax species, suggesting they were well established beds. While throw traps were the most effective gear type, they also were the most labor and time intensive to use. Continued state-wide monitoring is recommended to examine seasonal trends and track potential changes in population demographics of Dwarf Seahorses. Gear type should be a careful consideration depending on the goal(s) of continued monitoring. Further analysis of the preliminary genetic results will help determine the exact extent that migration is occurring among Texas bays and the mechanisms that may be supporting that genetic connectivity.

Introduction

Dwarf Seahorses (*Hippocampus zosterae*) are found in shallow waters along the Atlantic Ocean ranging from Bermuda to the Bahamas and within the Gulf of Mexico (Bohlke and Chaplin 1966, Ginsberg 1937, Irey 2004). Dwarf Seahorses have a form similar to most other seahorse species, with a head at a right angle to their body, a prehensile tail that lacks a caudal fin, and bony plates that appear as rings underneath their thin skin (Irey 2004). This species can vary in coloration, appearing beige, yellow, green, and black, with some exhibiting white or dark markings (Lourie et al. 2004). Other identifiable features include the presence of 10 to 13 dorsal and pectoral fin rays, nine to 10 trunk rings, a snout that is one-third its head length, skin covered in small warts, and a knob-like coronet without spines or projections (Lourie et al., 2004). The Dwarf Seahorse is a member of the Syngnathidae family is the smallest species of seahorse in the Western Hemisphere, with other species in the Western Hemisphere being significantly larger and not possessing the distinctive short snout (Lourie et al. 2004). Their small size makes them distinguishable from other seahorses, averaging about two centimeters with a maximum recorded length (height) of 2.5 centimeters (Lourie et al., 2004). One of the challenges in monitoring Dwarf Seahorse populations is gear selectivity due to their small size (Masonjones et al. 2017). As a result, instances of incidental catch from coastal monitoring projects and other research studies are rare.

Dwarf Seahorses have historically been found in highest abundances along the Florida coast, particularly Florida Bay which represents the best studied population in their range (Irey 2004, Carlson et al. 2019, NMFS 2020). A population analysis in Florida estimated there are approximately 2.1 million individuals present in the Florida Bay and Cedar Key areas alone (Carlson et al. 2019). Dwarf Seahorse are captured extensively in Florida for the pet trade, with an average of 17,000 individuals being legally harvested each year (Carlson et al. 2019). While most of these individuals are captured by divers, some are caught as bycatch in shrimp trawlers (CBD 2011). Direct capture is the only legal way to obtain Dwarf Seahorses and legal only in Florida. Any other Dwarf Seahorses in the trade can be considered poached, including any individuals captured from Texas (Carlson et al. 2019). While bycatch from commercial bottom trawling is a threat to larger seahorse species, it may not be so to Dwarf Seahorses due to their smaller size. Dwarf Seahorses may be caught as bycatch if netting is fine enough, but a study in Florida indicated they are not captured at the same quantities as their larger cousin, *H. erectus* (Baum et al. 2003). Estimations based on this data indicate that approximately 150 Dwarf Seahorses are captured as bycatch each year by shrimping boats in Florida (Carlson et al. 2019). Less research has been done on Dwarf Seahorse within Texas despite historical evidence indicating they reside along the entire Texas Coast (Bruckner 2005, Hoese et al., 1998).

Seagrass beds provide essential nursery habitat for multiple species, including the Dwarf Seahorse, allowing for recruitment and development of larval and juvenile life forms (Jackson et al. 2001, Pulich and Onuf 2002). Additionally, they act as feeding areas and provide refuge from predation (Jackson et al. 2001). Indirectly, seagrass beds maintain sea life by providing organic matter which is an important part of nutrient cycling and the detrital food web (Jackson et al.

2001). Their extensive root and shoot systems stabilize sediments and slow water velocity, which in turn increases water clarity and reduces erosion (TPWD 2017). Dwarf Seahorses prefer seagrass bed habitats, because grass blades provide anchors for the seahorse's prehensile tail as well as cover from predators. Bed characteristics and species composition are regulated by water depth, light and nutrient availability, sediment type, hydrodynamic regimes, and local faunal activity (Robbins and Bell 1994). Research has also shown that seagrass beds can cause a 50% reduction in the amount of potential bacterial pathogens capable of causing disease in humans and marine organisms (Lamb et al. 2017). Seagrass species that form seagrass beds are considered foundation species, meaning they are crucial to maintaining species assemblages within associated ecological communities in bays along the Texas Coast and around the world (Hughes et al 2009). Of the 50 species of seagrass identified worldwide, the Texas Parks and Wildlife Department (TPWD) has confirmed the presence of five species on the Gulf Coast (TPWD 2017) (Table 1).

Table 1.Summary table of the types of seagrass and macroalgae encountered in Texas coastal waters.

Common Name	Scientific Name	Species Code	Physical Characteristics
Shoal Grass	*Halodule wrightii*	HAWR	▪ thin, flattened, tinsel-like leaves ▪ blade tip with three points ▪ 10-30 cm in length
Star Grass	*Halophila engelmannii*	HAEN	▪ leaves oriented in star-like whorl ▪ leaves in clusters of 4-8 blades ▪ < 10 cm in length
Manatee Grass	*Syringodium filiforme*	CYFI	▪ cylindrical and more ridged leaves ▪ leaves have rounded blade tip ▪ 50+ cm in length
Turtle Grass	*Thalassia testudinum*	THTE	▪ broad, flat, ribbon-like leaves ▪ prefers high salinity, calm water ▪ 20-50 cm in length
Widgeon Grass	*Ruppia maritima*	RUMA	▪ alternating leaf blades along stems ▪ wide salinity range (including fresh) ▪ 10-30 cm in length
Macroalgae	N/A	MACRO	▪ species of green, grown, and red algae ▪ typically, mat forming ▪ dominated by *Gracilaria* spp. and *Digenea* spp.

All species of seagrass worldwide seem to be experiencing widespread declines, based on anecdotal and quantitative analyses (Hughes et al. 2009; Pulich and White 1991; Handley et al. 2007). Decreased seagrass survival and growth are most likely due to a variety of stressors, such as reduced water quality, overgrazing, and physical damage (Hughes et al. 2009). While there is no consistent, long-term, monitoring of seagrass that covers the entire Texas Coast, previous studies have assessed seagrass coverage and health in four of the bay systems within the Dwarf Seahorses' range (Aransas, Corpus Christi, and the Upper and Lower Laguna Madre Bay systems) (Congdon and Dunton 2016). Overall, seagrass health was found to be spatially variable but generally stable, though shifts in water quality were observed in times of drought. The Upper Laguna Madre can shift to hypersaline conditions, which are well above the physiological thresholds of some species of seagrass (Congdon and Dunton 2016). Increases in suspended sediments can decrease light attenuation and has the potential to bury seagrass beds, especially in instances of dredging and extreme weather events (e.g., hurricanes or floods) (Congdon and Dunton 2016). Increased nitrogen enrichment due to input by wastewater treatment facilities may impair water quality and promote micro- and macroalgae species that can outcompete seagrasses (Congdon and Dunton 2016, Dunton et al. 2011). Due to these environmental and anthropogenic hydrologic impacts, as well as natural variability in water depth, seagrasses are unevenly distributed along the Texas coast. The primary factors affecting the gradient of seagrass bed coverage and species composition along the Texas coast are freshwater inflow and water temperature (Pulich and Onuf 2002). Declines in seagrass as a foundation species can lead to declines in the many species that depend on this habitat, including Dwarf Seahorses.

Dwarf Seahorses are relatively short-lived with a life span of 2 years and reaching sexual maturity at 3 months (Strawn 1958, Lourie et al. 2004). Like most *Hippocampus* species, Dwarf Seahorses are thought to be monogamous due to the nature of their reproduction and their limited active movement potential. Males possess a pouch where the female deposits unfertilized eggs. The male then fertilizes the eggs internally and provide the developing eggs with nutrients and oxygen for the remainder of gestation. In Dwarf Seahorses, gestation is roughly 12-14 days, with only 4-20 hours of recovery between broods (Rose et al. 2014; Masonjones 2001). The largest documented brood size for a male Dwarf Seahorse was 55 young, but this is considered exceptional (Strawn 1958). The annual protracted iteroparous breeding season for Dwarf Seahorses occurs between February and October, but during the beginning and end of the breeding season, larger individuals represent a larger proportion of the breeding population (Strawn 1958). In tropical environments they can exhibit constant iteroparous breeding. The monogamous nature of Dwarf Seahorses limits gene flow, though no studies have been conducted to determine if Dwarf Seahorses are serially monogamous, where monogamy only occurs during one breeding event, and a new mate is chosen for the next breeding event, which would increase gene flow over true monogamy (Woodall et al. 2011). Studies to discover if Dwarf Seahorses are truly monogamous would be difficult due to the small size and cryptic nature of Dwarf Seahorses, which makes them difficult to mark, recapture, or observe. For example, during mark and recapture surveys conducted in Tampa Bay, only a few individuals were recaptured out of hundreds of marked individuals (Masonjones et al. 2010; Rose et al. 2019).

Threats to Dwarf Seahorses include overharvesting, bycatch, and habitat destruction. Dwarf Seahorses are common in the pet trade, captive breeding of all *Hippocampus* species is currently experimental with no established facilities (Vincent and Koldewey 2006). This lack of viable captive stock has led to increased harvest pressure on wild populations (Vincent and Koldewey 2006, Vincent et al. 2011). Shrimp trawling using conventional otter trawl doors and weighted lead lines, can directly disturb the seagrass beds by uprooting vegetation and destroying the habitat. When roller trawls are used there is a lessened impact on the seagrass beds over a short time period, but long-term impacts have not been investigated (Meyer et al. 1999). Seahorse bycatch, which is not reported, is often part of the international trade of seahorses, which are harvested to be used in traditional eastern medicine or for the pet trade (Baum and Vincent 2005). Compounding these stressors, coastal development can directly and indirectly destroy seagrass beds through runoff of chemicals and debris leading to increased turbidity, reduced oxygen availability, and algal blooms (Lewis and Devereux 2009).

Dwarf Seahorse are listed as a species of least concern globally by the IUCN Red list, though they are currently a candidate species for federal listing due to existing data being deficient for the purposes of assessing population viability (Masonjones et al. 2017, NOAA 2012). The global population of Dwarf Seahorses is considered stable but insufficient data for the Texas population(s) resulted in inability to conduct statistical analysis for population trends, and recommendations for additional monitoring in the past (Masonjones et al. 2017). There have been a variety of studies of this species throughout their entire range, but durations, gear, and sampling frequencies have been inconsistent (Masonjones et al 2017). Dwarf Seahorse data in Texas consist primarily of historical observations such as incidental catch from TPWD routine monitoring, and sporadic occurrences documented during scientific studies throughout the coast (Masonjones et al 2017, NMFS 2020).

The life history of Dwarf Seahorse including short life span and extremely limited active mobility result in limited spatial dispersal (Vincent 1996, Fedrizzi et al. 2015). When juvenile seahorses are released from their father’s pouch they settle almost immediately, with little dispersal occurring (Strawn 1958). It is theorized that the primary methods for progeny dispersal is through rafting, where seagrass breaks away from the sediment and is transported to a new location in currents (Masonjones et al. 2010). Most studies of the genetic connectivity of Dwarf Seahorses within the Gulf of Mexico have occurred in Florida (Fedrizzi et al. 2015, Rose et al. 2014). Although rafting is an unreliable mode of dispersal, most Florida populations of Dwarf Seahorses are genetically similar, which shows that rafting must occur often enough for the populations to retain a level of genetic connectivity (Fedrizzi et al. 2015). However, as the distance between populations increases, the likelihood that a rafting event will connect populations decrease. The reliance on this unreliable dispersal method makes protecting Dwarf Seahorses even more important to ensure that populations can remain connected and genetically viable.

Genetics work with Dwarf Seahorses in Texas is minimal, with much of the work focusing on Florida, the mitochondrial genome, or small sample sizes (Fedrizzi et al. 2015, Rose et al. 2014). The full genome has been sequenced for other seahorse species, including the Lined Seahorse (*Hippocampus erectus)*, which is one of the closest living relatives of the Dwarf Seahorse (Lin et al. 2017). While this could be helpful for conducting nuclear genome sequencing of Dwarf Seahorses, the seahorse genome is one of the fastest evolving genomes of any marine fish, which can cause even relatively closely related species to have high genetic divergence (Lin et al. 2016).

Our study is the first state-wide assessment aimed at characterizing the distribution, abundance, demographics and genetic connectivity of Dwarf Seahorse populations along the Texas Coast. Additionally, a gear comparison study was conducted to investigate the selectivity of various gears used to target small cryptic species as well as some more widely used standardized gear types used for non-selective coastal monitoring. This information will provide critical information needed to assess the health and distribution of Texas seagrass beds.

Methods

Site Selection

Sampling occurred at 80 sites along the Texas Coast. These sites were selected from those initially established by the Texas Seagrass Project so that sampling occurred in areas with studied and established seagrass beds (Dunton, 2022). Five sites were chosen in the Galveston Bay system, five in the Matagorda/East Matagorda Bay system, ten in the San Antonio Bay system, ten in the Aransas Bay system, ten in the Corpus Christi Bay system, and twenty in each the Upper and Lower Laguna Madre systems. Sites originally monitored as part of the Texas Seagrass Project were filtered to remove sites with an average depth greater than 1.22 meters (m) due to limitations of gear types. Then, historic occurrences of Dwarf Seahorse were spatially evaluated and assigned a seagrass monitoring site if one was present within the same contiguous seagrass bed and within a distance of 2,000 m. Additional seagrass monitoring sites were chosen to represent a range of seagrass species composition, percent cover, and spatial distribution throughout the bay to reach our target number of sites per bay system (Figure 1 and Table 2). A total of 91 backup sites meeting each criterion were also selected in case sampling could not be conducted at some sites upon arrival in the field. The Matagorda Bay system was not part of the Texas Seagrass Project, so sites were selected based on the TPWD seagrass map, with sites randomly generated on each side of the bay system within seagrasses.

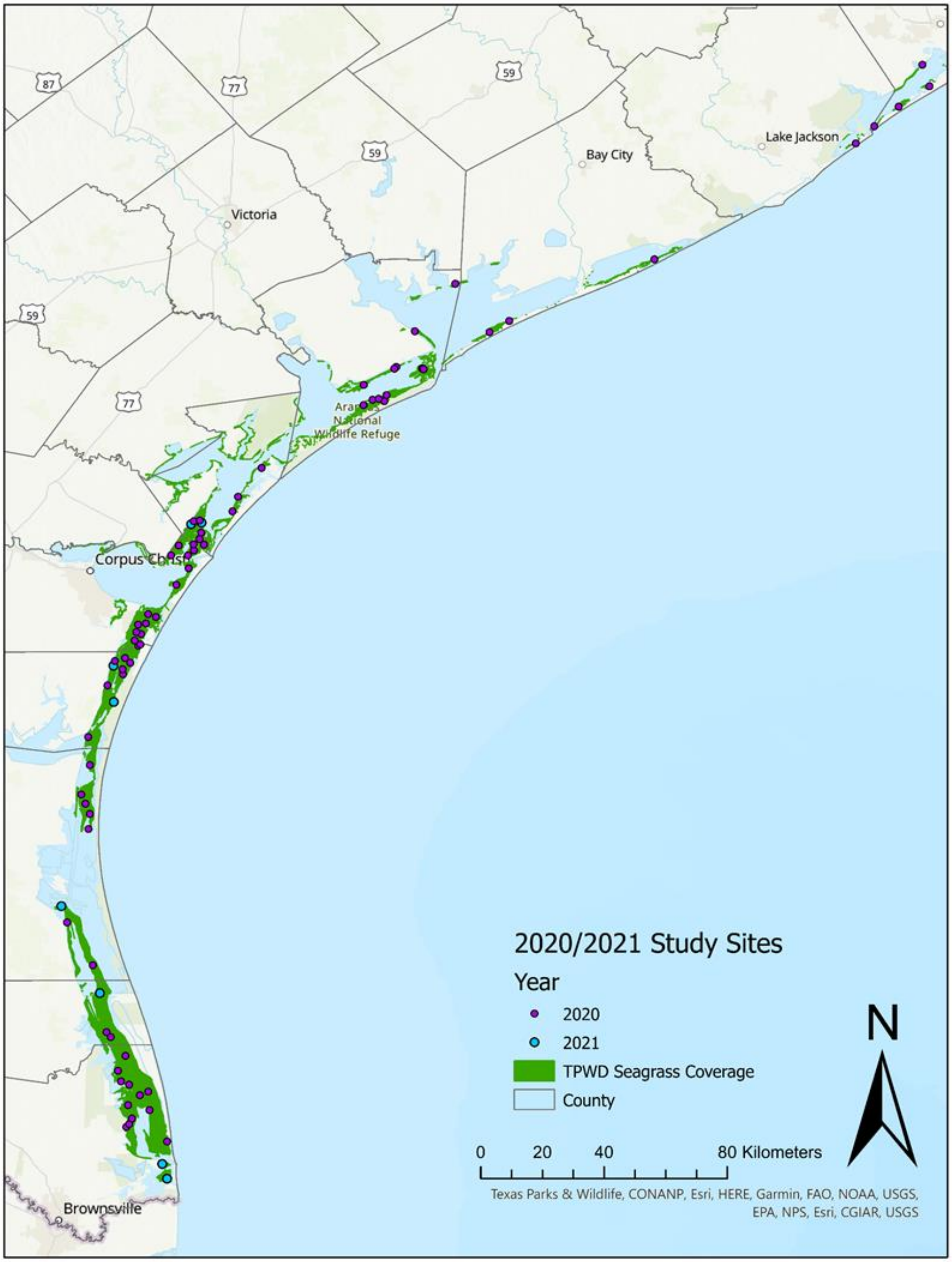


Figure 1. Map of study sites and seagrass coverage. All sites were included in the year-1 (2020) distribution and abundance study, and the sites in blue were chosen based on high catch of Dwarf Seahorse for an intensified gear comparison study in 2021.

Table 2. Summary of site selection criteria and final site distribution among the bay systems included in the study.

Major Bay System	Included Sub-bays	Open Water (km^2)	Seagrass Cover (km^2)	Seagrass % Cover	Number of sites	Historic Sightings
Galveston	West, Christmas	1531.66	3.13	0.20	5	1
Matagorda	Matagorda, East Matagorda	1141.26	25.29	2.22	5	0
San Antonio	Espiritu Santo, San Antonio	521.37	71.49	13.71	10	4
Aransas	Aransas, Mesquite	574.18	67.45	11.75	10	16
Corpus Christi	Corpus Christi	539.62	99.92	18.52	10	13
Upper Laguna	Upper Laguna, Baffin	561.81	199.80	35.56	20	44
Lower Laguna	Lower Laguna	693.81	461.74	66.55	20	15

Field Methods

Year-1: Coast-wide Distribution and Abundance Study

Site coordinates were recorded in NAD83 at each site using a handheld GPS unit (Garmin eTrex, Garmin LTD., Olathe, KS). Salinity (psu), pH, water temperature (C), turbidity (NTU), specific conductivity (uS), and depth (m) were measured with a multiparameter sonde (ProDSS, YSI Inc., Yellow Springs, OH) at each site. Photosynthetically active radiation (PAR) was measured using a LI-COR meter at the seagrass canopy height depth and on the deck of the boat at each site. Water transparency was also measured using a 1.2 m Secchi tube (m). Physical environmental conditions such as wind speed (mph) and direction were also recorded using a hand-held anemometer (Kestrel 2000, Kestrel Instruments, Boothwyn, PA).

At each site a 20 m by 20 m square plot (400 m^2) was delineated using pvc poles and used as the sampling area. Within this plot, four points were randomly selected using a random number generator function for vegetation quadrat placement (Figure 2). The 0.25 m by 0.25 m square (0.0625 m^2) vegetation quadrats were placed at each of the four random points. Percent coverage by seagrass species or other habitat types (e.g. macroalgae or oysters) and canopy height (cm) of seagrass were recorded within each quadrat. If seagrass were present, a biomass core (10 cm diameter) with a target depth of 20 cm below the sediment-water interface (total sample volume 78.5 cm^2) was used to collect whole seagrass biomass within the quadrat. The biomass core was processed at the site using a fine mesh bag to wash out sediment, and then stored on wet ice and returned to the lab for analysis.

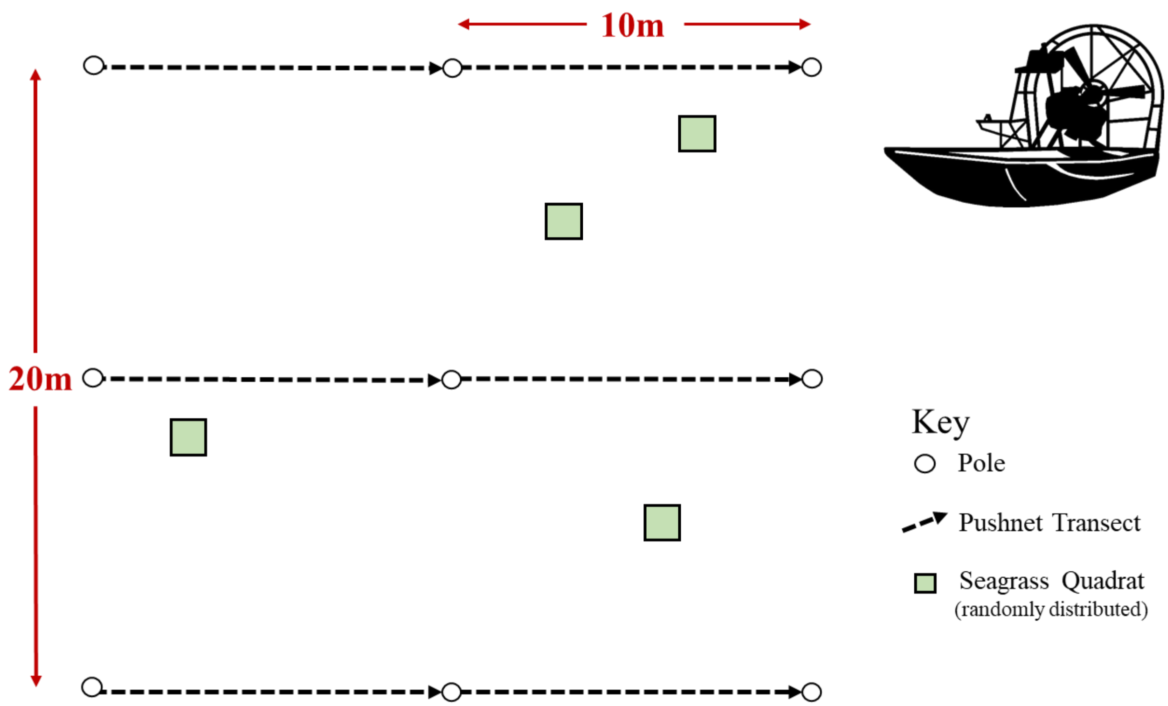


Figure 2. Example of site layout for the year-1 (2020) distribution and abundance study of Dwarf Seahorse. A 20 m by 20 m grid was laid out using poles, with careful attention to not disturb the push net transects. The green squares provide examples of the randomly distributed seagrass quadrat locations, although their exact placement varied at each site.

Within the site sampling area, six replicate 10 m pushes were performed using a 1 m^2 push net with 1/32" mesh (Figure 3) (Strawn 1958). The pushes were conducted facing into the prevailing movement of the water to ensure that the motion of the waves did not cause a loss of catch. Two pushes were conducted on each of the sides of the sampling area, and two were conducted through the middle. At the end of each tow, the net was lifted horizontally out of the water and the catch was sorted, identified, and recorded. Some pushes were stopped before the target 10 m distance when the net filled up with vegetation. When this occurred, the distance travelled was estimated to the nearest whole meter and recorded to calculate effort. Additionally, some sites had less than six pushes conducted due to time constraints. To ensure that each site was surveyed in a single day, a time limit of four hours per site was used. If the time limit was imminent, no more pushes were conducted at that site, provided a minimum of three replicate pushes had already been conducted. During field sorting of catch, all Dwarf Seahorses that were found were

Figure 3. Photo of push net used in the study.

placed in individual sample vials and assigned a unique identification number. Next, any specimens of other nekton species from the catch that were too large to archive were identified on site, photographed, and released. After the sample had been field-examined for Dwarf Seahorse, the remainder of the catch was placed into a sample bottle with seawater. All catch was treated with a lethal dose of MS-222 and then preserved. Formalin was added to the bottles containing other nekton species to create a solution of 10% formalin. The Dwarf Seahorse sample vials were preserved by placing them on wet ice. At the end of the day, the seahorses were blotted dry and frozen (-1^{o}C) for long-term preservation.

Year-2: Gear Comparison Study

During the summer of 2021, eight of the sites sampled in 2020 which contained some of the highest seahorse catches were revisited to compare the collection efficiency of multiple gear types. Four of the sites were in the Lower Laguna Madre and two each in the Upper Laguna Madre and Aransas Bay (Figure 1). Sampling was conducted using 5 different gear types: push net, throw trap, beam trawl, 15 ft straight seine, and 60 ft bag seine. The push net was the same design as was used in the year-1 (2020) study. The throw trap was a 1 m^2 trap constructed of aluminum with 1/32" mesh. The Renfro Beam Trawl "beam trawl" was manufactured by Sea-Gear Corporation of Melbourne, Florida with 333-micron mesh (Renfro 1963). The 15' x 4' straight seine with 1/8" bar width, and the 60' bag seine was built to the same specifications as the TPWD coastal fisheries monitoring net as described in Martinez-Andrade (2015). This sampling was conducted to compare Dwarf Seahorse capture rates between the different gear types and to determine which gear was the optimal method to maximize Dwarf Seahorse capture.

The same water quality and physical habitat data as collected in year-1 were recorded at each site. Seagrass community data and cores were not collected in year-2, however the presence of seagrass species was qualitatively noted. Before sampling began, the site was divided into lanes for sampling to ensure that no area was sampled twice and to designate areas where people could walk without disturbing sampling areas. Figure 4 shows an example of this setup, with the boat in the middle, throw traps thrown around the boat, and a ring of 9 lanes around the boat for the other sampling methods (not necessarily in that order). The throw trap gear type used an exhaustive sampling technique where once the trap was in place, a net was used to sweep the area inside the trap in alternating directions until there were three sweeps in a row with no catch. This small frame net was manufactured to fit snugly within the throw trap dimensions to prevent organisms from swimming around it. Each of the other gear types were sampled starting at the point furthest from the boat in a direction back toward the boat. Three replicates were conducted for each gear type, except for the 60' bag seine. When sampling at the site was finished the crew moved to the nearest shore-line and conducted a single 50ft pull of the 60' bag seine following the TPWD protocols (Martinez-Andrade 2015). The catch from each replicate was bottled with seawater and then a lethal dose of MS 222 was added followed by buffered formalin to create a 10% formalin solution for preservation.

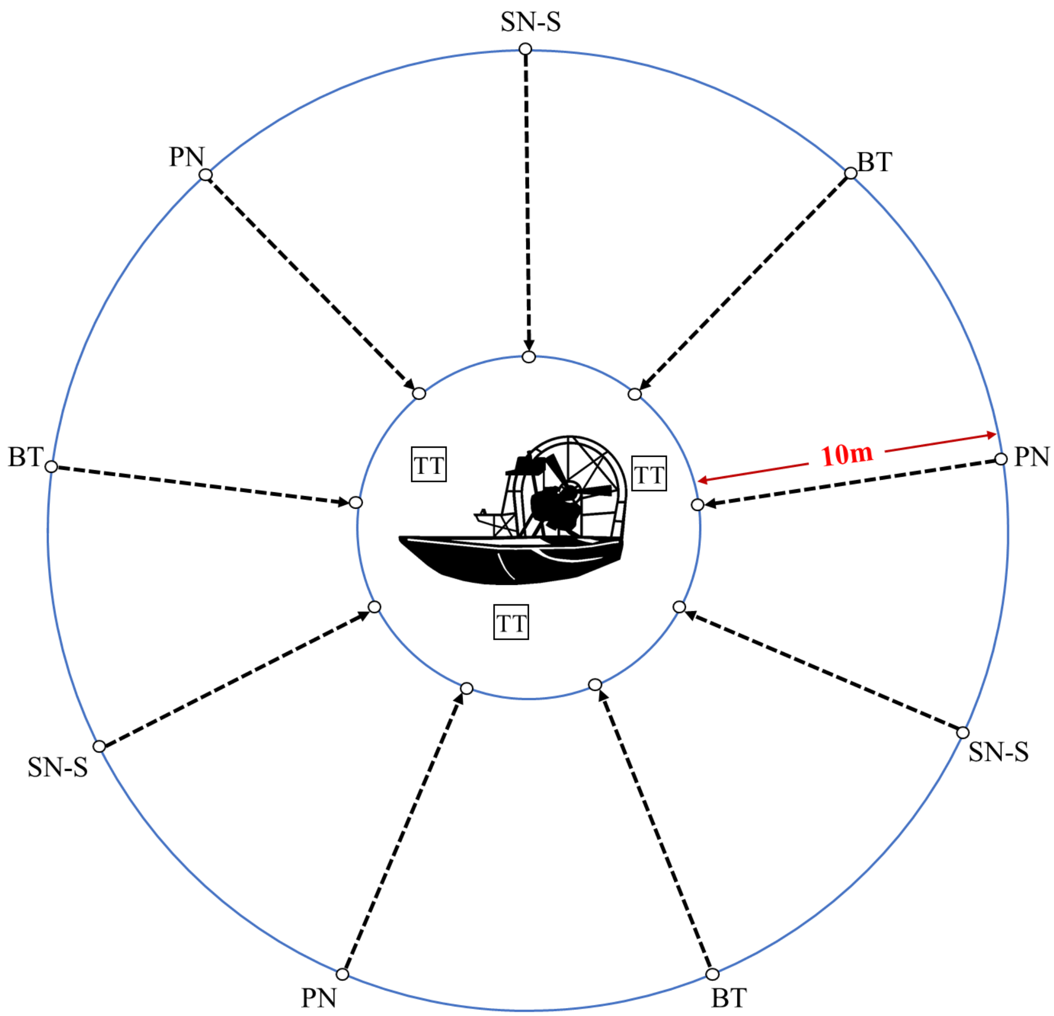


Figure 4. Example of site layout for the year-2 (2021) gear comparison study of Dwarf Seahorse. Three replicates of each gear type were conducted around the central location of the vessel. Throw trap (TT), push net (PN), beam trawl (BT), and 15' straight seine (SN-S).

Laboratory Methods

Dwarf Seahorses that were placed in sample vials and frozen were transported to the university laboratory at EIH for long-term storage. Each frozen seahorse was briefly thawed before being photographed, weighted, and measured using Olympus cellSens imaging software. The tail length (from tail tip to the posterior edge of the dorsal fin), total height (from tail tip to the tip of the coronet), and snout length (from the tip of the snout to the center of the eye) were measured, and then maturity and sexed were determined (Figure 5). The seahorses were then blotted by hand with a dry paper towel to remove moisture and refrozen for later genetic analysis. Any seahorses that were missed during the initial field sorting and instead had been preserved in formalin with the other species of nekton were still measured and photographed but afterwards were preserved in 70% ethanol instead of being frozen and were not included in the genetic analysis.

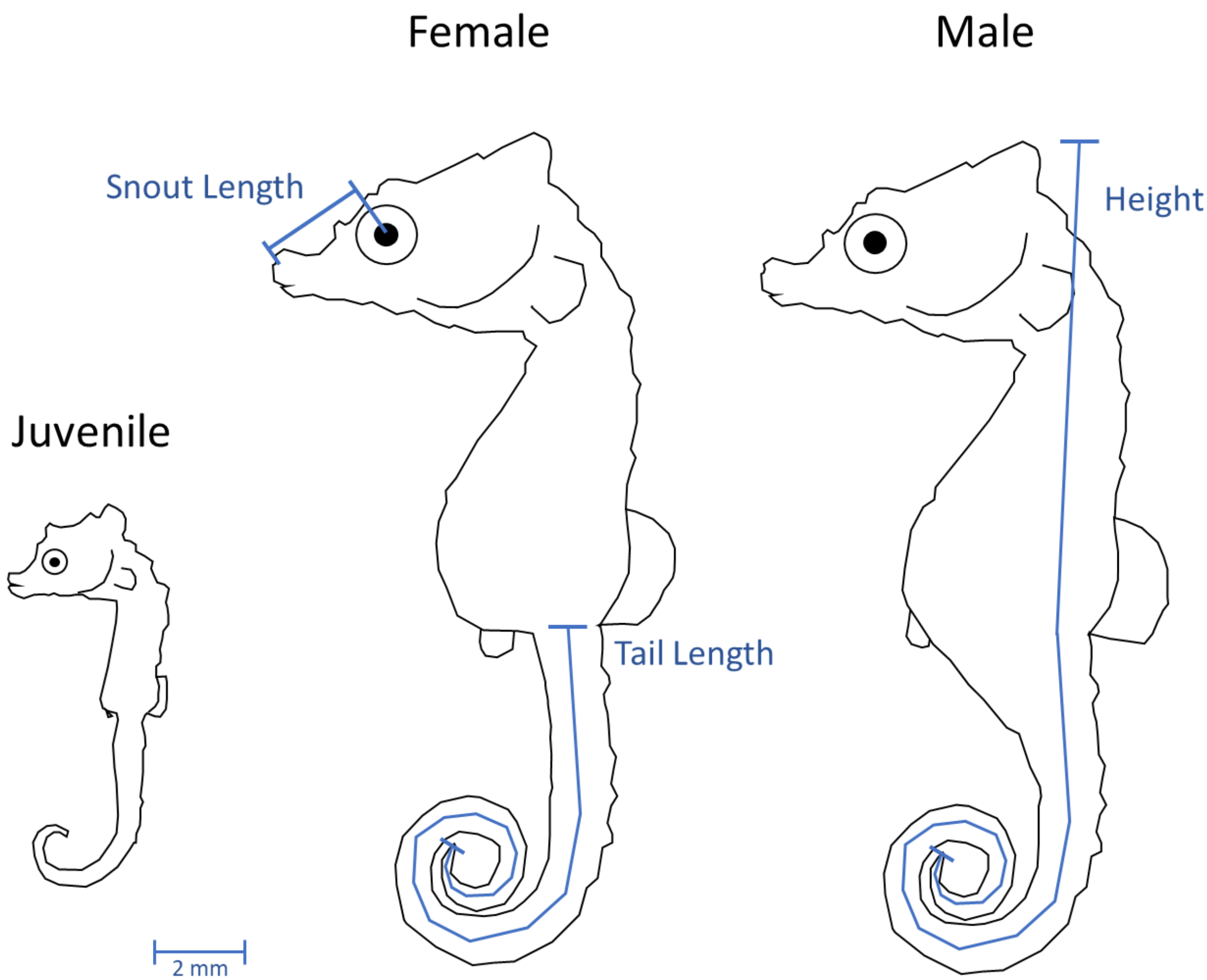


Figure 5. Developmental and sexual differences in body shape of Dwarf Seahorses and measurements.

The remainder of the nekton catch that was retained and preserved in buffered 10% formalin and brought back to the lab where they were sorted, identified to the lowest possible taxon, and enumerated. Most invertebrates were identified to family or genus. Spelling and format of scientific and common names followed the guidelines of the American Fisheries Society (McLaughlin et al. 2005; Cairns et al. 2003; Turgeon et al. 1998).

The biomass cores were cleaned under flowing water over a 33 µm sieve and the tentatively viable (i.e. intact fragments of green) seagrass were separated from dead seagrass (i.e. degraded, brown), algae, and other debris. The seagrass from each of the four replicates per site were combined and dried in an oven set at 60°C for three to five days. Once the seagrass was dried (determined by a consistent weight), it was weighed to the nearest 0.1 mg to obtain a total above and below ground biomass.

Genetics Methods

To determine genetic ecology of the Dwarf Seahorse, 72 individuals across 5 bay systems were used for genetic analyses (14 mature males, 39 mature females, and 19 juveniles). To isolate DNA, individuals were thawed and then a portion of the body was cut into pieces using a dissection scalpel. For larger mature Dwarf Seahorses, the tail-only was used, but for smaller individuals and juveniles the entire body was used for isolation with the goal of obtaining similar quantities of DNA. Between each individual a new scalpel blade was used, and the workspace was cleaned with 95% ethanol. Dwarf Seahorse DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD). The prepared tissue was placed into a 1.5 mL microcentrifuge tube with 180µL of Buffer ATL and 20µL of proteinase K. This resulting mixture was vortexed and incubated at 56°C while being monitored until all of the tissue cells were lysed and there were no tissue clumps remaining (the amount of time required varied by sample but generally took between 20 and 60 minutes). During incubation, samples were vortexed approximately every 5 minutes to prevent clumping and ensure efficient lysing. The remaining extraction methods followed the DNeasy Blood & Tissue Kit instructions (Qiagen, Germantown, MD).

DNA samples were prepared for sequencing following double digest restriction-site associated DNA sequencing (ddRADseq) (Peterson et al. 2012). This method of sequencing does not require a reference genome and uses two restriction enzymes simultaneously alongside a size selection protocol to create a library of DNA fragments within a specific size range (Peterson et al. 2012). One of the restriction enzymes used is a frequent cutter, which targets a short palindromic DNA sequence, such as CCGG, while the other restriction enzyme is an infrequent cutter which targets a longer palindromic sequence, such as CTGCAG. This method is an advancement over previous restriction-site associated DNA sequencing (RADseq) methods which required random shearing of the DNA subsequent repair of the ends of the DNA fragments, leading to loss of a large amount of genetic material (Peterson et al. 2012). The preservation of this genetic material is why ddRADseq can be optimal when working with small species like Dwarf Seahorses.

Dwarf Seahorses from Texas bays were prepared alongside 144 individuals captured from 7 bay systems in Florida. Individuals from Florida had previously been analyzed as part of a mitochondrial study, so the DNA was already isolated (Fedrizzi et al. 2015). The extracted DNA for each individual was quantified using a Qubit 3.0 Fluorometer. DNA samples were not diluted due to the low concentrations found in some of the samples. Two different restrictions enzymes (MspI and PstI) were added to the DNA samples and then the samples were placed within a thermal cycler for 18-24 hours. Three libraries of samples were standardized to 20 ng of DNA, and 2 libraries were standardized to 25 ng of DNA. This was done to ensure the same amount of DNA from each sample was going into the library. The samples were size selected using the Pippin Prep, which uses agarose gel and electrophoresis to filter out all DNA fragments which are not within 325 and 425 bp. This size was selected based on the sizes of the genomes of other seahorse species, and includes the 79 bp adapters (Lin et al. 2016; Lin et al. 2017).

The libraries were sent to the Midwest Fisheries Center in Wisconsin for 150bp single-end sequencing on an Illumina NextSeq 500. Preliminary analysis was run at the Southwestern Native Aquatic Resources and Recovery Center on the sequences generated. The programs STACKS and ADMIXTURE were used to organize the data and get a preliminary look at the population structure by first filtering the samples down to 1 SNP per locus present in 50% of the individuals and then that SNP matrix was run through ADMIXTURE. In ADMIXTURE the matrix was run for between one and ten genetic clusters and each number of genetic clusters was tested 20 times. The resulting cross-validation values were compared to select the number of genetic clusters which best predicted the genetic diversity seen, then these data were run through the program CLUMPAK to crease visuals.

Data Analysis

Nekton and seagrass community structure were characterized by calculating total taxa abundance (N), relative abundance (%), taxa richness (S), Shannon Weiner Diversity (H), and catch per unit effort (CPUE). Catch per unit effort was defined by the number of individuals captured per m^2, regardless of the gear type. Community assemblage data as CPUE were log+1 transformed and a Bray-Curtis similarity index was generated between sample events using the PRIMER 7 statistical software package (Bray and Curtis 1957, Clarke and Warwick 2001). An analysis of similarity (ANOSIM) was conducted to ensure no differences in catch between replicates at each site. Replicates were then pooled and site groupings based on similar species assemblages were further investigated using ANOSIMs to test for significant patterns in community structure. Non-metric multidimensional scaling (nMDS) plots of assemblages were constructed in PRIMER 7 to visualize assemblage similarities. Pearson Correlations were conducted to determine which species were contributing to the data spread on MDS1 and MDS2 ordinations.

All physicochemical and habitat variables were tested for normality prior to statistical analysis (Shapiro and Wilk 1965). If data were determined to be non-normal, nonparametric statistical methods were used. Statistical analyses were conducted using R 4.1.2 (RStudio Team 2021). The relationship between catch per unit effort (CPUE) of Dwarf Seahorse and multiple variables were evaluated using either zero-inflated binomial or Poisson linear models (R, package pscl). The relationship between the presence/absence of Dwarf Seahorse and categorical variables were evaluated using the Kruskal-Wallis one-way analysis of variance on ranks with subsequent post-hoc Dunn’s or Mann-Whitney tests adjusted to reduce false discovery rates when applicable (Mann and Whitney 1947, Dunn 1964). For all statistical tests we used $\alpha = 0.05$. While we refer to some analyses in terms of the presence and absence of Dwarf Seahorse we cannot confirm absence, rather that we did not detect them at the site.

Results

Distribution and Abundance of Dwarf Seahorse (Year-1 2020)

A total of 80 sites were visited between Galveston Bay and the Lower Laguna Madre between June 2 and August 20, 2020. As expected, the upper Texas coast had generally lower salinity and higher turbidity water (Table 3). The lowest average turbidity levels were observed in the Aransas Bay system, although all three of the southern bay systems had average turbidity as measured with the handheld YSI datasonde less than 10 NTU. The average depth of the study sites was 0.76 m and ranged between 0.24 to 1.38 m.

Table 3. Water Quality data collected from the bottom of the water column (0.1 m from the sediment), from year-1 (2020) abundance and distribution study, averaged by bay system.

Major Bay System	Number of Sites	Avg. Total Depth (m)	Avg. Water Temp. (°C)	Avg. Salinity (psu)	Avg. DO (mg/L)	Avg. pH	Avg. NTU	Avg. PAR Difference (µmol of photons/m2*s)	Avg. Secchi (m)
Galveston	5	0.82	28.3	19.75	7.77	8.11	13.09	1178.75	0.23
Matagorda	5	0.68	28.6	23.85	7.11	8.18	30.19	1086.26	0.38
San Antonio	10	0.64	28.4	27.54	7.64	8.23	12.88	530.46	0.37
Aransas	10	0.77	28.8	30.51	5.04	8.16	12.15	434.26	0.63
Corpus Christi	10	0.65	30.5	34.59	5.24	8.12	4.06	478.58	0.93
Upper Laguna	20	0.90	30.2	34.73	5.23	8.05	7.31	667.42	0.64
Lower Laguna	20	0.72	30.9	23.84	7.05	8.41	9.01	809.09	0.62
Grand Total	**80**	**0.76**	**29.7**	**28.43**	**6.40**	**8.20**	**11.08**	**692.08**	**0.59**

Dwarf Seahorses were captured at 30 of the 80 sites representing 37.5 % of the sites surveyed (Table 4). Dwarf Seahorses were not captured in the Galveston Bay system. A total of 79 Dwarf Seahorses were collected in 2020 with the highest CPUE by bay recorded in Aransas Bay at 0.038 individuals per m^2 (Table 4 and Figure 6). The average CPUE of Dwarf Seahorse for all sites was 0.017 per m^2 and CPUE ranged from 0.017 (which is 1 individual per 60 m^2) to 0.136 (from a site in the Upper Laguna Madre where three individuals were captured in just 22 square meters of effort). There was no significant difference in the CPUE of Dwarf Seahorse by bay system (p-value = 0.259) (Figure 6).

A Dwarf Seahorse was captured at one of the five sites in the Matagorda Bay system with a CPUE of 0.017 per m^2 (Figure 7). In the San Antonio Bay system (which focused on Espiritu Santo Bay), Dwarf Seahorses were captured at three of the ten sites sampled with a CPUE of 0.017 per m^2 at one of the sites and 0.033 per m^2 at two of the sites (Figure 8). Aransas Bay sites focused around Redfish Bay had some of the highest CPUE for Dwarf Seahorse (Figure 9). Dwarf Seahorse were captured at six of the ten sites surveyed in Aransas Bay. In Corpus Christi Bay they were captured at four of the ten sites surveyed, with CPUE ranging from 0.017

to 0.033 per m^2 (Figure 10). Dwarf Seahorse were captured at seven of the twenty sites sampled in the Upper Laguna Madre with positive detections distributed throughout the spatial extent of the bay (Figure 11). Within the Lower Laguna Madre system, they were captured at nine of the twenty sites surveyed (Figure 12).

Table 4. Summary of Dwarf Seahorse capture data from year-1 (2020) abundance and distribution study by bay system. Catch per unit effort (CPUE) as number of Dwarf Seahorse captured per meter^2.

Major Bay System	Number of sites	Number of Dwarf Seahorse Captured	Percent of Sites with Dwarf Seahorse Detection	CPUE of Dwarf Seahorse
Galveston	5	0	0	0.000
Matagorda	5	1	20	0.003
San Antonio	10	5	30	0.008
Aransas	10	20	60	0.038
Corpus Christi	10	6	40	0.011
Upper Laguna	20	19	35	0.017
Lower Laguna	20	28	45	0.023
Grand Total	**80**	**79**	**37.5**	**0.017**

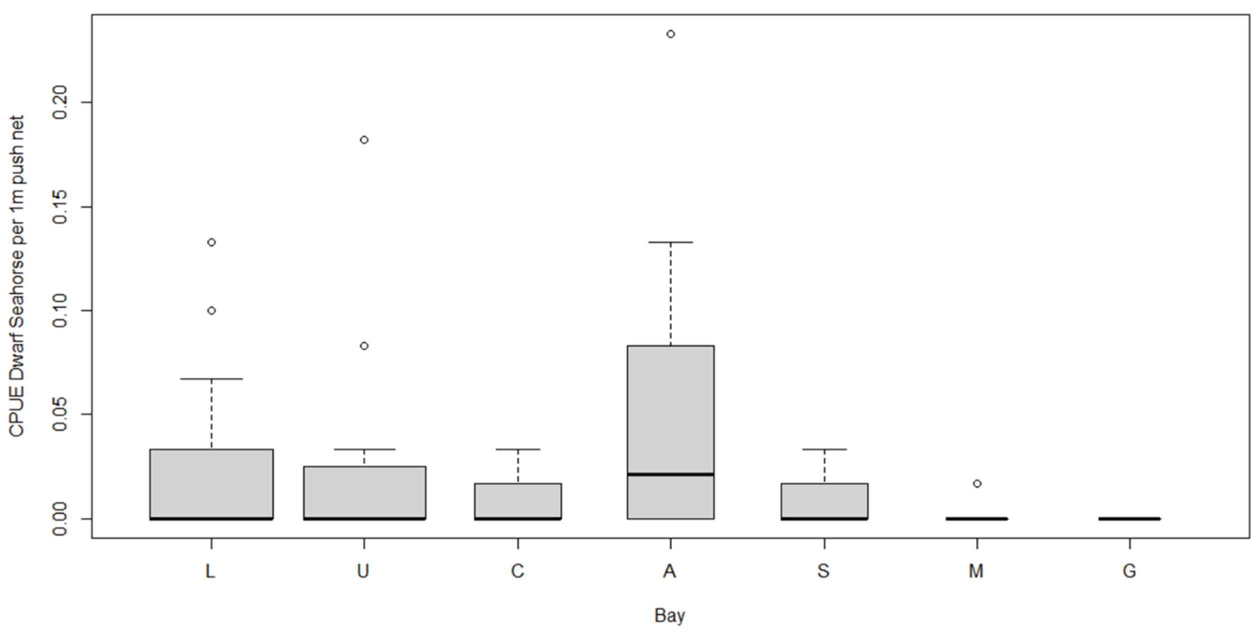


Figure 6. Boxplot of the catch per unit effort (CPUE) in number of Dwarf Seahorse captured per meter^2 by bay system. Lower Laguna Madre (L), Upper Laguna Madre (U), Corpus Christi Bay (C), Aransas Bay (A), San Antonio Bay (S), Matagorda Bay (M), and Galveston Bay (G).

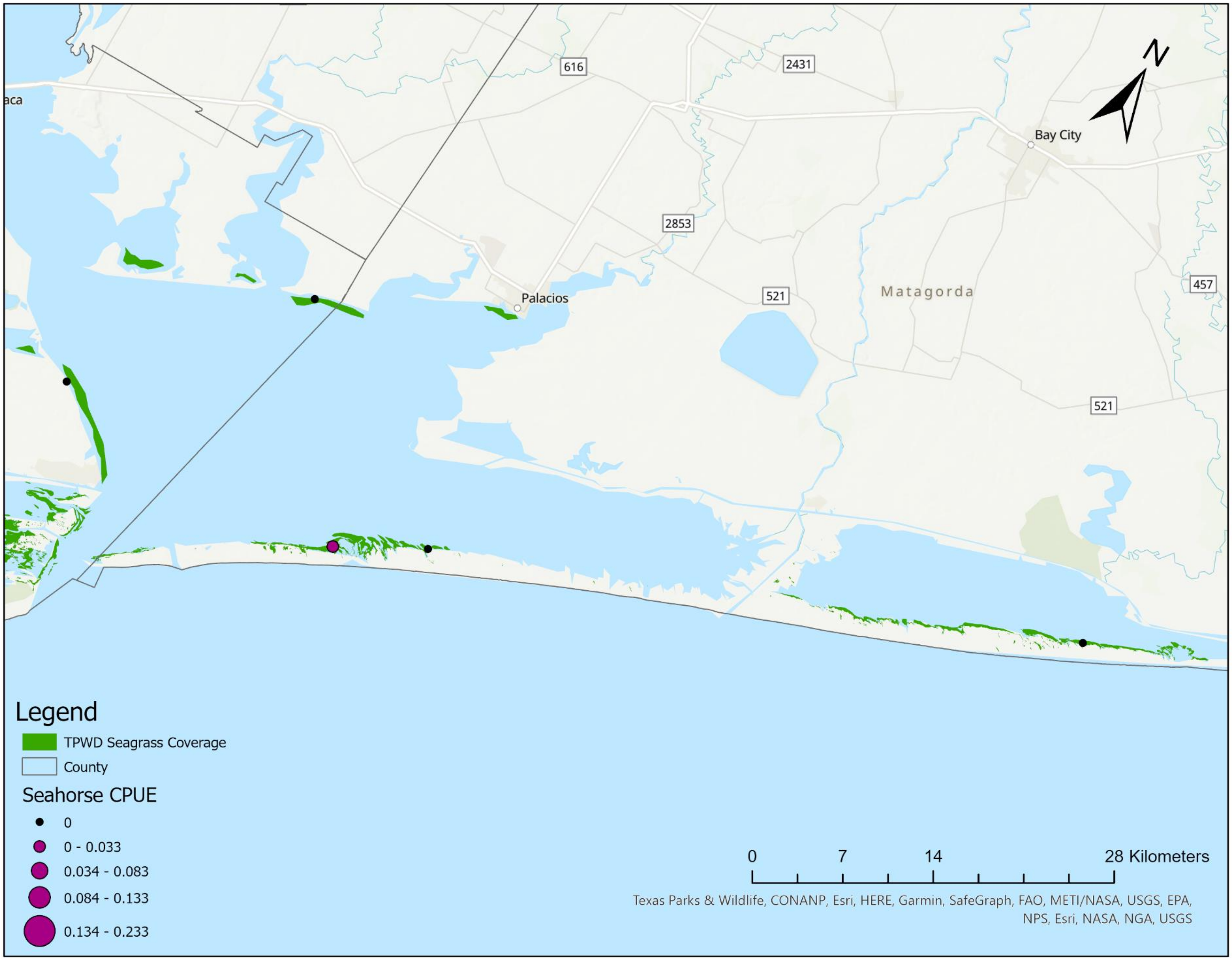


Figure 7. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in Matagorda Bay and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter^2.

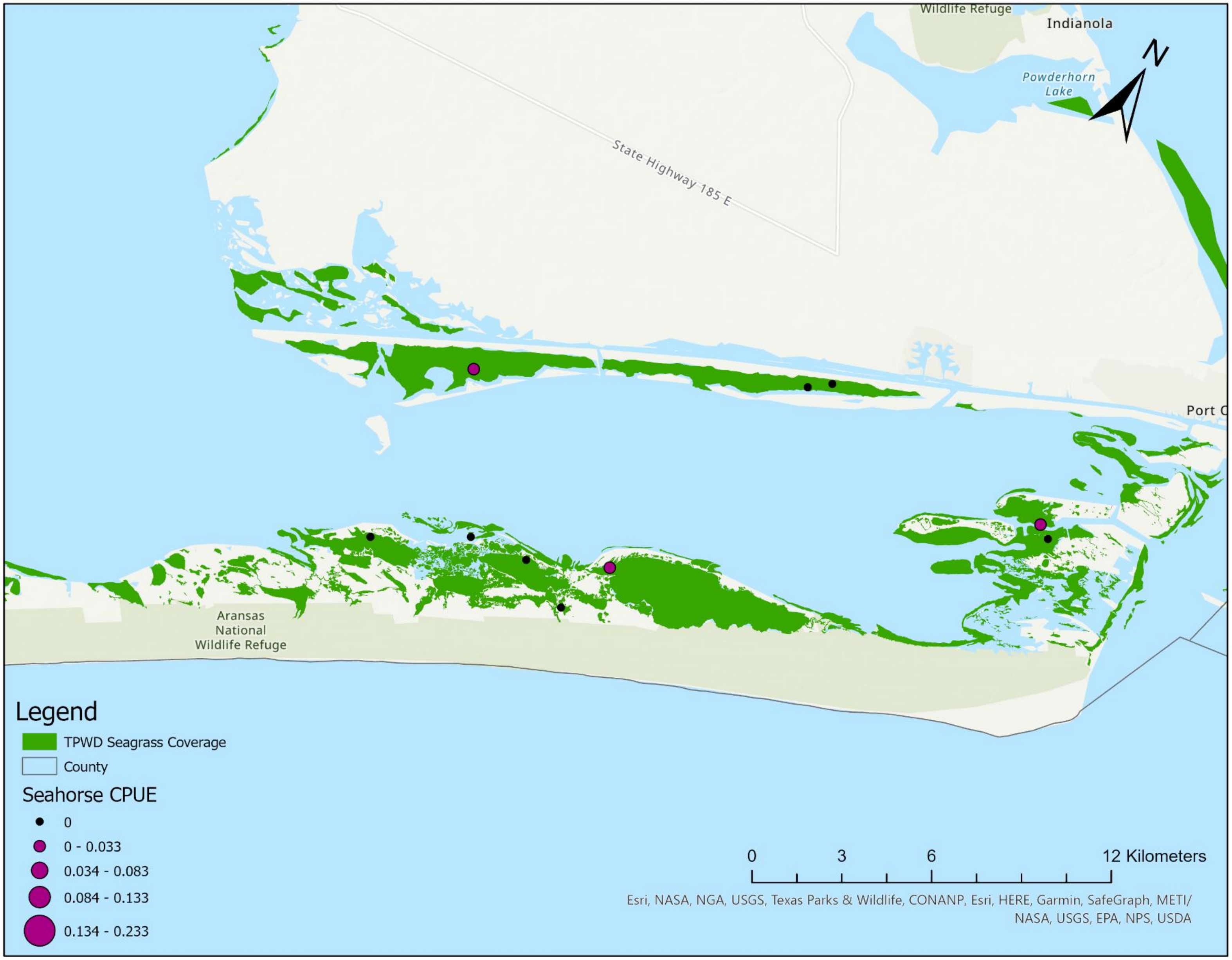


Figure 8. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in San Antonio Bay and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter^2.

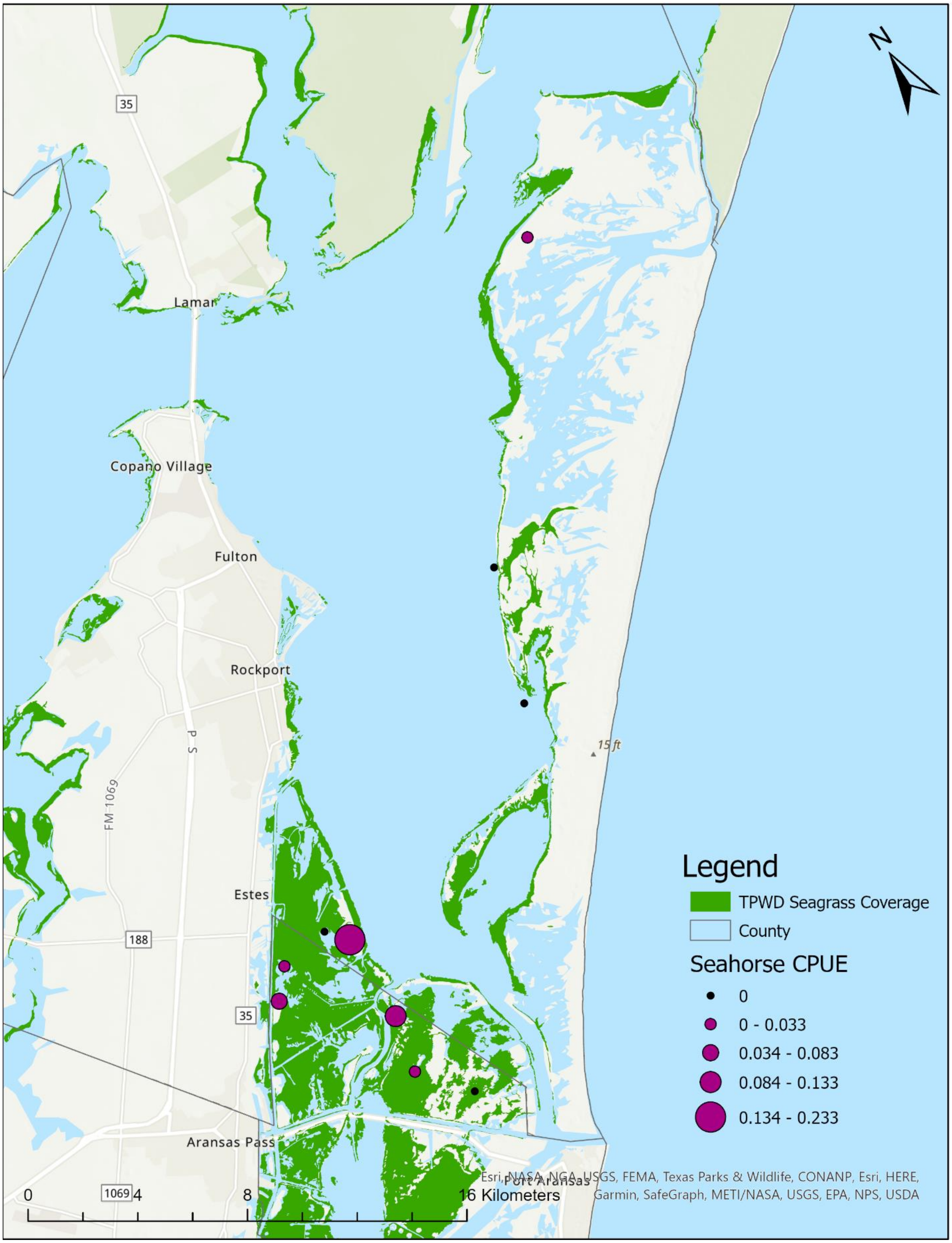


Figure 9. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in Aransas Bay and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter^2*.*

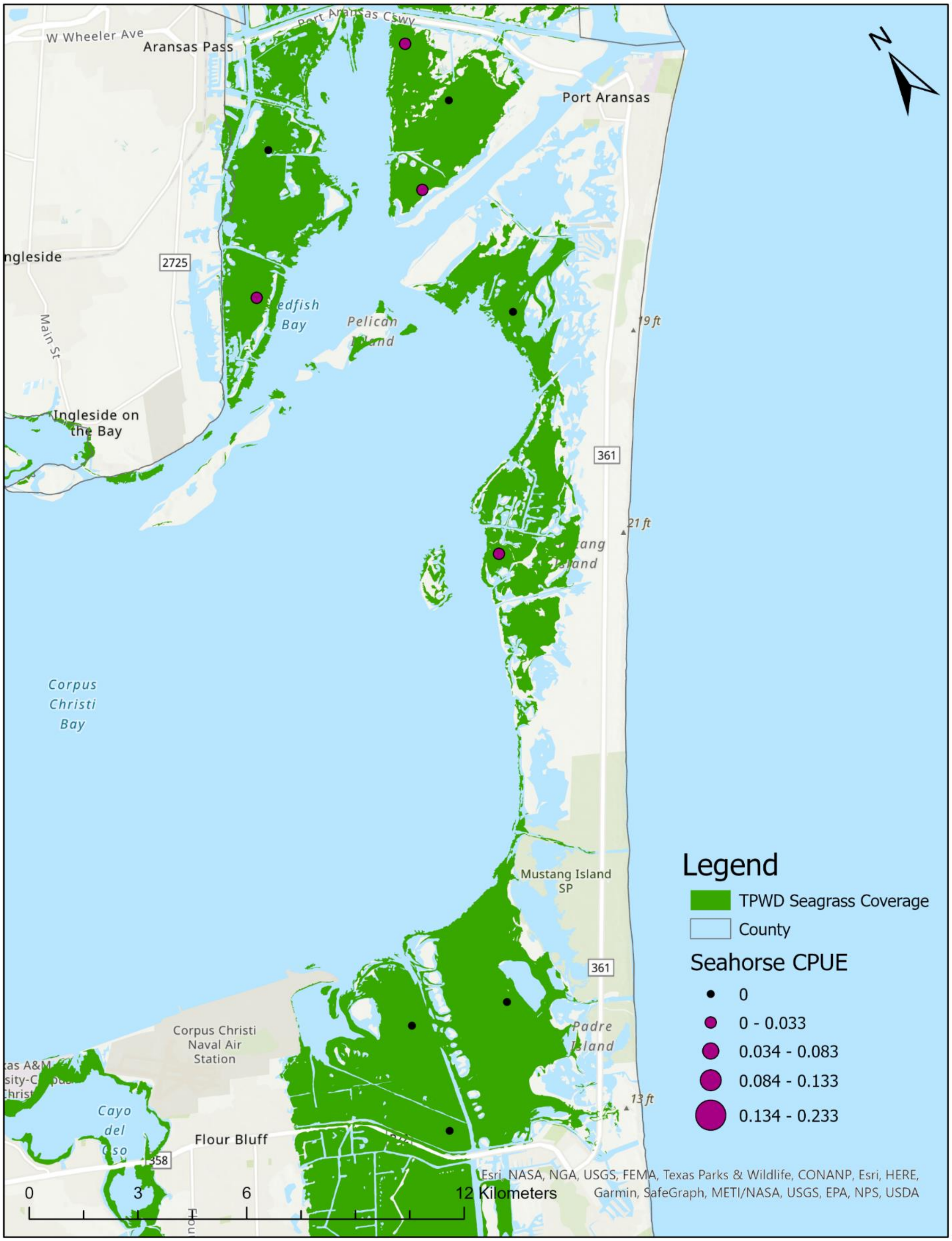


Figure 10. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in Corpus Christi Bay and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter^2.

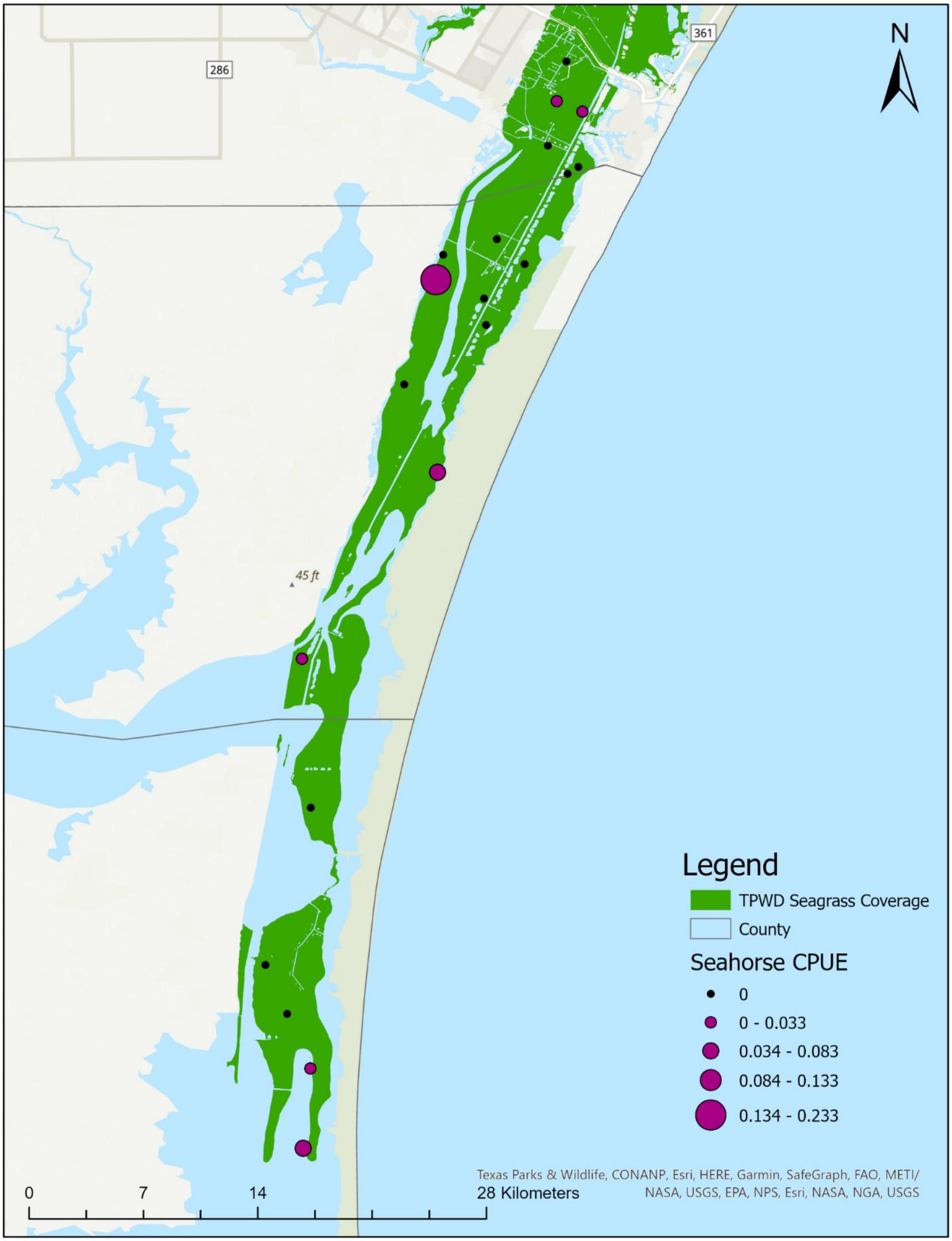


Figure 11. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in the Upper Laguna Madre and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter^2.

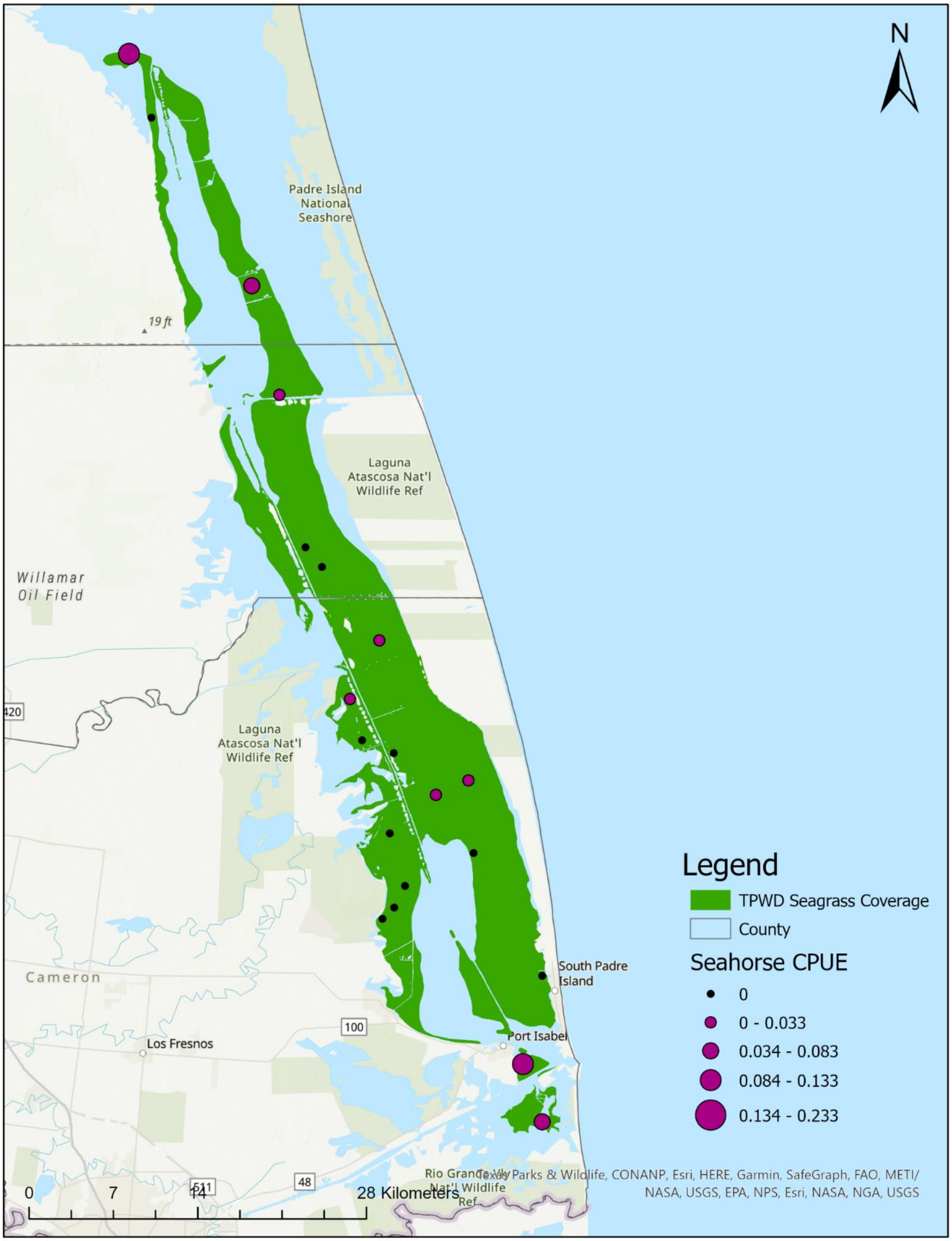


Figure 12. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in the Lower Laguna Madre and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter^2.

Habitat Associations of Dwarf Seahorse

Each of the five seagrass species were observed along the Texas coast. There were no sites in which all 5 species were observed together but, Corpus Christi Bay was the only system in which all five species were observed (Table 5). There are geographic shifts observed in the seagrass community with the lower coast (Corpus Christi to the Lower Laguna Madre) being the only bay systems where *S. filliforme* was present, but *H. wrightii* was observed in all bay systems. Seagrass biomass (g), canopy height (cm), and total percent cover was generally lower along the upper coast, and increased towards the coastal bend. Macroalgae coverage was highest in Corpus Christi Bay.

Table 5. Summary of average seagrass percent cover by species (species codes correspond to Table 1), biomass of seagrass from core samples, canopy height of seagrass, and percent cover of macrophytes and bare ground by bay system. Values presented as Averages. Data were lost for some of the Galveston sites biomass samples so summary data are not reported here.

Major Bay System	Number of Sites	% Cover HAWR	% Cover THTE	% Cover HAEN	% Cover CYFI	% Cover RUMA	Biomass (g)	Canopy Height (cm)	% Cover Seagrass	% Cover MACRO	% Bare
Galveston	5	13.2	0.0	0.3	0.0	5.3	N/A	3.0	18.7	0.0	79.3
Matagorda	5	21.0	0.0	0.0	0.0	0.0	0.3	4.6	21.0	0.1	77.0
San Antonio	10	35.0	0.0	10.4	0.0	0.0	0.9	10.3	45.4	8.0	46.6
Aransas	10	9.6	17.4	15.9	0.0	7.9	1.5	18.6	50.8	5.1	43.9
Corpus Christi	10	18.1	11.9	0.2	18.8	0.1	2.1	20.1	49.1	27.5	23.1
Upper Laguna	20	25.7	0.0	20.6	11.2	0.0	1.2	20.9	57.5	11.2	28.5
Lower Laguna	20	23.9	30.1	1.2	8.2	0.0	3.2	18.2	63.4	6.4	29.7
Grand Total	**80**	**22.4**	**11.2**	**8.8**	**7.2**	**1.3**	**1.8**	**16.4**	**50.9**	**9.5**	**38.5**

Dwarf Seahorse CPUE as well as presence/absence was analyzed for each of the water quality and physical habitat variables collected and there were no significant correlations detected. When comparing the CPUE of Dwarf Seahorses with the species richness of the seagrass community observed there appears to be a trend with increasing CPUE at sites where an increasing number of seagrass species were present (p-value = 0.0466), but post hoc analysis did not determine any significant differences among the seagrass species richness groups (Figure 13). However, when we evaluated the Shannon H Diversity of the seagrass community at sites where Dwarf Seahorses were captured versus sites where we did not detect them, the seagrass diversity was significantly higher (p-value < 0.001) (Figure 14). Using a fitted binomial GLM we can predict that at sites where there is a seagrass community diversity (H) of 0.6, the probability that we would detect Dwarf Seahorse is modeled at 70% (Figure 14). While not significant at the α=0.05 level, there appeared to be a potential correlation between the canopy height (cm) of the seagrass community present at the site and the likelihood that we would capture a Dwarf Seahorse (p-value = 0.0777). Maps illustrating the percent composition of the seagrass community by species for each site where Dwarf Seahorses were captured by bay system are provided in Appendix 4.

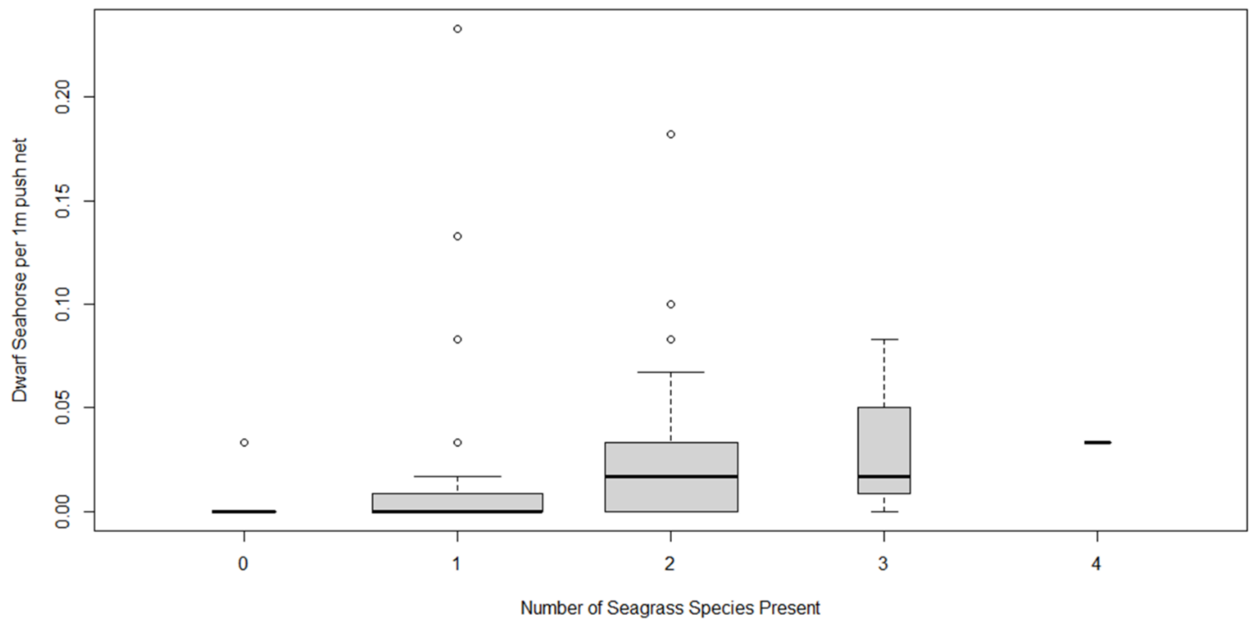


Figure 13. Boxplot of the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter^2 by the species richness of seagrass observed at the site.

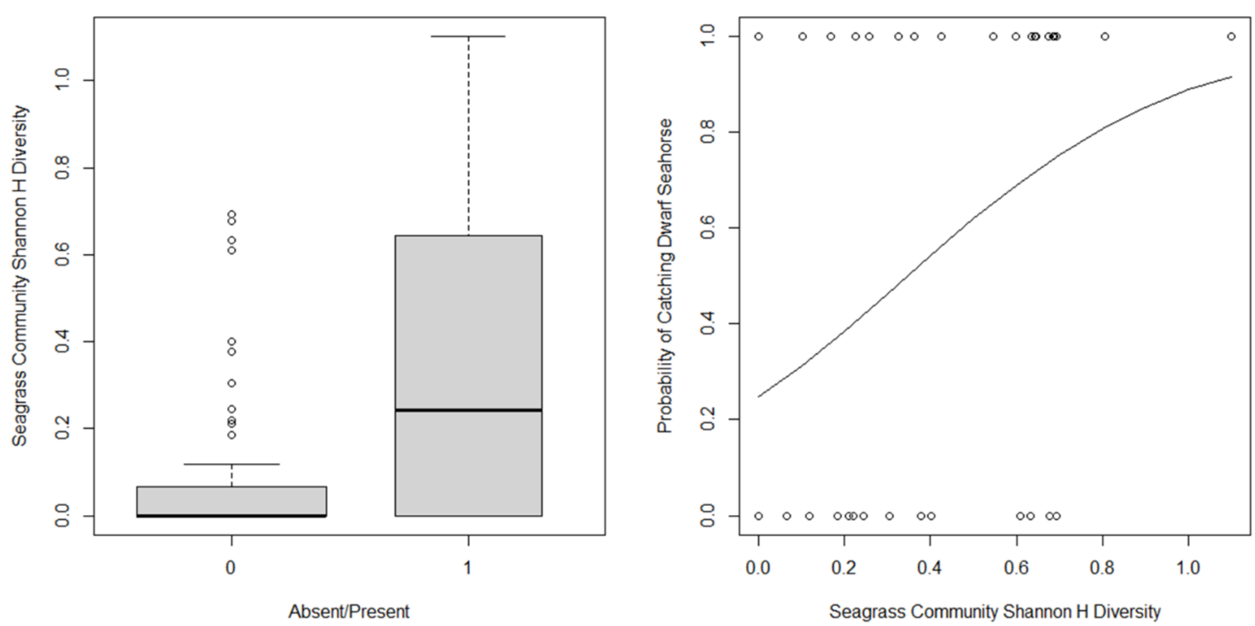


Figure 14. Boxplot of the diversity (Shannon H Diversity) of the seagrass community present at sites where Dwarf Seahorses were present versus not captured, in all sampling events throughout year-1 (2020) distribution and abundance study and fitted binomial GLM applied to the presence/absence of Dwarf Seahorses by the diversity of the seagrass community present at the site.

The percent coverage of each species of seagrass and macroalgae were compared to the presence or absence of Dwarf Seahorses (Figure 15). While a visual trend existed with a general increase in detections of Dwarf Seahorses when the percent cover of the seagrass and macroalgae was higher, the only instance with a statistically significant relationship was with Turtle Grass (*T. testudium*) (p-value = 0.0095). Similar analysis was conducted for the CPUE of Dwarf Seahorse at sites where each species of seagrass or macroalgae were present or absent (Figure 16) and once again, the only statistically significant result was for the presence of Turtle Grass (*T. testudium*) (p-value = 0.0031). While not significant at the $\alpha = 0.05$ level, sites where Manatee Grass (*S. fileforme*) was present did show an increase in the CPUE of Dwarf Seahorse (p-value = 0.0559).

Nekton Community Structure

The push nets proved an effective method for catching not only Dwarf Seahorse, but also other smaller, less mobile fishes and invertebrates in shallow seagrass habitats. A total of 72,630 organisms from 47 different species (or higher taxonomic groups when identification to species was not possible) from 32 families were captured (Appendix 6). Dwarf Seahorse was the tenth most abundant fish species captured with an overall CPUE of 0.065 individuals per m^2 (Table 6). The most abundant fish species caught was the Gulf Pipefish (*Syngnathus scovelli*) with a CPUE of 0.91 per m^2 and it was captured in every bay system. Gobies (Darter Goby [*Ctenogobius boleosoma*] and Code Goby [*Gobisoma robustrum*] as well as larval gobies labeled as "Gobiidae" which where were too small to identify to species) were also among the most abundant fishes. The most abundant organisms captured with the push net were invertebrates including the group of Grass Shrimp from the genus *Palaemonetes*, the Mysid Shrimp (*Taphromysis louisianae*), Comb Jellyfish from the phylum Ctenophora, and Arrow Shrimp (*Tozeuma carolinense*) which were each caught in all of the bay systems sampled.

The nekton community data were analyzed for their similarities and dissimilarities by site and while there appears to be a tighter grouping of the nekton communities in which Dwarf Seahorse were captured, and ANOSIM conducted on the resemblance matrix did not show a significant difference (p-value = 0.287) (Figure 17). Person correlations were run to examine which species were contributing to the variance observed along each of the 2D non-metric MDS plot ordinations, and the Dwarf Seahorse showed the second highest correlation to MDS2 ordination (-0.368). All of the correlation values for MDS2 that were greater than 0.25 are displayed in Figure 17. When these data are displayed by the CPUE of the Dwarf Seahorse using a heat color output, there appears to be a gradient along the MDS 2 ordination (Figure 18). There is an observable and significant grouping of nekton community structure by bay system (ANOSIM p-value = 0.001) with the bay systems in closest geographic proximity to one another having the most similar nekton communities. P-values for similarity comparisons between bay systems are included in Figure 19.

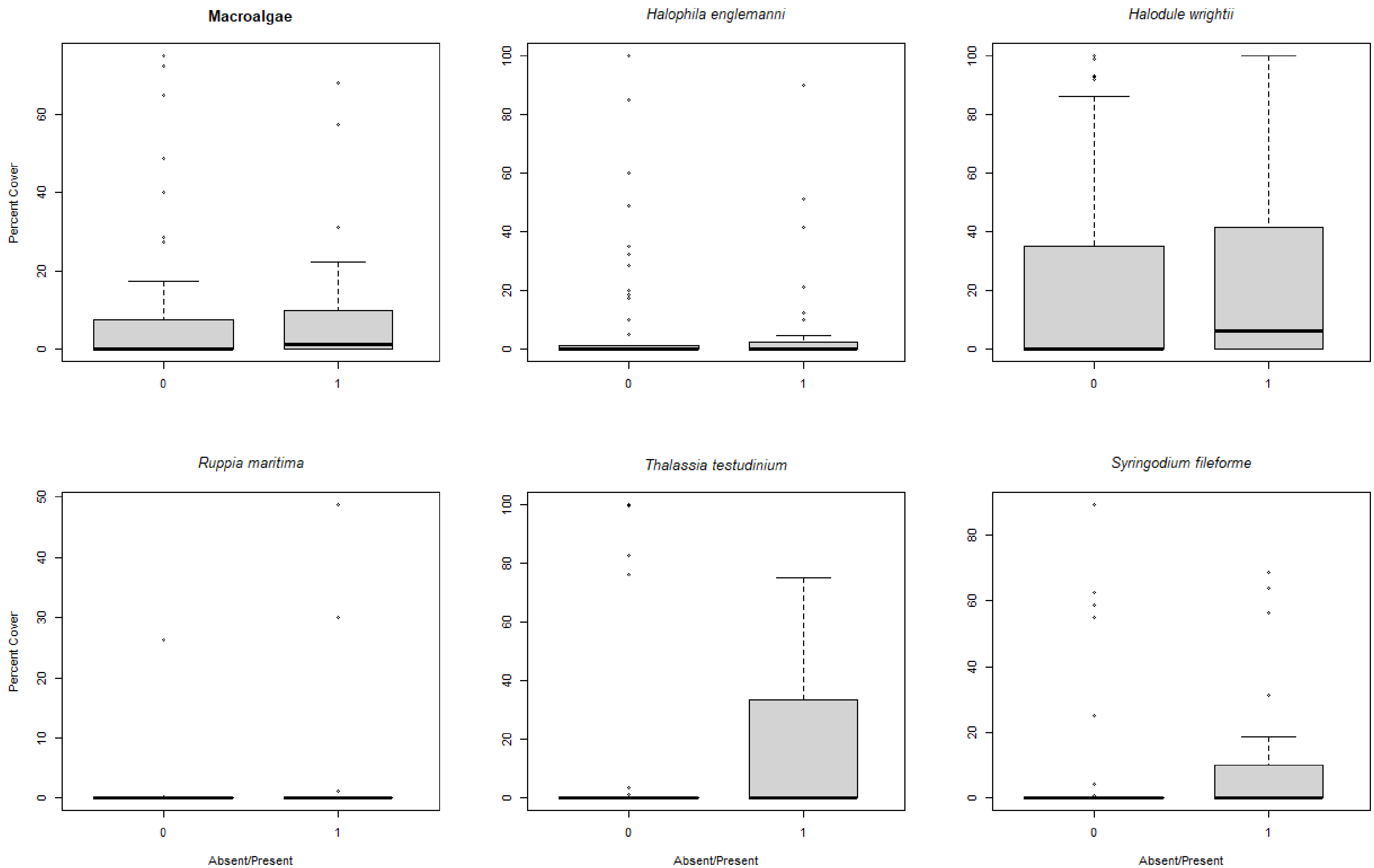


Figure 15. Boxplots of percent cover of seagrass species and macroalgae at sites where Dwarf Seahorse were present versus not captured in all sampling events throughout the year-1 (2020) distribution and abundance study.

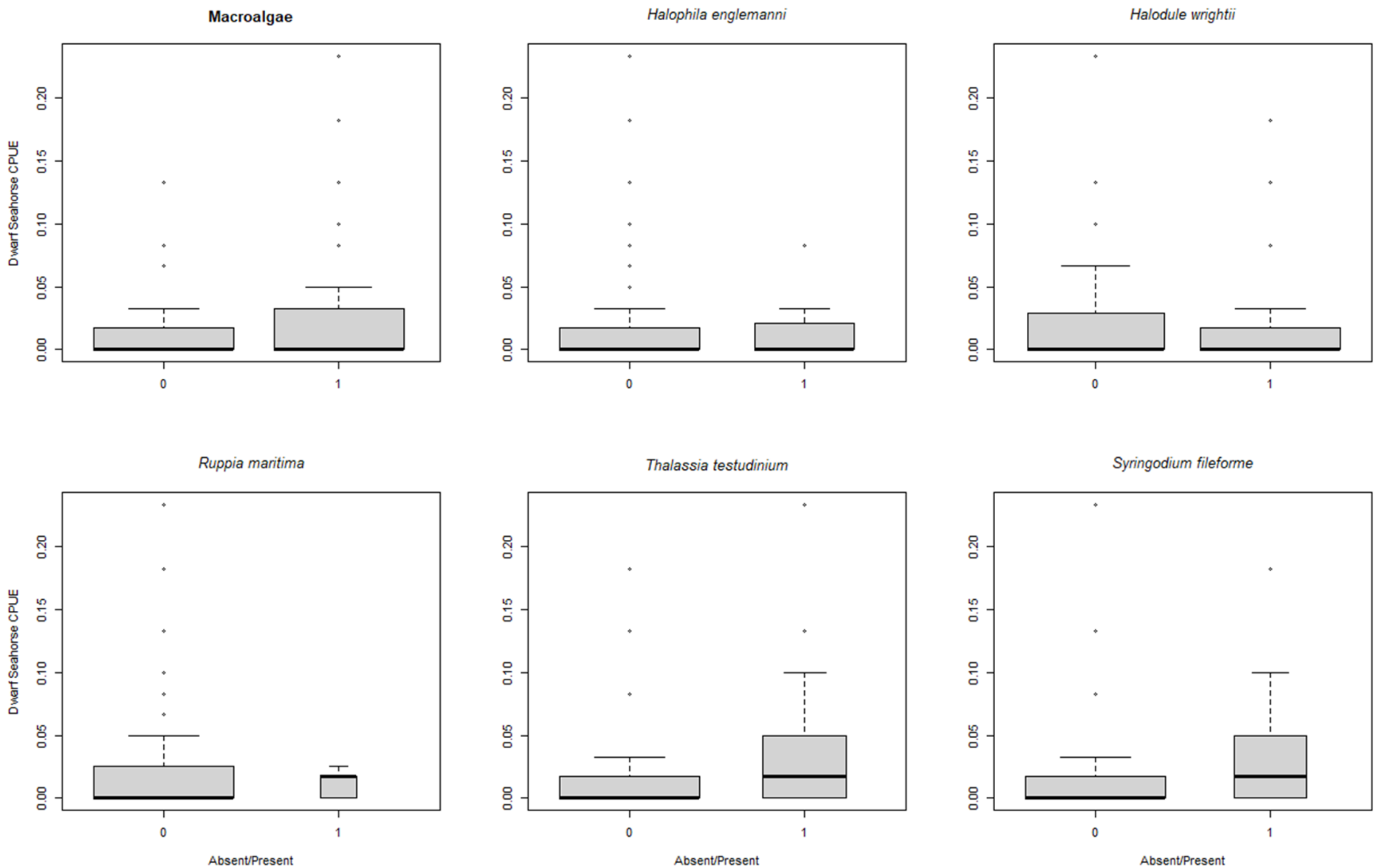


Figure 16. Boxplots of catch per unit effort (CPUE) of Dwarf Seahorses per meter^2 at sites where seagrass species and macroalgae were present versus not, in all sampling events throughout the year-1 (2020) distribution and abundance study.

Table 6. Catch per unit effort (CPUE) in number of individuals caught per meter² of the top 10 fish species captured in the push nets during year-1 (2020) distribution and abundance study.

Scientific Name	Common Name	Galveston	Matagorda	San Antonio	Aransas	Corpus Christi	Upper Laguna Madre	Lower Laguna Madre	Grand Total
Syngnathus scovelli	Gulf Pipefish	0.037	0.020	0.158	0.115	0.077	0.251	0.501	0.910
Ctenogobius boleosoma	Darter Goby	1.033	0.053	0.040	0.046	0.200	0.001	0.020	0.428
Gobiosoma robustrum	Code Goby	-	0.010	0.042	0.113	0.193	0.205	0.061	0.413
Gobiidae	Gobiidae	-	0.273	0.335	0.015	0.002	0.009	-	0.252
Lucania parva	Rainwater Killifish	-	-	-	0.288	0.186	0.006	0.025	0.244
Lagodon rhomboides	Pinfish	-	-	0.158	0.048	0.163	0.021	0.024	0.221
Opsanus beta	Gulf Toadfish	-	0.003	0.005	0.075	0.095	0.047	0.025	0.149
Syngnathus louisianae	Chain Pipefish	0.017	0.010	0.090	0.092	0.016	0.018	0.029	0.145
Eucinostomus melanopterus	Flagfin Mojarra	-	-	-	0.004	0.028	-	0.088	0.103
Hippocampus zosterae	Dwarf Seahorse	-	0.003	0.008	0.038	0.011	0.017	0.023	0.065

Table 7. Catch per unit effort (CPUE) in number of individuals caught per meter² of the top 10 invertebrate species/groups captured in the push nets during year-1 (2020) distribution and abundance study.

Scientific Name	Common Name	Galveston	Matagorda	San Antonio	Aransas	Corpus Christi	Upper Laguna Madre	Lower Laguna Madre	Grand Total
Palaemonetes	Palaemonetes	0.220	4.460	0.232	14.046	6.552	4.390	3.734	18.238
Taphromysis louisianae	Mysid Shrimp	2.183	3.303	10.618	19.740	0.298	0.076	2.271	17.717
Ctenophora	Comb Jellyfish	0.523	5.847	0.120	0.402	0.016	5.052	2.075	8.528
Tozeuma carolinense	Arrow Shrimp	0.007	0.007	0.003	0.960	0.708	0.237	2.103	3.077
Panopeid	Mud Crab	-	0.007	0.073	0.683	1.408	0.665	0.182	1.794
Penaeid	Penaeid sp	0.657	0.503	0.002	0.252	0.434	0.110	0.776	1.483
Callinectes sapidus	Blue Crab	0.550	0.047	0.205	0.031	0.021	0.009	0.014	0.298
Alpheus heterochaelis	Bigclaw Snapping Shrimp	-	-	0.020	0.019	0.039	0.008	0.014	0.058
Salpidae	Salp	-	-	0.035	-	-	-	0.003	0.020
Clibanarius vittatus	Thinstripe Hermit Crab	0.007	-	0.003	0.006	0.002	-	0.002	0.008

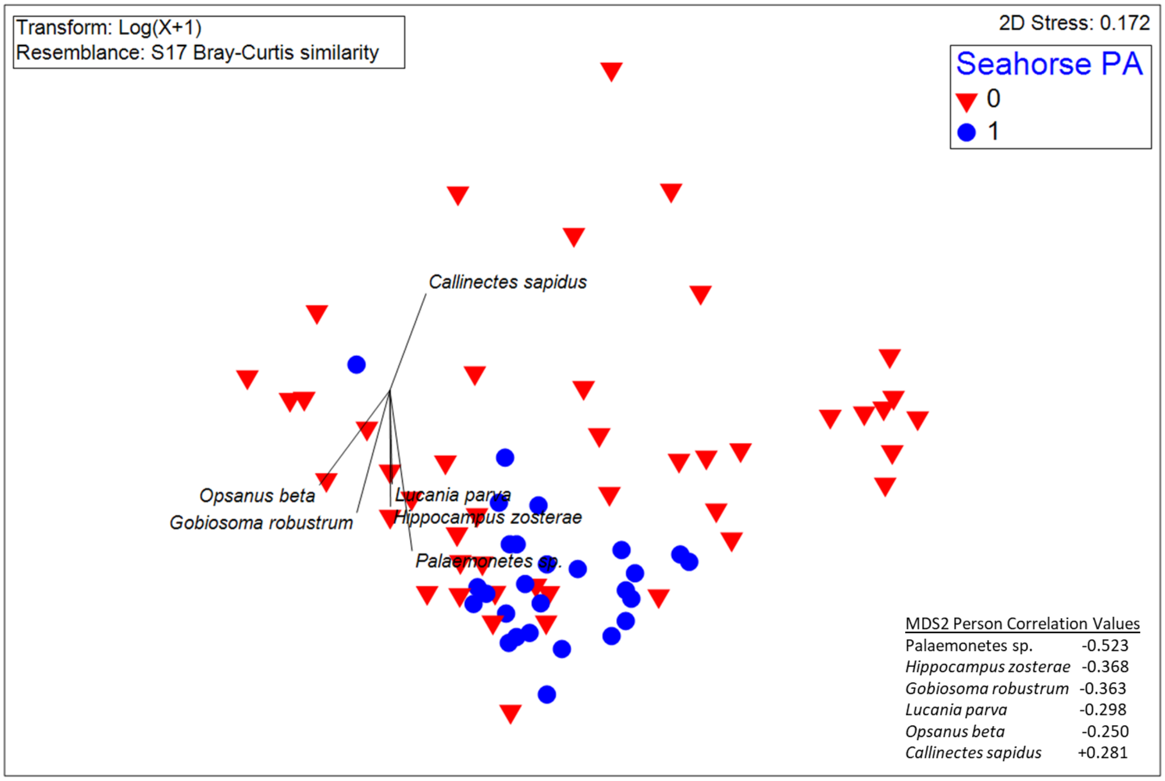


Figure 17. Non-metric MDS plot of nekton community catch by site from year-1 (2020) distribution and abundance study. Events labeled by blue circles for sites where Dwarf Seahorse(s) were captured, and red triangles for sites where Dwarf Seahorse were not observed. Ordinations and Pearson Correlation values included for six species with the highest correlation to MDS 2.

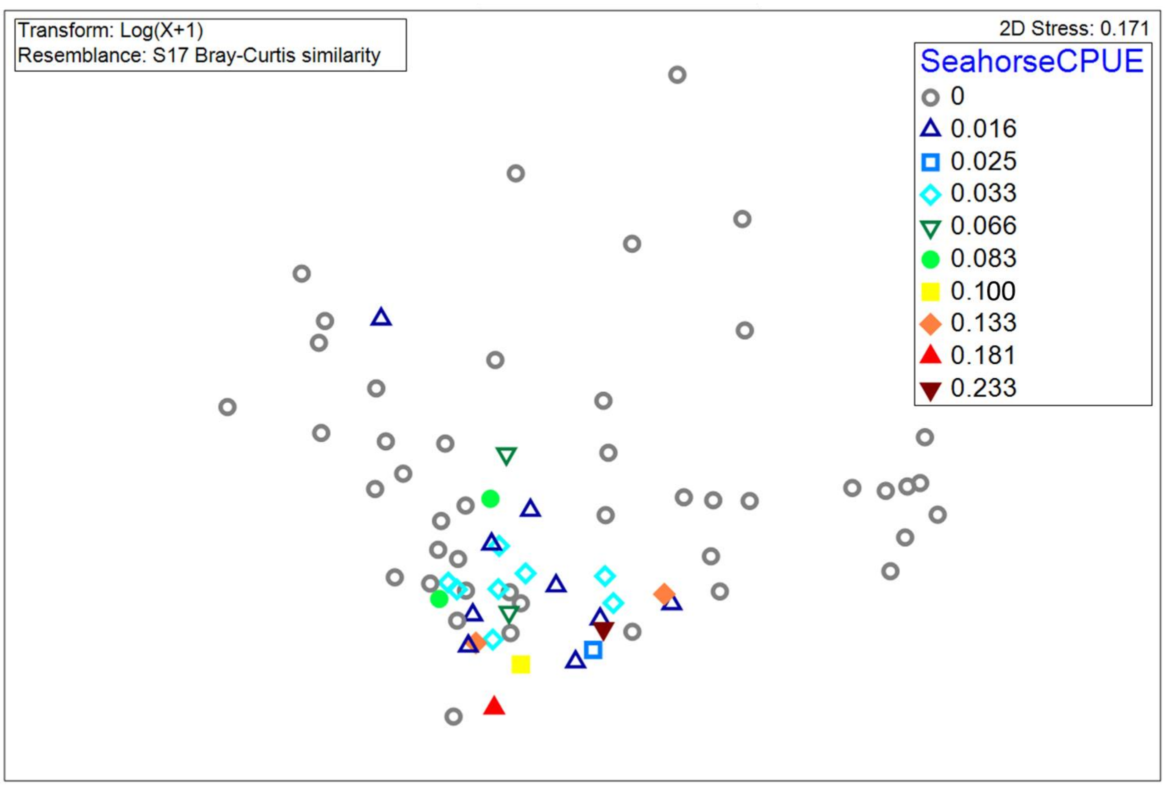


Figure 18. Non-metric MDS plot of nekton community catch by site from year-1 (2020) distribution and abundance study. Events labeled by the catch per unit effort (CPUE) of Dwarf Seahorse per meter^2.

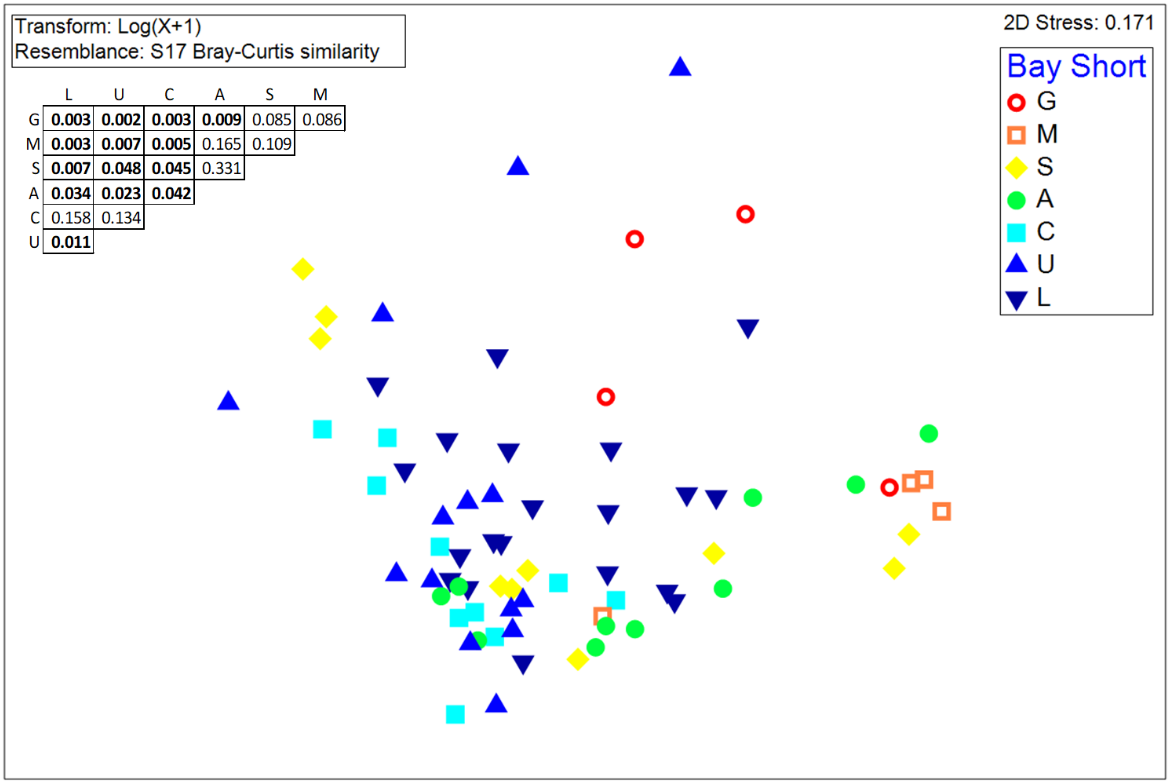


Figure 19. Non-metric MDS plot of nekton community catch by site from year-1 (2020) distribution and abundance study. The p-value results from an Analysis of Similarities (ANOSIM) by Bay system are displayed in the top left corner of the figure. Events labeled by bay system. Lower Laguna Madre (L), Upper Laguna Madre (U), Corpus Christi Bay (C), Aransas Bay (A), San Antonio Bay (S), Matagorda Bay (M), and Galveston Bay (G).

Demographics of Dwarf Seahorse

A total of 90 Dwarf Seahorses were collected throughout both years of the study. A total of 33 individuals were juveniles (37% of the total catch), 40 individuals were mature females (44%), and 17 individuals were mature males (19%). Juveniles display quick height growth with minimal weight gain making them appear very thin, which is a criterion for visual maturity determination (Figure 5 and Figure 20). A power curve was fitted to the height/weight data which resulted in an R^2 of 0.9635. The smallest individual collected had a height of 6.52 mm and the largest had a height of 31.18 mm with an average weight of 41.25 mg (Table 8).

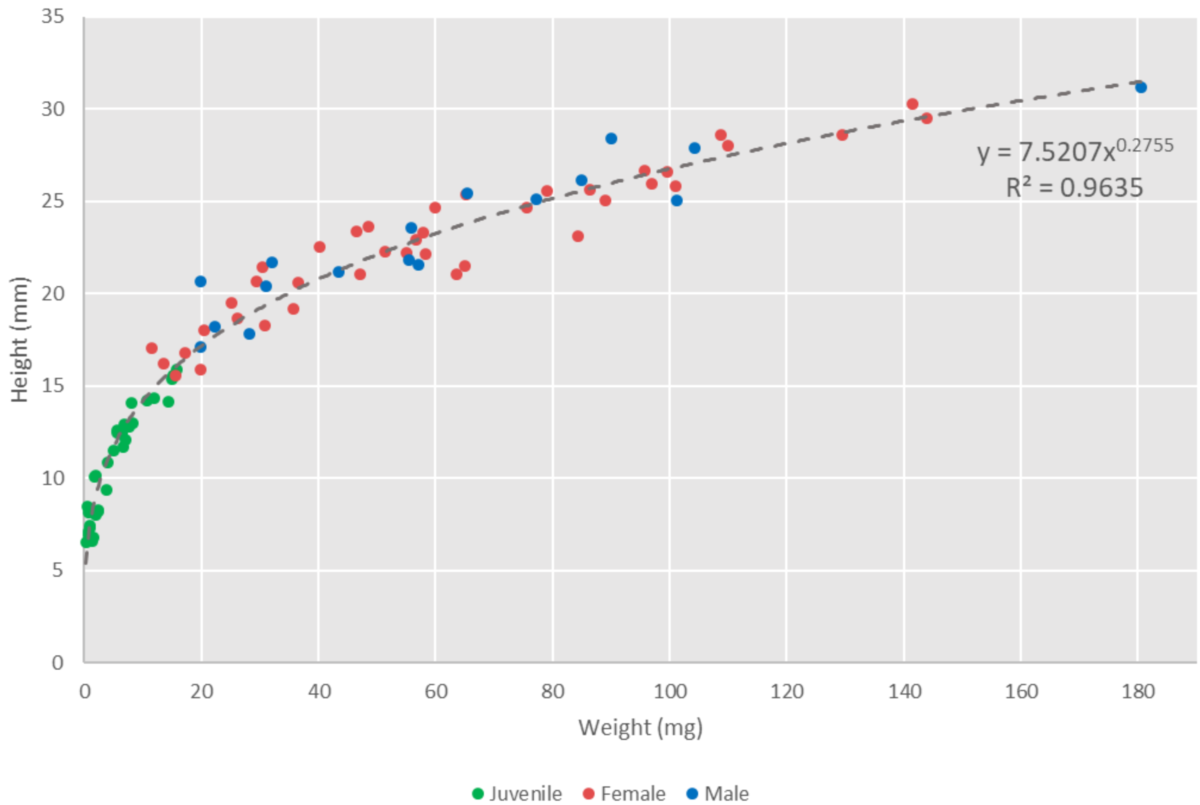


Figure 20. Height and weight plot of the 90 Dwarf Seahorse collected throughout both year-1 (2020) distribution and abundance study, and year-2 (2021) gear comparison study by maturity and sex. The R^2 and the equation for the plotted power curve is displayed in the top right of the graph.

Table 8. Summary table of Dwarf Seahorse morphometrics from the 90 individuals collected in both year-1 (2020) distribution and abundance study, and year-2 (2021) gear comparison study.

	Height (mm)	Tail Length (mm)	Snout Length (mm)	Weight (mg)
Min	6.52	4.07	0.57	0.30
Q2	12.63	7.61	1.17	6.95
Median	19.35	11.35	1.68	28.85
Mean	18.36	11.11	1.67	41.29
Q3	23.62	14.22	2.18	64.73
Max	31.18	20.46	2.77	180.60

Comparison of tail to height ratio of Dwarf Seahorses by maturity and sex was investigated and there were statistically significant differences among each, with males having significantly larger tail to height ratio (due to the presence of the brooding pouch) than juveniles (p-value = 0.0132) and females (p-value < 0.001) (Figure 21). Juveniles also had a significantly higher tail to height ratio compared to females (p-value = 0.0004). For the majority of the juveniles (largest juveniles excluded) and the larger mature individuals, the tail to height ratio diverges providing a tool for confirming visual sex and maturity determinations (Figure 22).

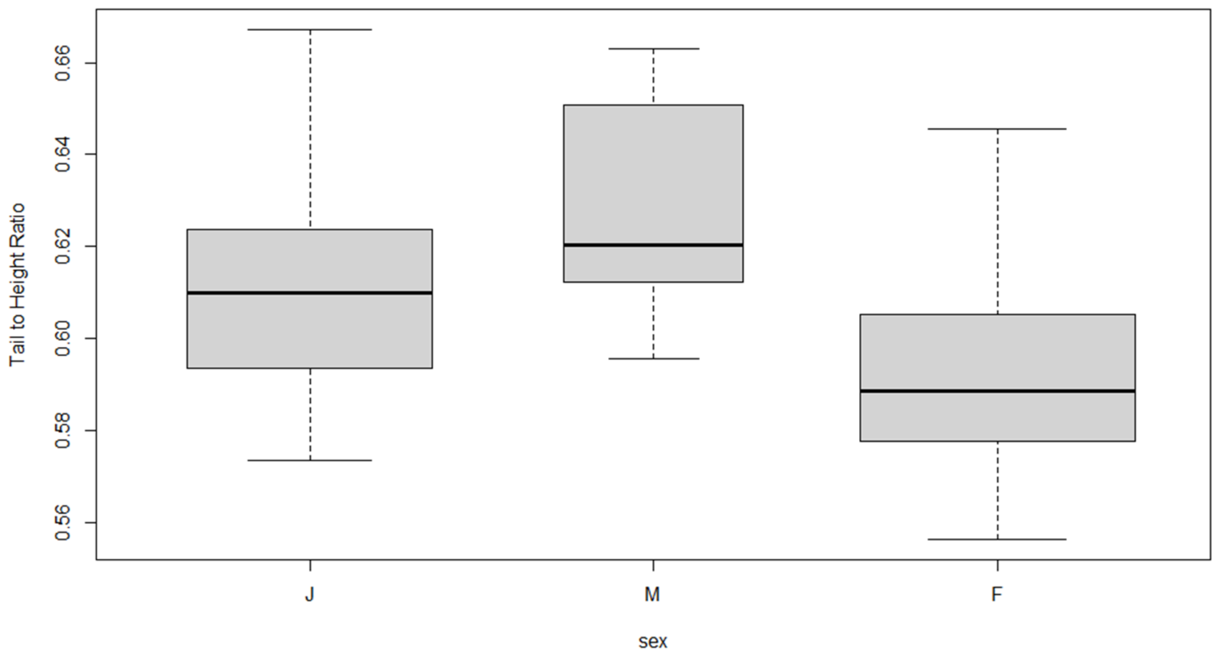


Figure 21. Boxplots of the tail to height ratio for all Dwarf Seahorses captured throughout the entire study by sexual maturity and sex. Juvenile (J), male (M), and female (F).

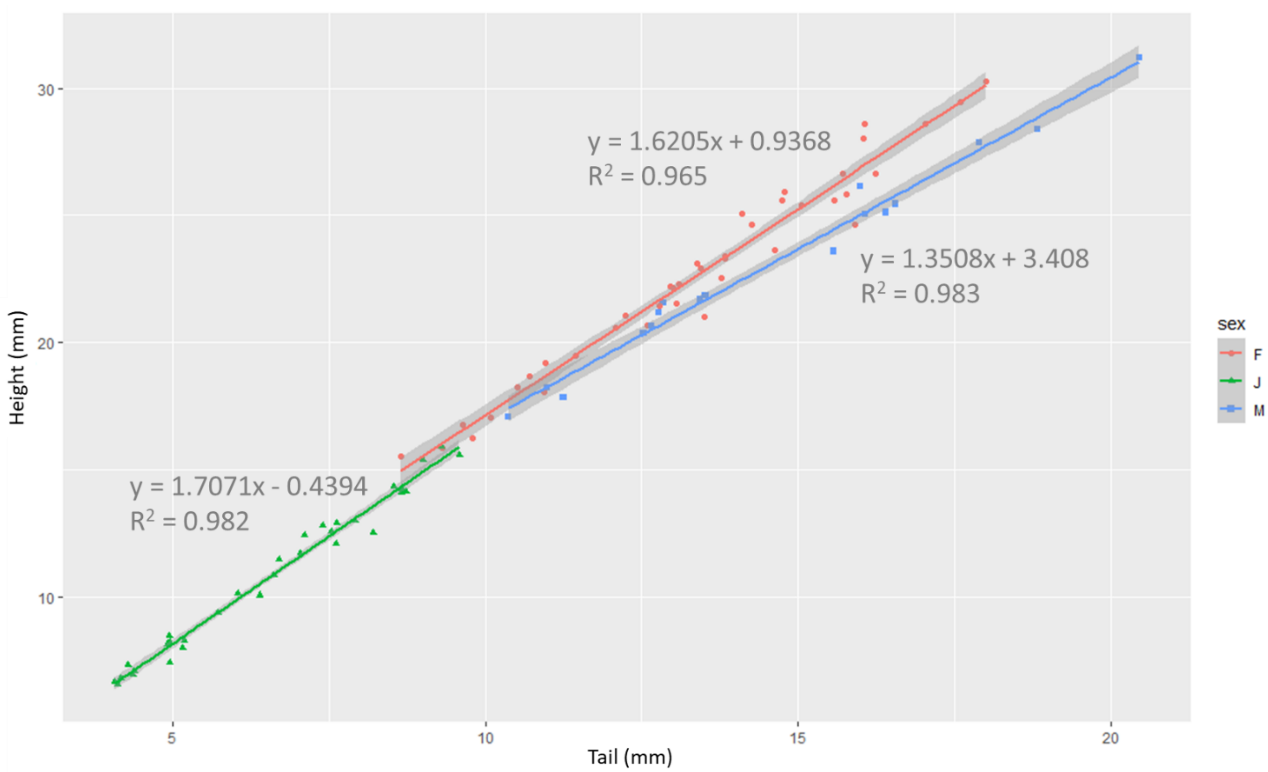


Figure 22. Tail to height values of all Dwarf Seahorses captured throughout the study by sex and sexual maturity. The R^2 and the equation for the linear trend lines are displayed near each set of data.

Comparison of snout to height ratio of Dwarf Seahorses by maturity and sex were investigated and there were statistically significant differences between juveniles and mature individuals (p-value = 0.0023) but no significant difference between mature males and females (p-value = 0.5649) (Figure 23). For the majority of the juveniles, the snout to height plot is notably lower than mature individuals although, especially for mature individuals, there is considerable variability in the snout to height ratio, with an R^2 of 0.738 (Figure 24).

Analysis evaluating correlation with maturity, sex, and morphometrics and the water quality and habitat variables were investigated and there were no statistically significant differences. There appears to be some differences in the proportion of the catch that were mature, with a significantly smaller proportion of juveniles (smaller snout to height ratio) captured in Aransas Bay compared to the Lower Laguna Madre (p-value = 0.0067), the Upper Laguna Madre (p-value = 0.0309), and while not statistically significant at the $\alpha = 0.05$ level, Corpus Christi Bay (p-value = 0.0695) (Figure 25). Maps depicting the percent of catch of juvenile, male, and female Dwarf Seahorses by bay system are provided in Appendix 5.

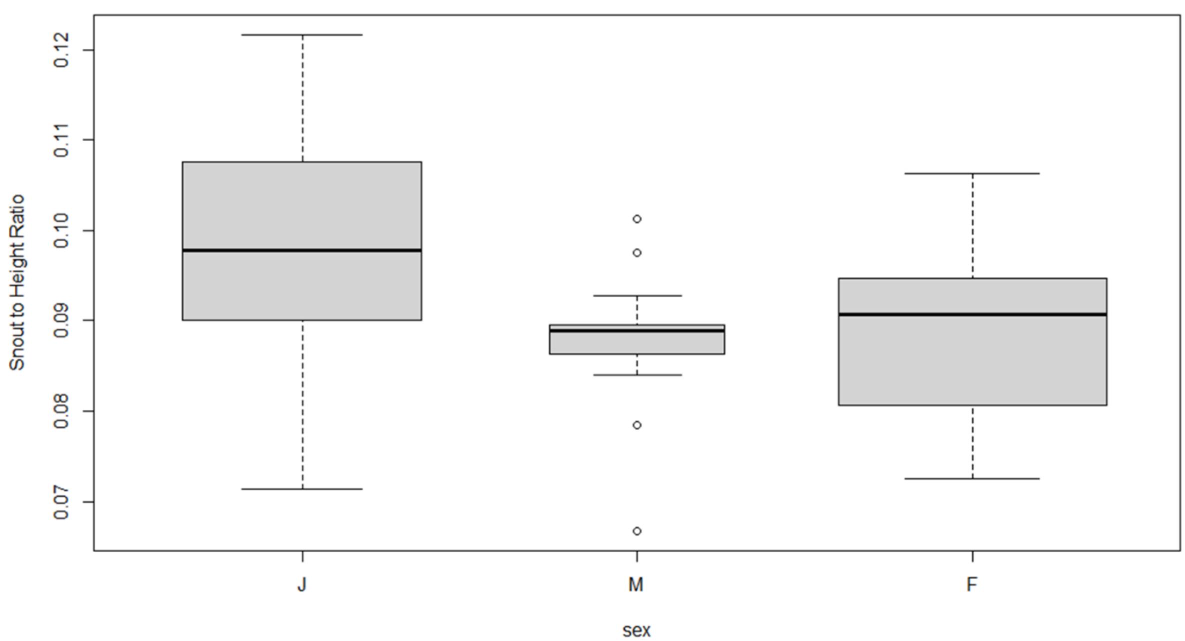


Figure 23. Boxplots of the snout to height ratio for all Dwarf Seahorses captured throughout the entire study by maturity and sex. Juvenile (J), Male (M), and Female (F).

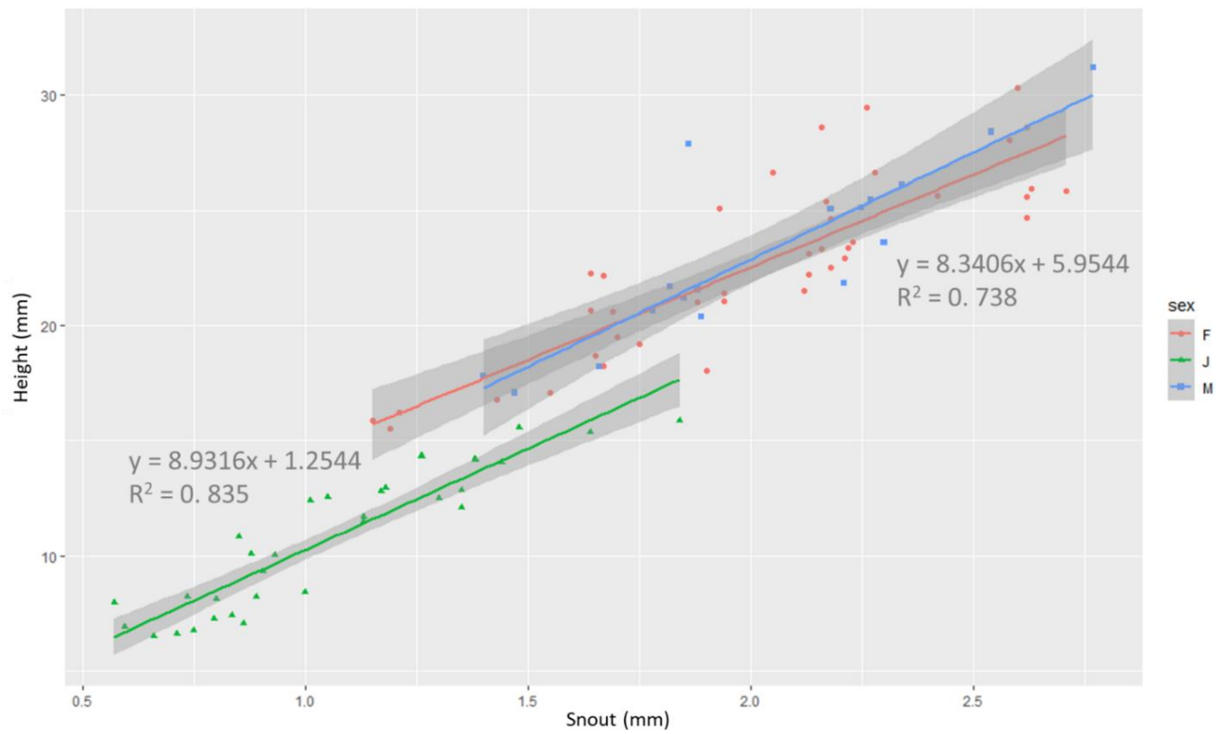


Figure 24. Snout to height values of all Dwarf Seahorses captured throughout the study by sex and maturity. A single linear trend was created for all mature individuals and is included along with the R^2 values next to the corresponding maturity level data.

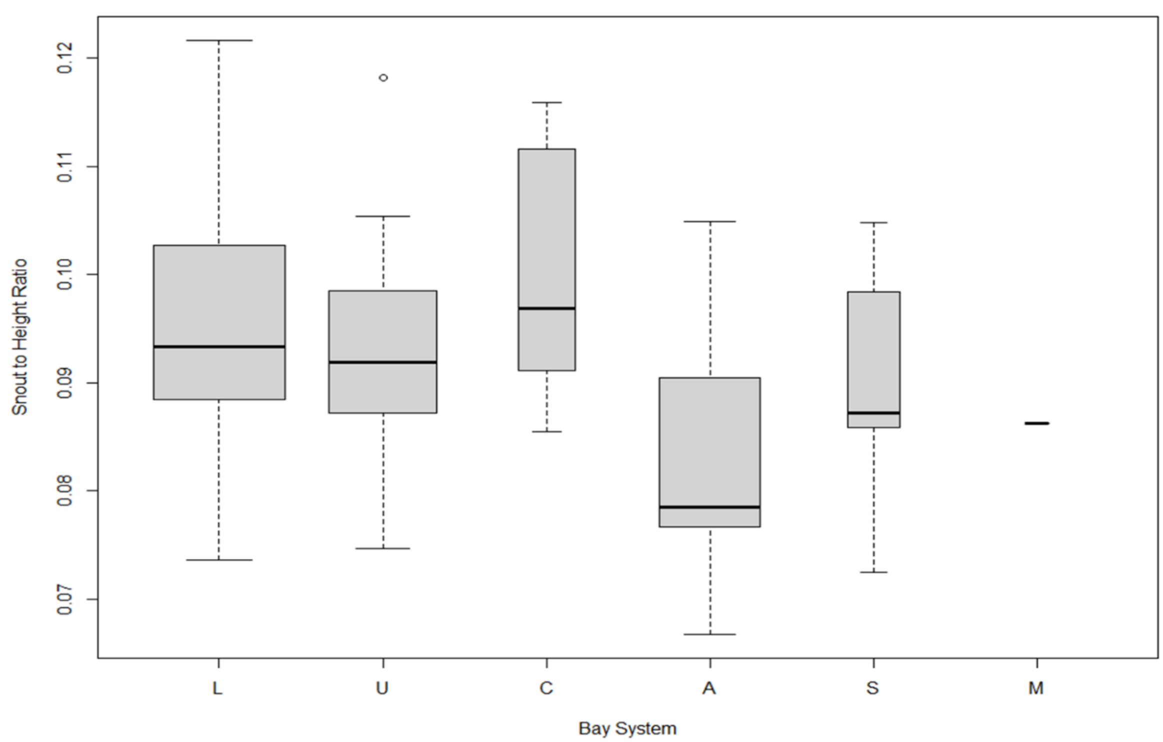


Figure 25. Boxplots of the snout to height ratio for all Dwarf Seahorses captured throughout the entire study by bay system. Lower Laguna Madre (L), Upper Laguna Madre (U), Corpus Christi Bay (C), Aransas Bay (A), San Antonio Bay (S), Matagorda Bay (M), and Galveston Bay (G).

Gear Comparison (Year-2 2021)

A total of 12 Dwarf Seahorses were captured during the year-2 (2021) gear comparison study at 7 of the 8 sites sampled. Three of the five sampling gears studied (throw trap, push net, and beam trawl) captured Dwarf Seahorse(s), but none were captured with either the 60’ bag seine or the 15’ straight seine (Appendix 7). The throw trap provided significantly higher CPUE of Dwarf Seahorses per m^2 than the push net (p-value = 0.0285) and the beam trawl (p-value = 0.019) (Figure 26). The overall CPUE of Dwarf Seahorses for all sites and replicates combined using the throw trap gear was 0.222 per m^2, while the push net (0.019 per m^2) and the beam trawl (0.003 per m^2) were notably lower. The amount of time required to complete each gear type (excluding the 60’ bag seine gear) sampling was recorded for five of the sites sampled in the Lower Laguna Madre. The average amount of time it took a team of three to complete three replicate throw trap samples was highest at 79 minutes (nearly 4 man-hours). The next most time-consuming gear type was the push net (59 minutes), then the 15’ straight seine (37 minutes), and finally the beam trawl (28 minutes). Anecdotally, these time estimates would be elevated at sites with high percent cover of macroalgae but were not officially measured.

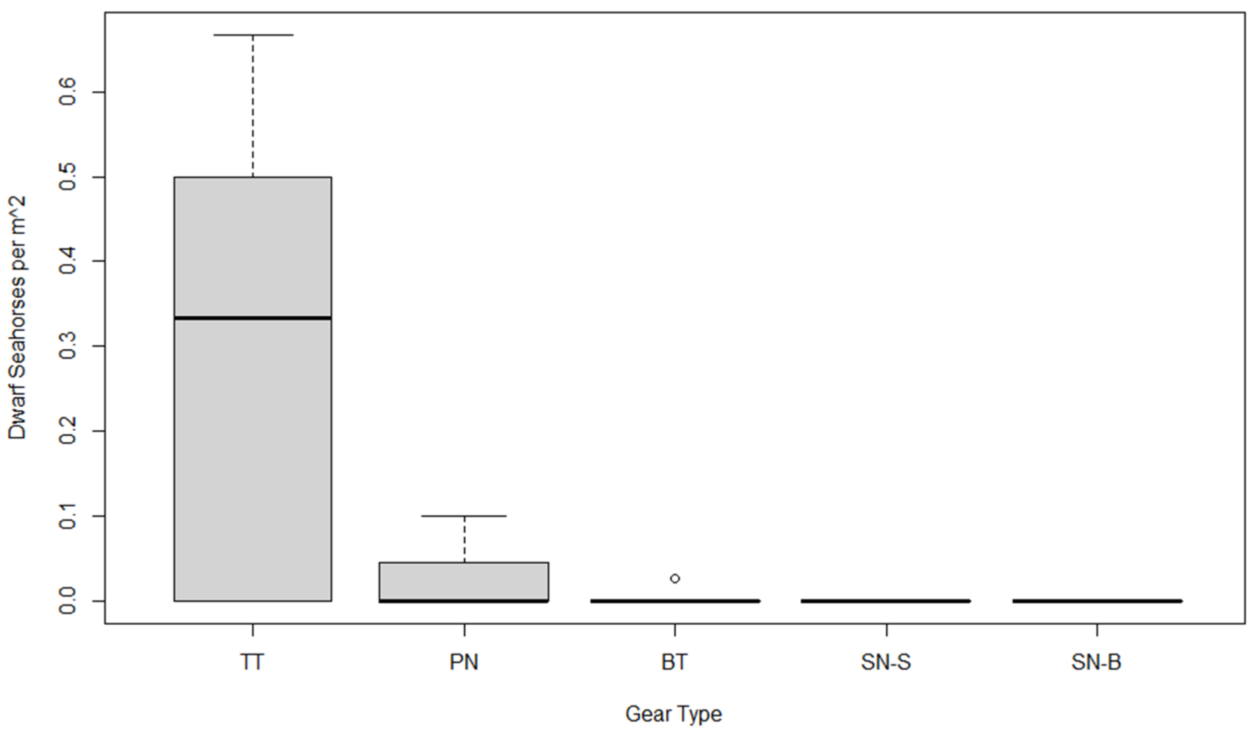


Figure 26. Box plots of catch per unit effort (CPUE) of Dwarf Seahorse captured per meter^2 by gear type from year-2 (2021) gear comparison study. Throw trap (TT), push net (PN), beam trawl (BT), 15’ straight seine (SN-S), and 60’ bag seine (SN-B).

A total of 9,826 individuals from 47 different species or groups representing 30 different families were captured in the year-2 (2021) gear comparison study. A total of 8 species or groups were caught using all five gear types (Appendix 7). The top ten fish species with the highest overall CPUE are presented in Table 9. The fish species with the highest overall CPUE

(0.079 per m^2) was Pinfish (*Lagodon rhomboids*) followed by Bay Anchovy (*Anchoa mitchilli*). The most abundant groups of invertebrates captured included Grass Shrimp (*Palaemonetes spp.*), and Mud Crabs (family Panopeidae) (Table 10). The overall most abundant invertebrate group was Grass Shrimp with a total CPUE of 0.321 per m^2.

Table 9. Catch per unit effort (CPUE) per meter^2 of the top ten highest abundance fish species caught by gear type from year-2 (2021) gear comparison study. Throw trap (TT), push net (PN), beam trawl (BT), 15' straight seine (SN-S), and 60' bag seine (SN-B).

Scientific Name	**Common Name**	**TT**	**PN**	**BT**	**SN-S**	**SN-B**	**Grand Total**
Lagodon rhomboides	Pinfish	4.704	0.013	0.046	0.365	0.052	0.079
Anchoa mitchilli	Bay Anchovy	0.074	-	0.040	0.215	-	0.024
Leostomus xanthurus	Spot	-	-	-	-	0.047	0.022
Brevoortia patronus	Gulf Menhaden	4.333	0.018	-	0.020	0.003	0.021
Syngnathus scovelli	Gulf Pipefish	0.815	0.009	0.003	0.002	-	0.006
Ctenogobius boleosoma	Darter Goby	0.630	0.005	-	-	-	0.004
Lucania parva	Rainwater Killifish	0.185	0.001	0.003	0.019	-	0.003
Bairdiella chysoura	Silver Perch	0.037	-	0.006	0.018	-	0.002
Cyprinodon variegatus	Sheepshead Minnow	-	-	-	-	0.005	0.002
Opsanus beta	Gulf Toadfish	0.407	0.001	0.003	0.002	0.001	0.002
Hippocampus zosterae	Dwarf Seahorse	0.222	0.001	0.003	-	-	0.001

Table 10. Catch per unit effort (CPUE) per meter^2 of the top ten highest abundance invertebrate species caught by gear type from year-2 (2021) gear comparison study. Throw trap (TT), push net (PN), beam trawl (BT), 15' straight seine (SN-S), and 60' bag seine (SN-B).

Scientific Name	**Common Name**	**TT**	**PN**	**BT**	**SN-S**	**SN-B**	**Grand Total**
Palaemonetes spp.	Grass Shrimp	29.222	0.526	0.519	0.241	0.005	0.321
Panopeid	Mud Crab	22.481	0.089	0.162	0.064	0.005	0.100
Acetes americanus	Sergestid Shrimp	18.556	0.018	0.006	0.299	-	0.080
Tozeuma carolinense	Arrow Shrimp	2.222	0.101	0.088	0.136	-	0.062
Penaeid spp.	Penaeid Shrimp	2.926	0.048	0.083	0.078	0.036	0.053
Squilla spp.	Mantis Shrimp	0.519	0.002	0.006	0.113	-	0.014
Taphromysis louisianae	Mysid Shrimp	0.037	0.007	-	-	-	0.003
Callinectes sapidus	Blue Crab	0.481	0.002	0.003	0.001	0.001	0.003
Alpheus heterochaelis	Bigclaw Snapping Shrimp	0.630	-	-	-	0.001	0.002

The species richness, Shannon H Diversity, and Evenness of the nekton community were compared by gear type and while the diversity did not differ significantly by gear, the other two community metrics did display significant differences (Figure 27). The throw trap catch had significantly higher species richness compared to the beam trawl (p-value = 0.0012), and the 60' bag seine (p-value = 0.0064). The throw trap and push net had significantly lower Evenness compared to all other gears except for the 15' straight seine.

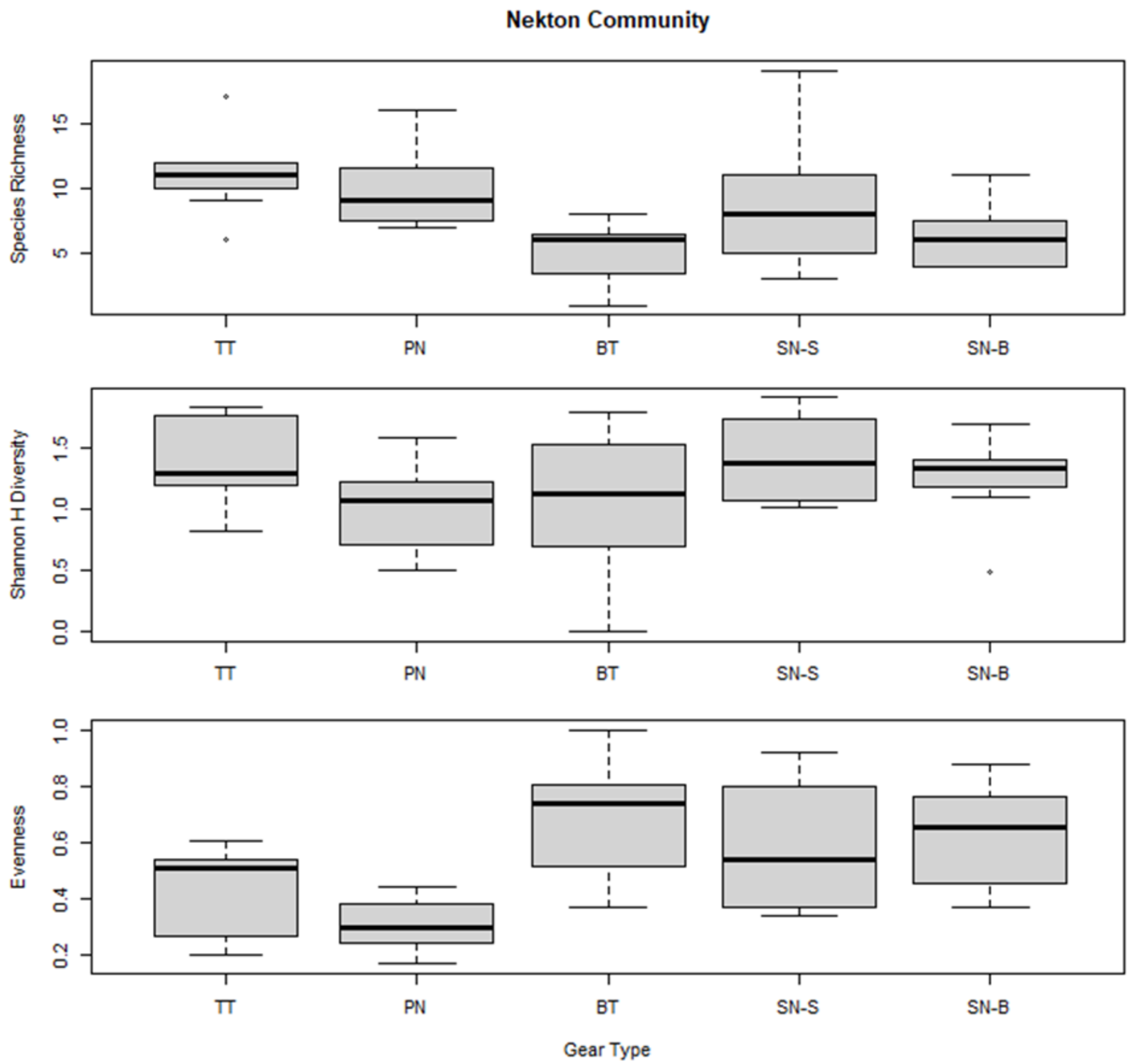


Figure 27. Overall nekton community species richness, Shannon H Diversity, and Evenness by gear type from year-2 (2021) gear comparison study. Throw trap (TT), push net (PN), beam trawl (BT), 15' straight seine (SN-S), and 60' bag seine (SN-B).

The nekton community data were analyzed for their similarities and dissimilarities by gear type and an ANOSIM was conducted on the resemblance matrix showing a significant difference between all gear types (p-values ≤ 0.003) except the beam trawl and the 15' straight seine (Figure 28). The sample events where Dwarf Seahorse(s) were captured are clustered to the right along the MDS1 ordination. The most spread in community similarly within a gear type is observed for both of the seine gears, while the throw trap communities were most tightly clustered (Figure 28).

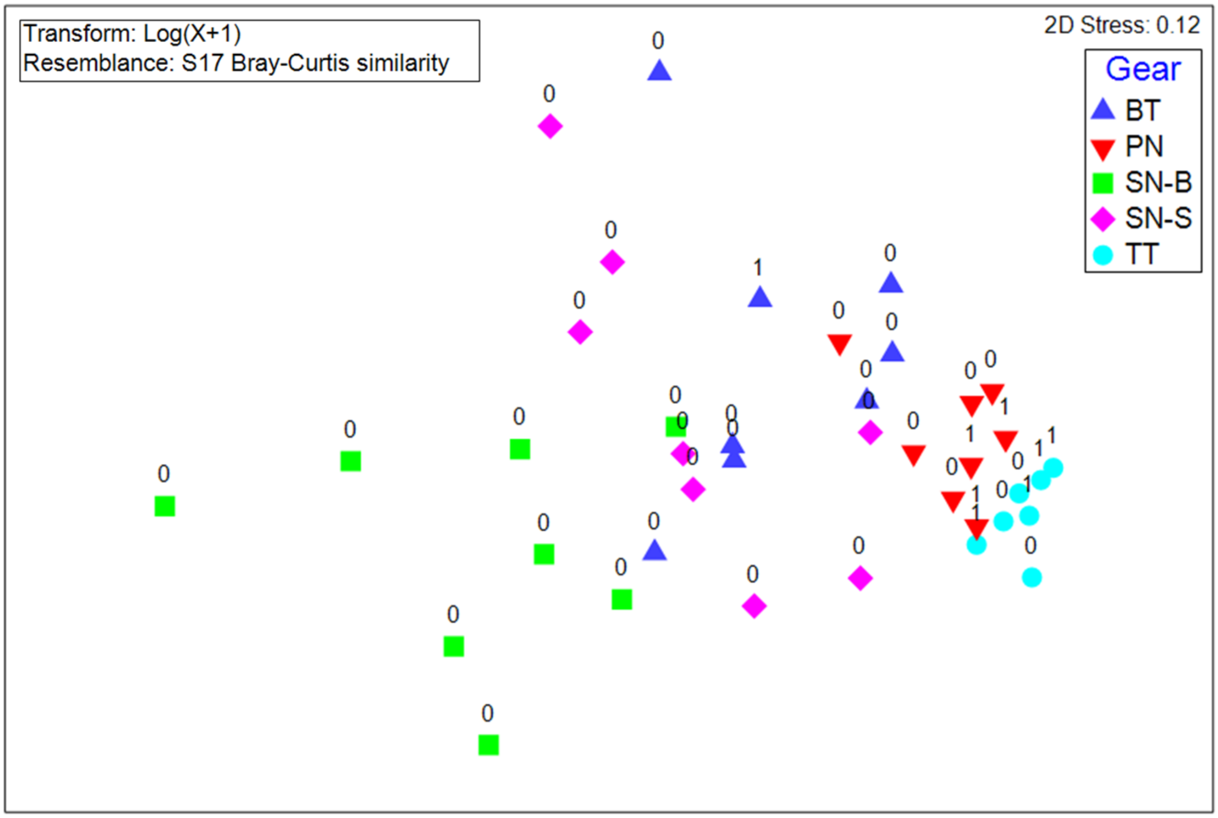


Figure 28. Non-metric MDS plot of nekton community catch by site from year-2 (2021) gear comparison study. Events labeled by gear type. Throw trap (TT), push net (PN), beam trawl (BT), 15' straight seine (SN-S), and 60' bag seine (SN-B).

Preliminary Genetic Assessment

The results of the preliminary population structure analysis show the likely ancestries of the Dwarf Seahorses found across the Florida and Texas coasts (Figure 29). Six genetic clusters were determined to best represent the data per the cross-validation analysis provided by the ADMIXTURE program. Each color represents a different genetic cluster, and each vertical bar represents an individual. A genetic cluster represents genetic sequences which are similar or shared between multiple individuals. Many individuals possess a mix of genetic sequences which belong to multiple genetic clusters. When this occurs, it is represented by multiple colors within the bar for the individual. For the Texas Coast individuals, there are two genetic clusters which are represented by light pink and orange. While there are some individuals with only a single genetic cluster represented in their ancestry (see San Antonio Bay individuals), most contain a mixture of the two genetic clusters.

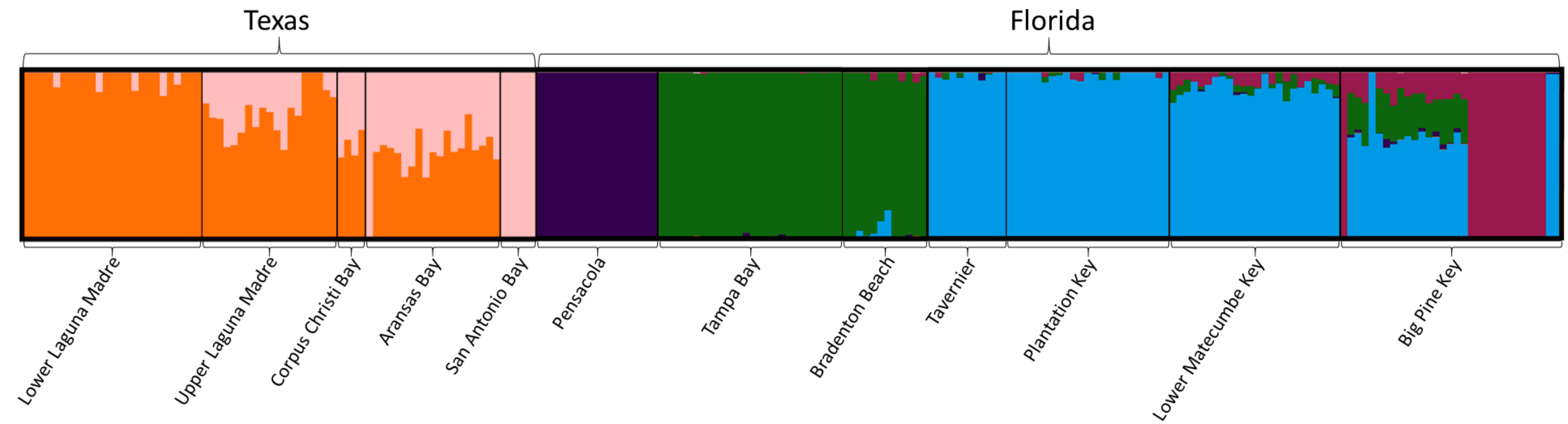


Figure 29. Preliminary results of genetic population structure analysis. Each color represents a distinct genetic cluster, and each bar represents an individual. Bars are grouped by geographic location of the samples and labeled. Sites correspond to the map provided in *Appendix 8*.

Discussion

Distribution and Abundance of Dwarf Seahorse (Year-1 2020)

This study represents the first coast-wide survey of Dwarf Seahorses in Texas. Previous studies and TPWD coastal routine monitoring have documented the presence of Dwarf Seahorse(s) as occasional or incidental catch with a total of 445 specimens observed throughout Texas from 1927 to 2012 (NMFS, 2020). While no Dwarf Seahorses were captured in Galveston Bay during this study, they were documented in all other bay systems studied. The bay system with the highest CPUE of Dwarf Seahorse was Aransas Bay, which was unexpected given the relatively low percent coverage of seagrass as well as low number of historic occurrences. The lower coast (Lower Laguna Madre) has been thought to support the highest abundance of Dwarf Seahorse in Texas (NOAA 2012). Sampling effort was not equal among all bay systems studied, more sites were distributed in the Upper and Lower Laguna Madre systems because of their higher percent coverage of seagrass and the higher number of historic occurrences in these areas. While all data were corrected for effort, additional surveys are recommended to further investigate the spatial differences in the abundance of Dwarf Seahorses along the Texas Coast.

While no statistically significant correlations were observed between Dwarf Seahorse abundance or presence and water quality variables, all sampling events for this study were conducted in the summer months of 2020, therefore conditions were relatively consistent. However, sampling in the Upper Laguna Madre Bay system occurred following a category 1 hurricane "Hanna" which made landfall at Padre Island on July 25, 2020 (Brown et al. 2021). No sampling occurred while the storm passed over the study area. However, scheduled biological sampling occurred just 3 days after landfall and for the following week the field crew experienced difficulties due to higher than usual water levels. These types of extreme meteorological events (hurricanes and floods) can cause disturbance to Dwarf Seahorse populations by displacing them during periods of high wave energy and erosion of seagrass beds, but also may be an important process facilitating genetic mixing for this, otherwise relatively sedentary, species (NMFS 2020).

Habitat Associations of Dwarf Seahorse

Based on a study conducted in Florida, Dwarf Seahorses are seagrass generalists at depths shallower than one meter (Masonjones et al. 2010) and they are most abundant in bay systems with warmer water temperatures and available seagrass habitats (Lourie et al. 2004, Masonjones et al. 2010, Carlson et al. 2019). While this expands their potential habitat range, it also means that all seagrass beds threatened by human activity along the Texas Coast may contain Dwarf Seahorses. Seagrass beds occur in shallow, sun-lit waters which are valued for recreational activities such as boating, swimming, and fishing. Boating can be especially impactful to seagrass beds, as propeller scarring and anchoring can cause physical damage, which take time to recover (Bell et al. 2002). No studies have been conducted on the effects that scarring have on seahorse species, but scarring is thought to disrupt available habitat for Dwarf Seahorses because of their limited ability to actively migrate within or between seagrass beds. Propellers from commercial and recreational boat traffic can re-suspend sediment which increases turbidity, and with prolonged disturbance can inhibit photosynthesis leading to declines in seagrass, and may reduce the ability of seahorses to find mates or feed.

Diversity of the seagrass community was a significant factor in the likelihood that Dwarf Seahorses would be detected. The higher the species richness and Shannon H diversity of the seagrass community the higher the CPUE and detection likelihood of Dwarf Seahorse at the site. Furthermore, at sites where Turtle Grass was present, a significantly higher CPUE of Dwarf Seahorse was observed. Seagrass communities like many other habitats undergo succession following disturbance or initial establishment/introduction into a new habitat. Shoal Grass (*Halodule wrightii*) is an efficient colonizing species (Zieman and Zieman, 1989) and is the only species that was found in all of the studied bay systems. As seagrass is able to establish, it can change the physical characteristics of the area by slowing water velocity, increasing organic deposition, and stabilizing the sediment (NMFS 2020). These processes improve conditions for the establishment of the other species of seagrass with Turtle Grass being considered a climax species of seagrass along the Gulf Coast (Zieman and Zieman 1989, Dunton, 2022, Arellano-Méndez et al. 2019).

The establishment of Turtle Grass increases overall biomass and the area available for epiphytic growth because of their broad and long leaves, which provides a more robust food base for the food web utilizing the seagrass bed (Zieman and Zieman, 1989, Arellano-Méndez et al. 2019). While at this point we are unable to determine what aspect(s) of the presence of Turtle Grass may be influencing Dwarf Seahorse presence, we know that seagrass beds with Turtle Grass tend to be more established beds and associated nekton communities have likely existed with minimal disturbance allowing for succession and diversification of the overall community (Arellano-Méndez et al. 2019). Further research is needed to evaluate micro-habitat associations of Dwarf Seahorse in Texas (for example within a mixed bed, is there an association with a specific seagrass species), which will require strict pairing of seagrass community assessment and seahorse selective gear deployment by replicate.

Macroalgae was present in all bay systems except for Galveston Bay at varying levels. Corpus Christi Bay had the largest average percent cover of macroalgae and a relatively reduced CPUE of Dwarf Seahorses. The presence of macroalgae complicated sampling with the push net, at times requiring the target length of the push to be shortened due to macroalgae filling the net. These instances required increased effort to sort through the samples in the field. As a result, the target number of reps were not always reached due to time (a single push net full of macroalgae could take a team of three up to one hour to sort through equaling three man hours). Therefore, Dwarf Seahorse abundance in areas with high macroalgae presence may be underrepresented in this study. The presence of macroalgae may be an important factor in Dwarf Seahorse passive dispersal, as the majority of the species of macroalgae are not attached to the substrate (NMFS 2020, Masonjones et al. 2010).

Nekton Community Structure

A number of fishes and invertebrates from a range of families were captured throughout the study using the push nets. In general, the fish species with the highest CPUE were smaller, less mobile types such as pipefish and gobies. The individuals that were caught from generally larger bodied species with higher mobility were typically juveniles (e.g. pinfish). The presence of certain species in the overall nekton community captured by sampling event correlated with the CPUE of Dwarf Seahorses. Specifically, when Grass Shrimp, Code Goby, and Rainwater Killifish were present in high numbers, the number of Dwarf Seahorse detected increased. Alternatively, when Blue Crab were present in high numbers the number of Dwarf Seahorse decreased. As expected, nekton community structure varied by bay system with similarities in community structure highest between spatially near bay systems and dissimilarities highest between spatially distant bay systems. But, these association data are complicated due to the natural spatial differences in community assemblage within the Dwarf Seahorses' range. Assessing the nekton community that Dwarf Seahorses are found in association with can provide additional information to help define their habitat preferences.

Demographics of Dwarf Seahorse

Of the 90 Dwarf Seahorses captured throughout both years of this study only 19% of them were mature males. Because males are primarily responsible for caring for eggs through gestation, and they give birth, the relatively low percentage of the captured Dwarf Seahorse as males is a concern for their population conservation (NMFS 2020, Carlson et al. 2019). Sex and maturity data were examined for correlation with all habitat and water quality variables and no statistically significant relationships were discovered. A study in Florida documented significantly female-biased sex ratios at sites near open water, and general female-biased populations overall (Rose et al. 2019). Continued monitoring of Dwarf Seahorse demographics is recommended to confirm or dispute and better determine the source of this apparent sex disparity in Texas.

Morphometrics, particularly ratios of the tail to height and snout to height were helpful at discerning maturity and sex of Dwarf Seahorses. Due to the presence of their brood pouch, males have a significantly larger tail to height ratio than both juveniles and females. Because of their small size, precise measurements and in many cases sex determination required euthanizing the captured Dwarf Seahorse in order to observe them under a dissecting microscope. Difficulty in taking measurements in the field due to their size are compounded by their prehensile tail and the difficulty in straightening it for ease of measurement. All measurements were taken using computer software of the tails in natural position (not extended straight). The ability to photograph individuals in the field with appropriate scale and image quality would allow for continued demographic analysis without sacrificing the individuals and should be considered for future monitoring. Snout to height ratio was helpful in confirming visually assessed maturity with juveniles displaying a larger snout to height ratio than mature individuals. Like many species, juvenile Dwarf Seahorse appear to "grow into their head".

Gear Comparison (Year-2 2021)

The most effective gear type for capturing Dwarf Seahorses in terms of CPUE was the throw trap followed by the push net. While the beam trawl captured a single Dwarf Seahorse, it is not recommended as a gear type for future studies targeting Dwarf Seahorses. The throw trap was the most time-consuming gear type, and samples the smallest area, but provides the most accurate estimates of abundance because of its exhaustive sampling technique. If the goal of future sampling is to monitor and determine Dwarf Seahorse abundance, the throw trap gear is recommended. However, due to the small sample area of the throw trap, care should be taken to account for the naturally patchy spatial distribution of Dwarf Seahorses and their seagrass habitats (NMFS, 2020), by increasing the number of replicates.

Alternatively, the push pet allows for coverage of a larger spatial area, but likely underrepresents the abundance of Dwarf Seahorse. If the goal of future sampling is to capture a large number of individuals for demographic analysis the push net gear is recommended. While there were insufficient data to determine statistical significance, there were no male Dwarf Seahorse captured in 2021 using the push net, while there were three captured using the throw trap. Further research is needed to determine if there may be a gear bias for sex with the push net.

As expected, the nekton community differed significantly by gear type. The mesh size, deployment technique, and general design of the gear types tested were all variable. Future community assemblage studies can benefit from including a combination of these and other gear types to assemble the most complete picture of what species are present within seagrass beds in Texas. Furthermore, the TPWD long-term coastal monitoring program which utilizes the 60’ bag seine, otter trawls, and gill nets are not able to quantitatively evaluate the abundance of Dwarf Seahorses, but occasional incidental catch has been helpful to provide background distribution information for the species.

Preliminary Genetic Assessment

When looking at the genetic population structure of Dwarf Seahorses on the Florida and Texas coasts, six genetic lineages are present. The preliminary results support previous work on the mitochondrial DNA of the Florida individuals, which also found that the Pensacola population is genetically unique (Fedrizzi et al. 2015). Genetic lineages of Dwarf Seahorses in Texas appear to be distinct from those found in Florida. The Texas lineages appear to follow a latitudinal gradient along the coast. Future work will include analysis of the Texas samples independently to determine the most accurate number of genetic lineages that best describe the Texas Dwarf Seahorse population(s). Beyond the physical distance between Texas and Florida, there are barriers to rafting between the two such as river deltas, where consistent freshwater inflow causes salinity in the nearshore waters to be too low for marine fishes such as seahorses to survive (Boehm et al. 2013). A previous study has indicated that there is gene flow between Texas and Florida populations, although this study only sampled 8 individuals from Redfish Bay, TX and 8 individuals from Tampa Bay, FL (Rose et al. 2014). However, other studies have found genetically distant populations within Florida, possibly due to different environmental conditions or geographic barriers (Fedrizzi et al. 2015). Further research should be conducted to determine the exact extent that migration is occurring between and beyond Texas bays and the mechanisms that may be supporting that genetic connectivity.

Future Work and Recommendations

The study design focused on a snapshot of Dwarf Seahorse distribution and abundance with only one sampling event at each site during the summer months of 2020. Continued periodic population monitoring is recommended to be able to track changes in the distribution, abundance, and demographics through time and address some of the data limitations outlined in this study. Additionally, year-round seasonal monitoring is needed to evaluate breeding timing, recruitment success, habitat utilization, and distribution and abundance variations on a temporal scale. Because of their small size and habitat association with shallow seagrass beds, sampling gear designed to target this and similar species are needed for continued monitoring. While incidental catch has historically occurred during routine coastal monitoring, it is likely that abundance estimates from these methods underestimate the actual population size. Specifically, throw traps and push nets are the most effective gear types for a continued monitoring program for Dwarf Seahorse in Texas. Additional sampling of deeper seagrass sites is recommended to further inform Dwarf Seahorse habitat suitability models which may include water depth. The challenge is incorporating a sampling gear that is selective for Dwarf Seahorses but also deployable in seagrass beds with water depths greater than 1.3m. Future work on Dwarf Seahorses in Texas should include more focused sampling efforts along the northern Texas coast, specifically the Matagorda and Galveston Bay systems, where seagrass coverage is the lowest and Dwarf Seahorse catch has been sporadic. Further analysis of Texas Dwarf Seahorse genetic data are on-going. Future work includes determining factors such as migration rates, loci under selection, and effective population size. This genetic work will provide a robust understanding of the genetic ecology of Dwarf Seahorse populations in both Texas and Florida. By understanding how the populations of each bay system are related, protections can be put in place to ensure that no major Dwarf Seahorse genetic diversity is lost, in Texas or for the species as a whole.

Deviations from Proposed Work

There were a few significant changes that were made to this project methodology from the initial proposal, though our overall goals and tasks did not change. One change was the distribution of sites within each bay system. Initially, sites were grouped into six major bay systems: Galveston, Matagorda, San Antonio, Aransas, Corpus Christi, and the Laguna Madre. The proposal for this project initially stated that we would sample 15 sites in the southern-most bays and only limited sampling would occur in Galveston Bay and Matagorda Bay. After evaluating the percent coverage of seagrass and historic occurrences of Dwarf Seahorse, we determined that it would be more efficient and would maximize our probability of detecting Dwarf Seahorse if our effort was weighted in bay systems that had the highest seagrass percent cover and historic Dwarf Seahorse sightings. Based on the percent cover of seagrass and historic sightings from agency records and published literature, we created a weighting regime with 80 total sites. We surveyed five sites in both Galveston and Matagorda Bays, 20 sites in both upper Laguna Madre and Lower Laguna Madre, which were split into two separate systems, and 10 sites in the remaining bay systems. More details about this site selection process are provided in our methodology.

We also deviated from our initial proposal by only sampling over one season rather than over the whole year. The decision to sample over the summer was made to reduce bias in sampling efficiency due to seasonality and to maintain consistent effort across the 80 sites. Sampling these sites over the course of multiple seasons would introduce a confounding seasonality affect to our data. Summer was chosen for sampling based on the idea that Dwarf Seahorse breeding season ranges from February to November (Strawn 1958). We hypothesized that we would have a higher probability of capturing Dwarf Seahorse during the summer months which fall directly in the middle of the breeding season.

Another change from the original proposal was that we only sampled biota using a 1x1 meter push net for the first season of this project. We discussed in our proposal also using additional gear to maximize the ability to compare new data with historical methods used to monitor shallow water nekton. The additional gear included a 10ft wide seine to sample at all sites and beam trawls, one-meter throw trap, a 60ft bag seine, and a 20ft wide otter trawls at selected sites. Instead we added a gear comparison study in year two of the project at the sites with the highest CPUE of Dwarf Seahorse from the first year of sampling. Some of the methods mentioned in the proposal were not applicable for the types of sites we sampled, all being less than four feet in depth. Therefore, the otter trawl was not utilized in the gear comparison study due to water depth and potential for damage to the seagrass beds being studied.

Finally, in the original proposal we stated that we would analyze seagrass community and habitat condition using modified Tier 1 and Tier 2 Texas Statewide Seagrass Monitoring Protocols (Dunton et al. 2009; Radloff et al. 2013). Tier 1 describes compiling status and trend maps of seagrass across the study area (Dunton et al. 2009). We used already created GIS layers of seagrass coverage from TPWD and Texas Seagrass Monitoring as well as historic seagrass coverage data from the Texas Seagrass Monitoring Program (http://www.texasseagrass.org/

(https://tpwd.texas.gov/arcgis/rest/services/GIS/Seagrass/MapServer) to characterize our study area and specific sites. Tier 2 protocols described specific data associated with the water column, seagrasses, and sediments that should be collected for more site-specific evaluation of the condition of seagrass habitat. We deviated from the protocols outlined in a few ways. First, we defined our site by a 20x20m square with our chosen GPS point within the square boundary of the site rather than a 10m radius centered on the GPS location. This was necessary to allow for sufficient area to conduct our targeted effort for push net sampling. We only recorded general "composite plant" average canopy height within a 0.25 m^2 area rather than canopy height and maximum leaf length. This was determined by tactile sensing of the "average" height of the canopy by hand due to lack of visibility. We used a coring device to obtain biomass and sediment characteristics, but did not estimate shoot density. Finally, instead of placing our quadrats on the four sides of the vessel, four randomly selected positions within our site for placement of sample quadrates were determined using a random number table, to get a more accurate unbiased representation of the entire site where the actual push net replicates were conducted.

Literature Cited

Arellano-Méndez, L. U., A. Mora-Olivo, C. Zamora-Tovar, E. de la Rosa-Manzano, J.A. Torres-Castillo, and J. Bello-Pineda. 2019. Structural complexity of tropical seagrasses meadows in a temperate lagoon in the Gulf of Mexico. A landscape ecology approach. Journal of Coastal Conservation 23: 969-976

Baum, J. K., J. J. Meeuwig, and A. C. Vincent. 2003. Bycatch of lined seahorses (*Hippocampus erectus*) in a Gulf of Mexico shrimp trawl fishery. Fishery Bulletin 101(4):721-731.

Baum, J. K., and A. C. J. Vincent. 2005. Magnitude and inferred impacts of the seahorse trade in Latin America. Environmental Conservation 32(4):305-319.

Bell, S. S., M. O. Hall, S. Soffian, and K. Madley. 2002. Assessing the impact of boat propeller scars on fish and shrimp utilizing seagrass beds. Ecological Applications 12(1):206-217.

Boehm, J., and coauthors. 2013. Marine dispersal and barriers drive Atlantic seahorse diversification. Journal of Biogeography 40(10):1839-1849.

Bohlke, J. E., & Chaplin, C. C. G. 1966. Fishes of the Bahamas and adjacent tropical waters. Wynnewood, PA: Livingston Publishing Co.

Bray, J. R. and J. T. Curtis. 1957. An ordination of upland forest communities of southern Wisconsin. Ecological Monographs 27, 325-349.

Brown, D.P., R. Berg, and B. Reinhart. 2021. National Hurricane Center Tropical Cyclone Report, Hurricane Hanna (AL082020). National Oceanic and Atmospheric Administration, National Hurricane Center. Available at: https://www.nhc.noaa.gov/data/tcr/AL082020_Hanna.pdf pp. 49.

Bruckner, A. W. 2005. The importance of the marine ornamental reef fish trade in the wider Caribbean. Revista de biología tropical, 53, 127-137.

Cairns, S.D., D.R. Calder, A. Brinkmann-Voss, C. Barreira e Castro, D. Fautin, P. Pugh, C.E. Mills, W.C. Jaap, M.N. Arai, S.H.D. Haddock and D.M. Opresko. 2003. Common and scientific names of aquatic invertebrates from the United States and Canada: Cnidaria and Ctenophora. 2nd edition. American Fisheries Society Special Publication 28. 126 pages. Bethesda, MD.

Carlson, J. K., C. S. Horn, K. L. Smith, and D. M. Bethea. 2019. Population Viability Analysis of the Dwarf Seahorse, *Hippocampus Zosterae*, in Florida. National Oceanic and Atmospheric Administration (NOAA) Technical Memorandum NMFS-SEFSC-739. doi:10.25923/pkdt-sg41. pp.41.

CBD (Center for Biologic Diversity). 2011. Petition to list the Dwarf Seahorse (Hippocampus zosterae) as threatened or endangered under the endangered species act. Center for Biological Diversity. 68 pp.

Clarke, K.R. and R.M. Warwick. 2001. Change in marine communities: an approach to statistical analysis and interpretation. 2nd edition. Plymouth, UK.

Congdon, V.M, and K.H. Dunton. 2016. Tracking Long-Term Trends in Seagrass Cover and Condition in Texas Coastal Waters. Final Report to the Texas Coastal Management Program, National Oceanic and Atmospheric Administration award no. NA14NOS4190139. pp. 43.

Dunn, O. J. 1964 Multiple comparisons using rank sums Technometrics, 6(3):241-252.

Dunton, K.H., K. Jackson, and C. Wilson (2009). Seagrass and Macroalgal Biomass. Tier 2 Seagrass Protocols, 10-11.

Dunton, K.H. 2022. Texas Statewide Seagrass Monitoring Program. On-line resource: https://www.texasseagrass.org/index.html accessed 1/11/2022.

Dunton, K. H., Pulich, W., & Mutchler, T. 2011. A seagrass monitoring program for Texas coastal waters: Multiscale integration of landscape features with plant and water quality indicators. Final Report to Coastal Bend Bays & Estuaries Program.

Fedrizzi, N., Stiassny, M. L. J., Boehm, J. T., Dougherty, E. R., Amato, G., and Mendez, M. 2015. Population Genetic Structure of the Dwarf Seahorse (*Hippocampus zosterae*) in Florida. PLOS ONE 10(7):e0132308.

Ginsberg, I. 1937. Review of the seahorses (*Hippocampus*) found on the coasts of the American continents and of Europe. *Proceedings of the United States National Museum.*, **83**, 497–594.

Handley, L., D. Altsman, and R. DeMay. 2007. Seagrass Status and Trends in the Northern Gulf of Mexico: 1940-2002. U.S. Geographical Survey Scientific Investigations Report 2006-5287.

Hoese, H.D., Moore, R. H., Sonnier, F., & Bowman, D. 1998. Fishes of the Gulf of Mexico: Texas. Louisiana, and adjacent waters: Texas A&M University Press College Station.

Hughes, A.R., S.L. Williams, C.M. Duarte, K.L. Heck Jr, and M. Waycott. 2009. Associations of concern: declining seagrasses and threatened dependent species. Frontiers in Ecology and the Environment, 7(5), 242-246.

Irey, B. 2004. "*Hippocampus zosterae*". University of Michigan – *Museum of Geology.* Retrieved from https://animaldiversity.org/accounts/Hippocampus_zosterae/.

Jackson, J. B., M.X. Kirby, W.H. Berger, K.A. Bjorndal, L.W. Botsford, B.J. Bourque, R.H. Bradbury, R. Cooke, J. Erlandson, J.A. Estes, T.P. Hughes, S. Kidwell, C.B. Lange, H.S. Lenihan, J.M. Pandolfi, C.H. Peterson, R.S. Steneck, M.J. Tegner, and R.R. Warner. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science, 293(5530): 629-637.

Lamb, J.B., J.A. Van De Water, D.G. Bourne, C. Altier, M.Y. Hein, E.A. Fiorenza, N. Abu, J. Jompa, and C. D. Harvell. 2017. Seagrass ecosystems reduce exposure to bacterial pathogens of humans, fishes, and invertebrates. Science, 355(6326): 731-733.

Lewis, M. A., and R. Devereux. 2009. Nonnutrient anthropogenic chemicals in seagrass ecosystems: fate and effects. Environmental Toxicology and Chemistry: An International Journal 28(3):644-661.

Lin, Q., Fan, S., Zhang, Y., Xu, M., Zhang, H., Yang, Y., Lee, A. P., Woltering, J. M., Ravi, V., Gunter, H. M., Luo, W., Gao, Z., Lim, Z. W., Qin, G., Schneider, R. F., Wang, X., Xiong, P., Li, G., Wang, K., Min, J., Zhang, C., Qiu, Y., Bai, J., He, W., Bian, C., Zhang, X., Shan, D., Qu, H., Sun, Y., Gao, Q., Huang, L., Shi, Q., Meyer, A., and Venkatesh, B. 2016. The seahorse genome and the evolution of its specialized morphology. Nature 540(7633):395-399.

Lin, Q., Qiu, Y., Gu, R., Xu, M., Li, J., Bian, C., Zhang, H., Qin, G., Zhang, Y., Luo, W., Chen, J., You, X., Fan, M., Sun, M., Xu, P., Venkatesh, B., Xu, J., Fu, H., and Shi, Q. 2017. Draft genome of the lined seahorse, *Hippocampus erectus*. GigaScience 6(6).

Lourie, S. A., Foster, S. J., Cooper, E. W., & Vincent, A. C. (2004). A guide to the identification of seahorses. Project Seahorse and TRAFFIC North America, 114.

Mann, Henry B.; Whitney, Donald R. 1947. On a Test of Whether one of Two Random Variables is Stochastically Larger than the Other. Annals of Mathematical Statistics 18, 50–60.

Martinez-Andrade, F. 2015. Marine Resource Monitoring Operations Manual. Texas Parks and Wildlife Department. Austin, TX. Available at: https://datadocs.bco-dmo.org/docs/Texas_Coastal_Fish/data_docs/CF-Mar-Res-Mon-Ops-Manual-2015.pdf. pp 133.

Masonjones, H. D. 2001. The effect of social context and reproductive status on the metabolic rates of dwarf seahorses (*Hippocampus zosterae*). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 129(2-3):541-555.

Masonjones, H. D., E. Rose, L. B. McRAE, and D. L. Dixson. 2010. An examination of the population dynamics of syngnathid fishes within Tampa Bay, Florida, USA. Current Zoology 56(1).

Masonjones, H., Hayashida-Boyles, A. & R. Pollom. 2017. *Hippocampus zosterae*. The IUCN Red List of Threatened Species 2017: e.T10089A46910143. https://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T10089A46910143.en. Downloaded on 26 October 2020.

McLaughlin, P. A., D. K. Camp, M. V. Angel, E. L. Bousfield, P. Brunel, R. C. Brusca, D. Cadien, A. C. Cohen, K. Conlan, L. G. Eldredge, D. L. Felder, J. W. Goy, T. Haney, B. Hann, R. W. Heard, E. A. Hendrycks, H. H. Hobbs III, J. R. Holsinger, B. Kensley, D. R. Laubitz, S. E. LeCroy, R. Lemaitre, R. F. Maddocks, J. W. Martin, P. Mikkelsen, E. Nelson, W. A. Newman, R. M. Overstreet, W. J. Poly, W. W. Price, J. W. Reid, A. Robertson, D. C. Rogers, A. Ross, M. Schotte, F. R. Schram, C.-T. Shih, L. Watling, G. D. F. Wilson, and D. D. Turgeon. 2005. Common and scientific names of aquatic invertebrates from the United States and Canada: crustaceans. American Fisheries Society, Special Publication 31, Bethesda, Maryland.

Meyer, D. L., Fonseca, M. S., Murphey, P. L., McMichael, R. H., LaCroix, M. W., Whitfield, P. E., and Thayer, G. W. 1999. Effects of live-bait shrimp trawling on seagrass beds and fish bycatch in Tampa Bay, Florida. Fishery Bulletin 97(1):193-199.

NOAA (National Oceanic and Atmospheric Administration). 2012 Endangered and threatened Wildlife; 90-day finding on a petition to list the Dwarf Seahorse as Threatened or

Endangered under the Endangered Species Act. Federal Register Volume 77 No. 876. Friday, May 4, 2012. Proposed Rules 50 CFR Parts 223 and 224.

NMFS (National Marine Fisheries Service). 2020. Status review report: dwarf seahorse (*Hippocampus zosterae*). Final Report to National Marine Fisheries Service, Office of Protected Resources. February 2020. 90 pp.Page, L. M., H. Espinosa-Pérez, L. T. Findley, C. R. Gilbert, R. N. Lea, N. E. Mandrak, R. L. Mayden, and J. S. Nelson. 2013. Common and scientific names of fishes from the United States, Canada, and Mexico, 7th edition. American Fisheries Society, Special Publication 34, Bethesda, Maryland.

Peterson, B. K., J. N. Weber, E. H. Kay, H. S. Fisher, and H. E. Hoekstra. 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLOS ONE 7(5):e37135.

Pulich, W., Jr., and C. Onuf. 2002. Statewide summary for Texas. Seagrass status and trends in the northern Gulf of Mexico. Available at: https://www.arlis.org/docs/vol1/234196545/statewidesummaryfortexas.pdf. pp. 10

Pulich, W.M., Jr., and W.A. White. 1991. Decline of submerged vegetation in the Galveston Bay system: chronology and relationship to physical processes. Journal of Coastal Research, 7:1125-1138.

RStudio Team. 2021. RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Radloff, P.L, C. Hobson, A. Whisenant, and J.M.B. Warren. 2013. Statewide seagrass monitoring protocol development – Phase 2 final report. Water Quality Technical Series Publication WQTS-2013-02. PWD RP V3400-1785. Texas Parks and Wildlife Department, Austin, TX.

Renfro, W.C. 1963. Small beam net for sampling post larval shrimp, U.S. Fish and Wildlife Service Circular 161. p 86-87.

Robbins, B.D. and S.S. Bell. 1994. Seagrass landscapes: a terrestrial approach to the marine subtidal environment. Trends in Ecology & Evolution, 9(8): 301-304.

Rose, E., M. Simmonds, A. L. Hayashida-Boyles, and H. D. Masonjones. 2019. Seasonal and spatial variation in the reproductive biology of the dwarf seahorse Hippocampus zosterae. Journal of fish biology 95(2):357-366.

Rose, E., C. M. Small, H. A. Saucedo, C. Harper, and A. G. Jones. 2014. Genetic Evidence for Monogamy in the Dwarf Seahorse, *Hippocampus zosterae*. Journal of Heredity 105(6):922-927.

Shapiro, S. S.; Wilk, M. B. 1965. An analysis of variance test for normality. Biometrika 52, 591–611.

Strawn, K. 1958. Life history of the pigmy seahorse, *Hippocampus zosterae* Jordan and Gilbert, at Cedar Key, Florida. *Copeia, 1958*(1): 16-22.

TPWD (Texas Parks and Wildlife Department). 2017. Seagrass Types. Online Resource: https://tpwd.texas.gov/landwater/water/habitats/seagrass/seagrass-types. Accessed 1/19/2022.

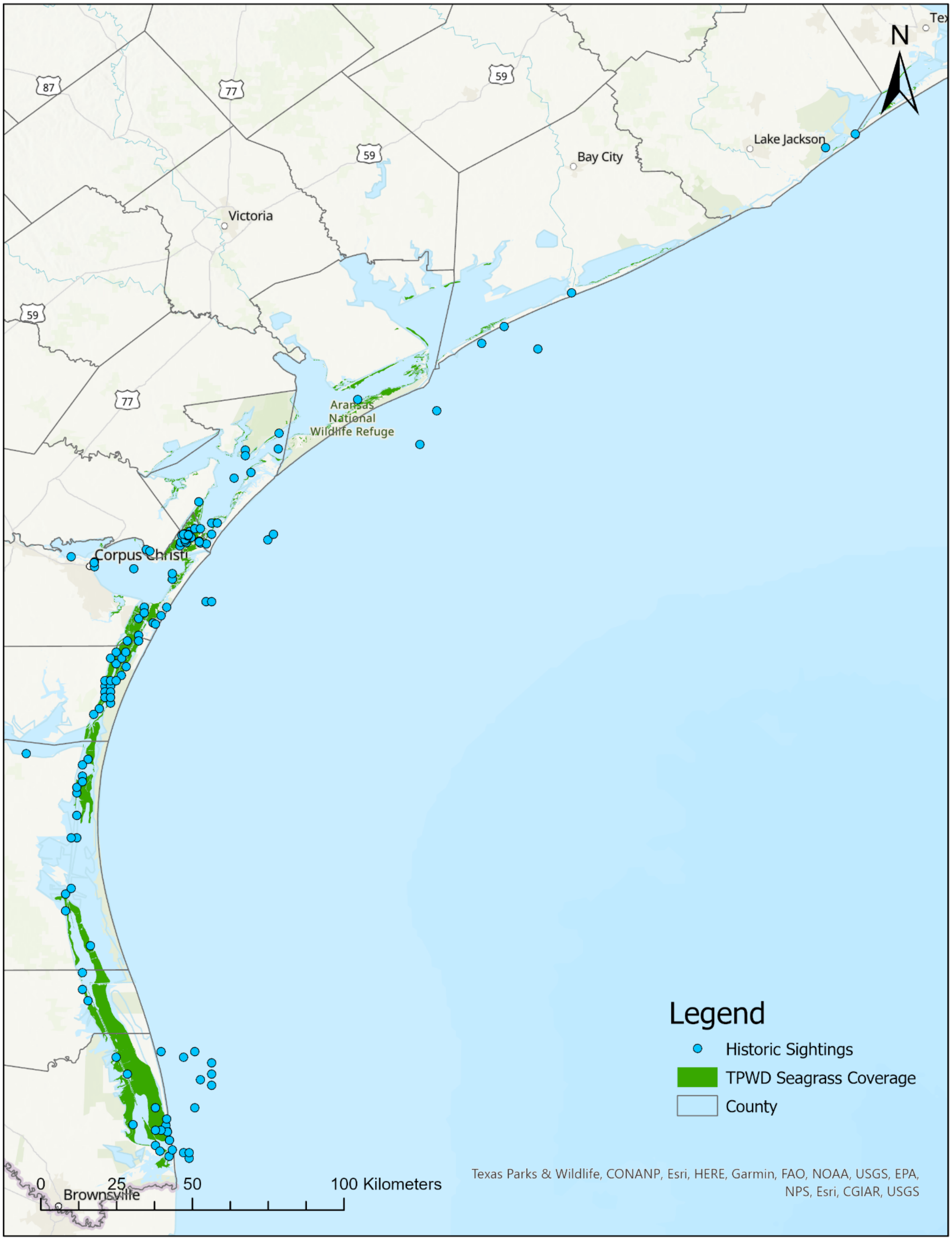
Turgeon, D. D., J. F. Quinn, Jr., A. E. Bogan, E. V. Coan, F. G. Hochberg, W. G. Lyons, P. M. Mikkelsen, R. J. Neves, C. F. E. Roper, G. Rosenberg, B. Roth, A. Scheltema, F. G. Thompson, M. Vecchione, and J. D. Williams. 1998. Common and scientific names of aquatic invertebrates from the United States and Canada: mollusks, 2nd edition. American Fisheries Society, Special Publication 26, Bethesda, Maryland. Vincent, A. C. J. 1996. The international trade in seahorses. p. 172. TRAFFIC International. Cambridge, UK. pp. 172. Vincent, A. C., S. Foster, and H. Koldewey. 2011. Conservation and management of seahorses and other Syngnathidae. Journal of fish biology 78(6):1681-1724.

Vincent, A. C., and H. J. Koldewey. 2006. An uncertain future for seahorse aquaculture in conservation and economic contexts. Pages 71-84 in Proceedings of the Regional Technical Consultation on Stock Enhancement for Threatened Species of International Concern, Iloilo City, Philippines, 13-15 July 2005. Aquaculture Department, Southeast Asian Fisheries Development Center.

Woodall, L. C., H. J. Koldewey, and P. W. Shaw. 2011. Serial monogamy in the European long-snouted seahorse *Hippocampus guttulatus*. Conservation Genetics 12(6):1645-1649.

Zieman, J.C., and R.T. Zieman. 1989. The ecology of the seagrass meadows of the west coast of Florida: A community profile. Biological Report 85(7.25) submitted to the Minerals Management Service and the U.S. Fish and Wildlife Service. Available at: http://www.tampabay.wateratlas.usf.edu/upload/documents/EcologySeagrassWestCoast1989.pdf. pp. 167.

Appendices



Appendix 1. Map of all historic accounts of Dwarf Seahorse in Texas.

Appendix 2. Table of all sites sampled during the year-1 (2020) distribution and abundance study with the number, effort and catch per unit effort (CPUE) of Dwarf Seahorse per meter squared.

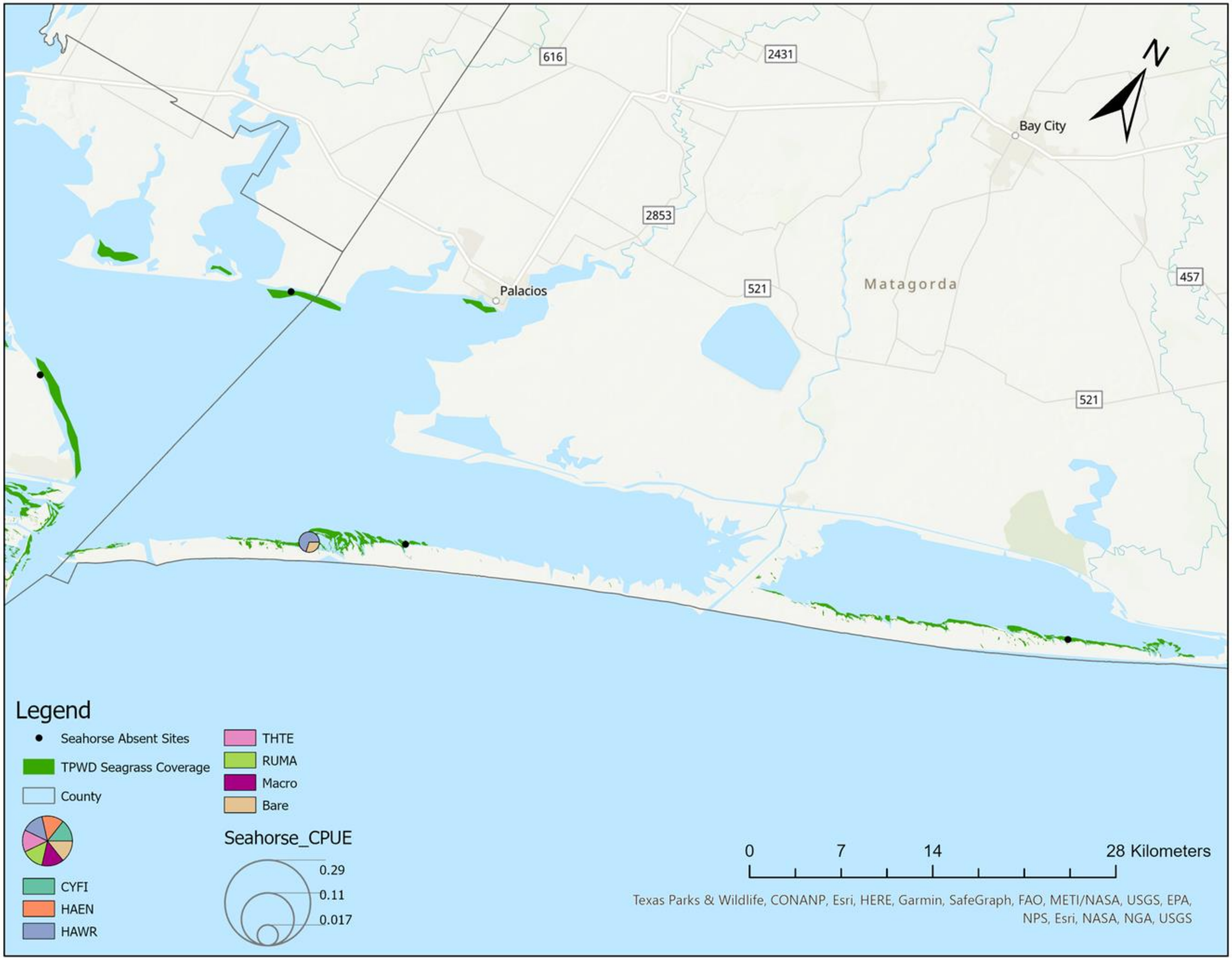
Bay System	Site ID	Latitude	Longitude	Dwarf Seahorse Catch	Effort (m^2)	Dwarf Seahorse CPUE
Galveston	GBAY21	29.21097	-94.95724	0	60	0
	GBAY1	29.09407	-95.11804	0	60	0
	GBAY42	29.04419	-95.17188	0	60	0
	GBAY6	29.15147	-95.04700	0	60	0
	GBAY35	29.27366	-94.97806	0	60	0
Matagorda	MAT2	28.70546	-95.75948	0	60	0
	MAT14	28.63405	-96.33977	0	60	0
	MAT9	28.52611	-96.18276	0	60	0
	MAT7	28.49329	-96.24024	**1**	60	**0.017**
	MAT19	28.49584	-96.45766	0	60	0
San Antonio	SABAY65	28.38541	-96.43242	0	60	0
	SABAY64	28.38791	-96.43662	**2**	60	**0.033**
	SABAY67	28.39182	-96.51183	0	60	0
	SABAY29	28.31010	-96.54050	**2**	60	**0.033**
	SABAY20	28.29237	-96.54687	0	60	0
	SABAY28	28.29922	-96.56319	0	60	0
	SABAY68	28.38711	-96.51760	0	60	0
	SABAY14	28.28084	-96.60674	0	60	0
	SABAY10	28.29642	-96.58095	0	60	0
	SABAY47	28.33996	-96.60619	**1**	60	**0.017**
Aransas	NERR28	27.93310	-97.10927	**5**	60	**0.083**
	NERR32	27.94177	-97.10159	**2**	60	**0.033**
	NERR48	28.01311	-96.97252	0	60	0
	NERR44	27.97052	-96.98873	0	60	0
	NERR33	27.94396	-97.08432	0	60	0
	NERR31	27.93720	-97.07894	**7**	30	**0.233**
	NERR1	27.87330	-97.07190	0	60	0
	NERR5	27.88945	-97.08469	**1**	40	**0.025**
	NERR18	27.90815	-97.08008	**4**	30	**0.133**
	NERR55	28.09729	-96.90403	**1**	60	**0.017**
Corpus Christi	CCBAY40	27.80412	-97.11668	0	60	0
	CCBAY34	27.75596	-97.15250	**2**	60	**0.033**
	CCBAY25	27.87424	-97.10255	**1**	60	**0.017**
	CCBAY14	27.85665	-97.10120	0	31	0
	CCBAY2	27.84171	-97.11886	**2**	60	**0.033**
	CCBAY3	27.84188	-97.16789	**1**	60	**0.017**
	CCBAY24	27.87086	-97.14538	0	60	0
	CCBAY72	27.67008	-97.23477	0	60	0
	CCBAY63	27.66203	-97.21184	0	60	0
	CCBAY49	27.64321	-97.24126	0	60	0

Appendix 2 cont. Table of all sites sampled during the year-2 (2021) gear comparison study with the number, effort and catch per unit effort (CPUE) of Dwarf Seahorse per meter squared.

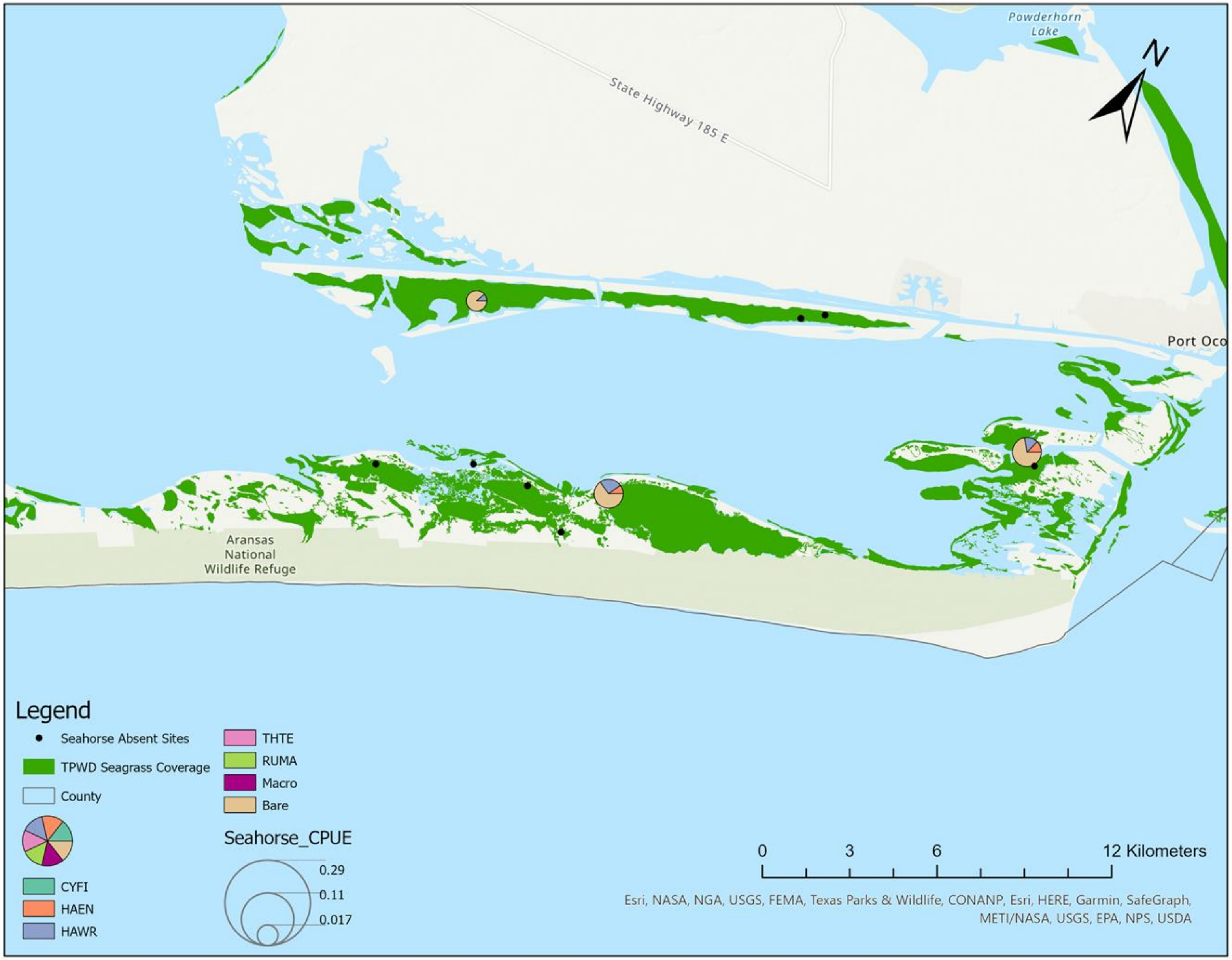
Bay System	Site ID	Latitude	Longitude	Dwarf Seahorse Catch	Effort (m^2)	Dwarf Seahorse CPUE
Upper Laguna Madre	ULM6	27.04207	-97.40866	**5**	60	**0.083**
	ULM16	27.08601	-97.40473	**1**	60	**0.017**
	ULM25	27.11603	-97.41747	0	60	0
	ULM31	27.14293	-97.42953	0	41	0
	ULM35	27.22939	-97.40449	0	60	0
	ULM46	27.31120	-97.40945	**1**	60	**0.017**
	ULM140	27.63978	-97.26398	0	60	0
	ULM100	27.54210	-97.30207	0	60	0
	ULM125	27.59339	-97.27413	0	60	0
	ULM119	27.57791	-97.26318	0	60	0
	ULM130	27.61208	-97.25508	**2**	60	**0.033**
	ULM133	27.61786	-97.26917	**2**	60	**0.033**
	ULM122	27.58169	-97.25727	0	40	0
	ULM88	27.50932	-97.30909	0	60	0
	ULM83	27.49486	-97.30800	0	34	0
	ULM91	27.51972	-97.33572	**4**	22	**0.182**
	ULM96	27.53342	-97.33164	0	60	0
	ULM98	27.52824	-97.28681	0	60	0
	ULM61	27.41384	-97.33475	**5**	60	**0.083**
	ULM72	27.46213	-97.35316	0	60	0
Lower Laguna Madre	LLM75	26.22108	-97.23058	0	60	0
	LLM62	26.19645	-97.28177	0	60	0
	LLM53	26.18044	-97.28973	0	60	0
	LLM49	26.17200	-97.29835	0	60	0
	LLM29	26.12955	-97.17947	0	60	0
	LLM6	26.06376	-97.19366	**6**	60	**0.100**
	LLM285	26.81692	-97.48744	**8**	60	**0.133**
	LLM277	26.76933	-97.47086	0	60	0
	LLM257	26.64411	-97.39602	**4**	60	**0.067**
	LLM238	26.56277	-97.37545	**1**	60	**0.017**
	LLM187	26.43436	-97.34367	0	60	0
	LLM197	26.44889	-97.35592	0	60	0
	LLM118	26.27509	-97.23434	**2**	60	**0.033**
	LLM109	26.26456	-97.25875	**2**	60	**0.033**
	LLM85	26.23566	-97.29315	0	60	0
	LLM127	26.29560	-97.29020	0	60	0
	LLM170	26.37967	-97.30075	**1**	60	**0.017**
	LLM134	26.30500	-97.31367	0	60	0
	LLM151	26.33593	-97.32266	**1**	60	**0.017**
	LLM3	26.02070	-97.17950	**3**	60	**0.050**

Appendix 3. Table of all sites sampled during the year-2 (2021) gear comparison study with the number, effort and catch per unit effort (CPUE) of Dwarf Seahorse per meter squared.

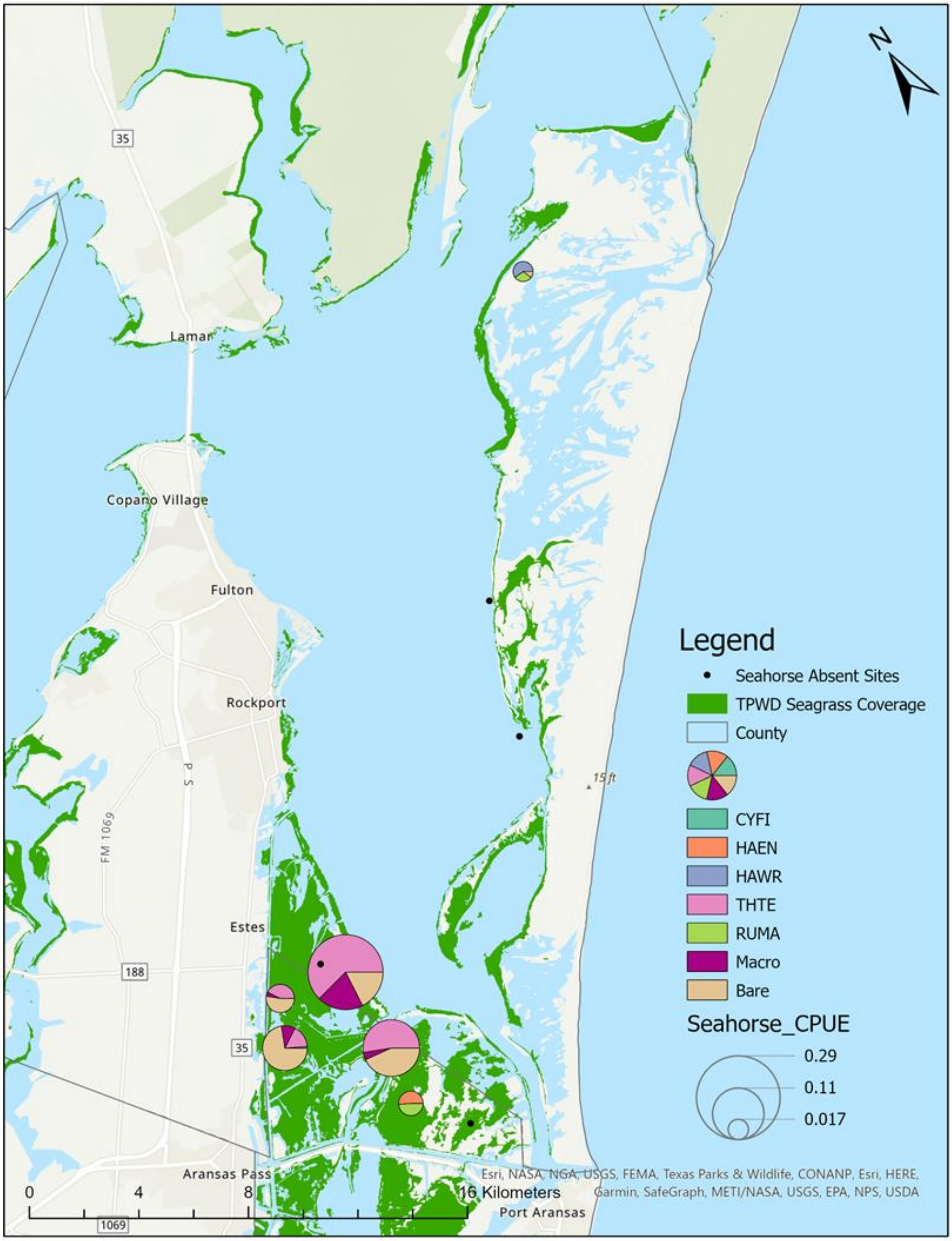
Bay System	Site ID	Gear Type	Dwarf Seahorse Catch	Effort (m^2)	Dwarf Seahorse CPUE
Lower Laguna Madre	LLM3	TT	0	3	0
		BT	0	39	0
		PN	**3**	60	**0.100**
		SN-S	0	137.2	0
		SN-B	0	613.2	0
	LLM6	TT	0	3	0
		PN	**1**	60	**0.033**
		BT	0	39	0
		SN-S	0	137.2	0
		SN-B	0	613.2	0
	LLM238	TT	**1**	3	**0.333**
		BT	0	39	0
		PN	0	60	0
		SN-S	0	137.2	0
		SN-B	0	613.2	0
	LLM285	TT	0	3	0
		BT	0	39	0
		PN	0	60	0
		SN-S	0	137.2	0
		SN-B	0	613.2	0
Upper Laguna Madre	ULM91	TT	**1**	3	**0.333**
		BT	0	39	0
		PN	0	22	0
		SN-S	0	137.2	0
		SN-B	0	613.2	0
	ULM61	TT	**2**	3	**0.667**
		BT	0	39	0
		PN	0	60	0
		SN-S	0	137.2	0
		SN-B	0	613.2	0
Aransas	NERR28	TT	0	3	0
		BT	**1**	39	**0.026**
		PN	0	60	0
		SN-S	0	137.2	0
		SN-B	0	613.2	0
	NERR31	TT	**2**	3	**0.667**
		BT	0	39	0
		PN	**1**	30	**0.056**
		SN-S	0	137.2	0
		SN-B	0	613.2	0



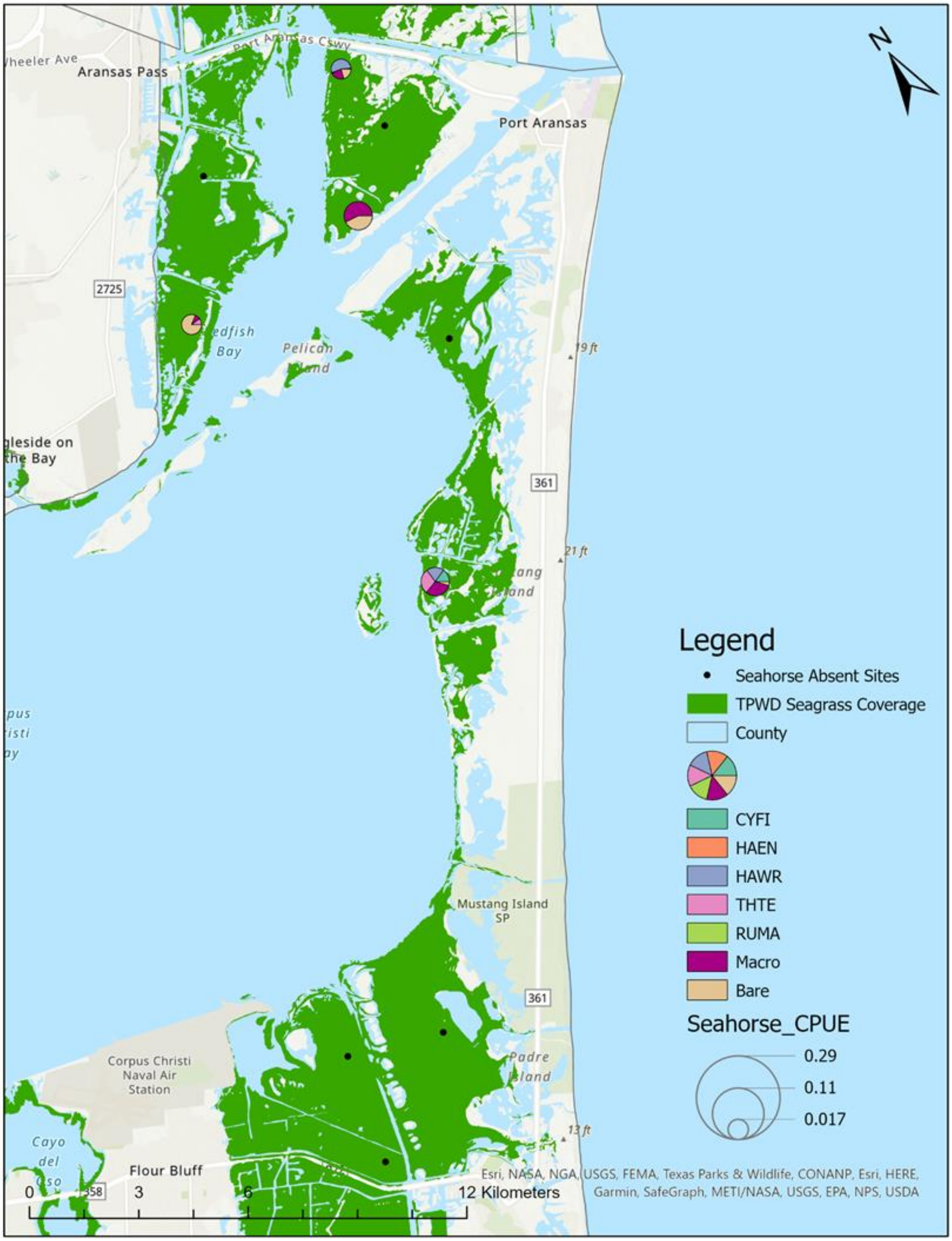
Appendix 4a. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in Matagorda Bay and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter squared with pie chart showing the percent coverage of seagrass species and macroalgae. .



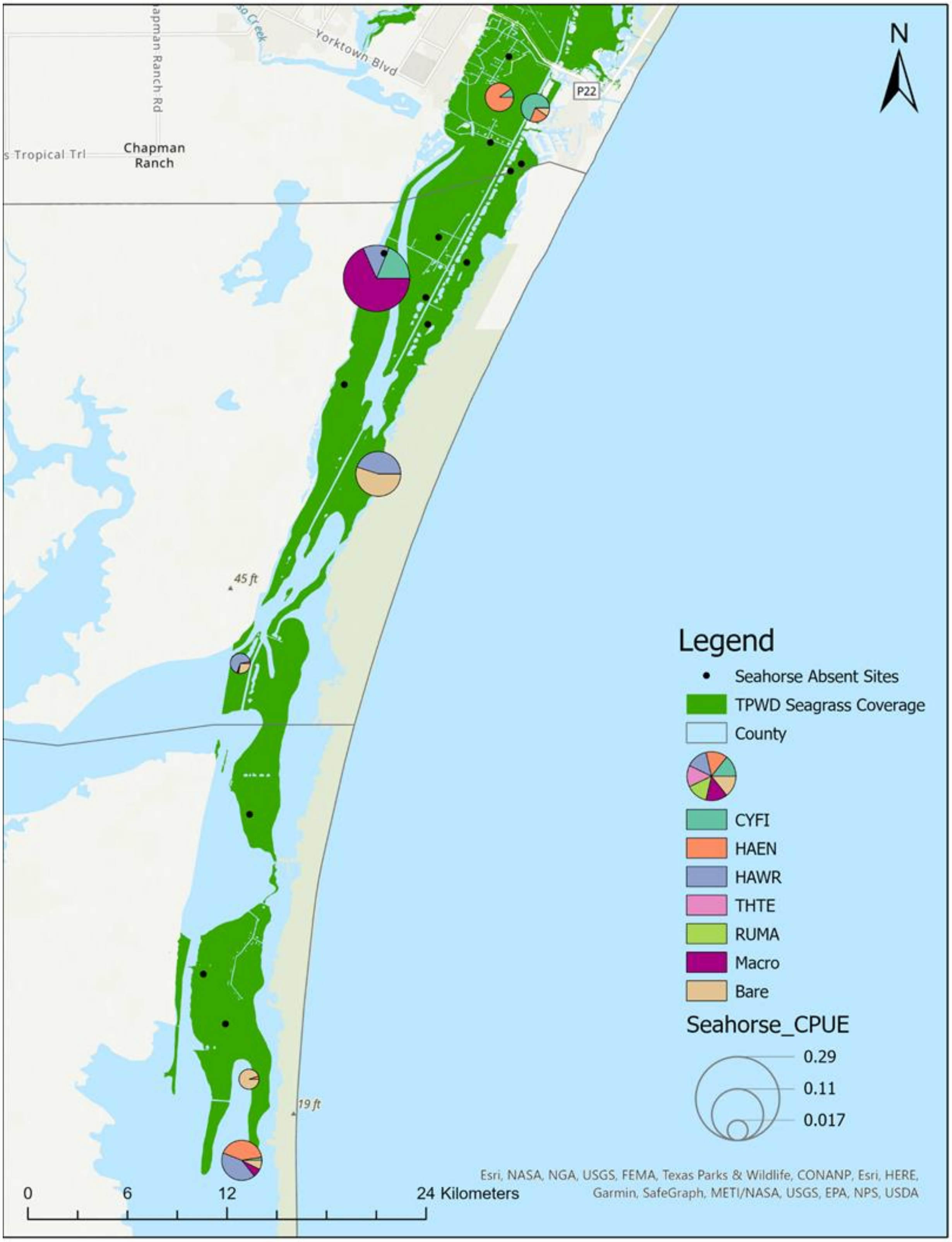
Appendix 4b. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in San Antonio Bay and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter squared with pie chart showing the percent coverage of seagrass species and macroalgae.



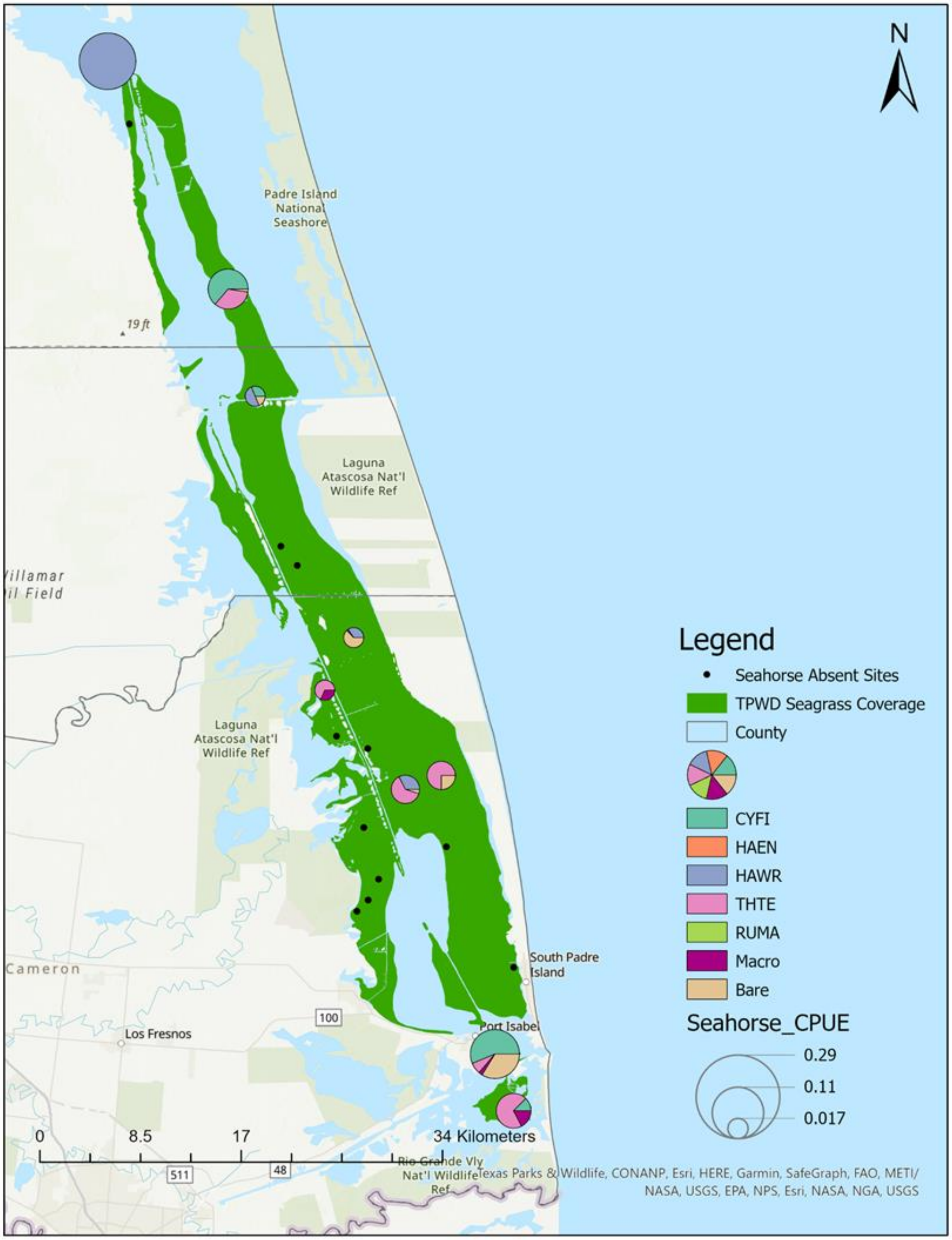
Appendix 4c. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in Aransas Bay and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter squared with pie chart showing the percent coverage of seagrass species and macroalgae.



Appendix 4d. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in Corpus Christi Bay and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter squared with pie chart showing the percent coverage of seagrass species and macroalgae.

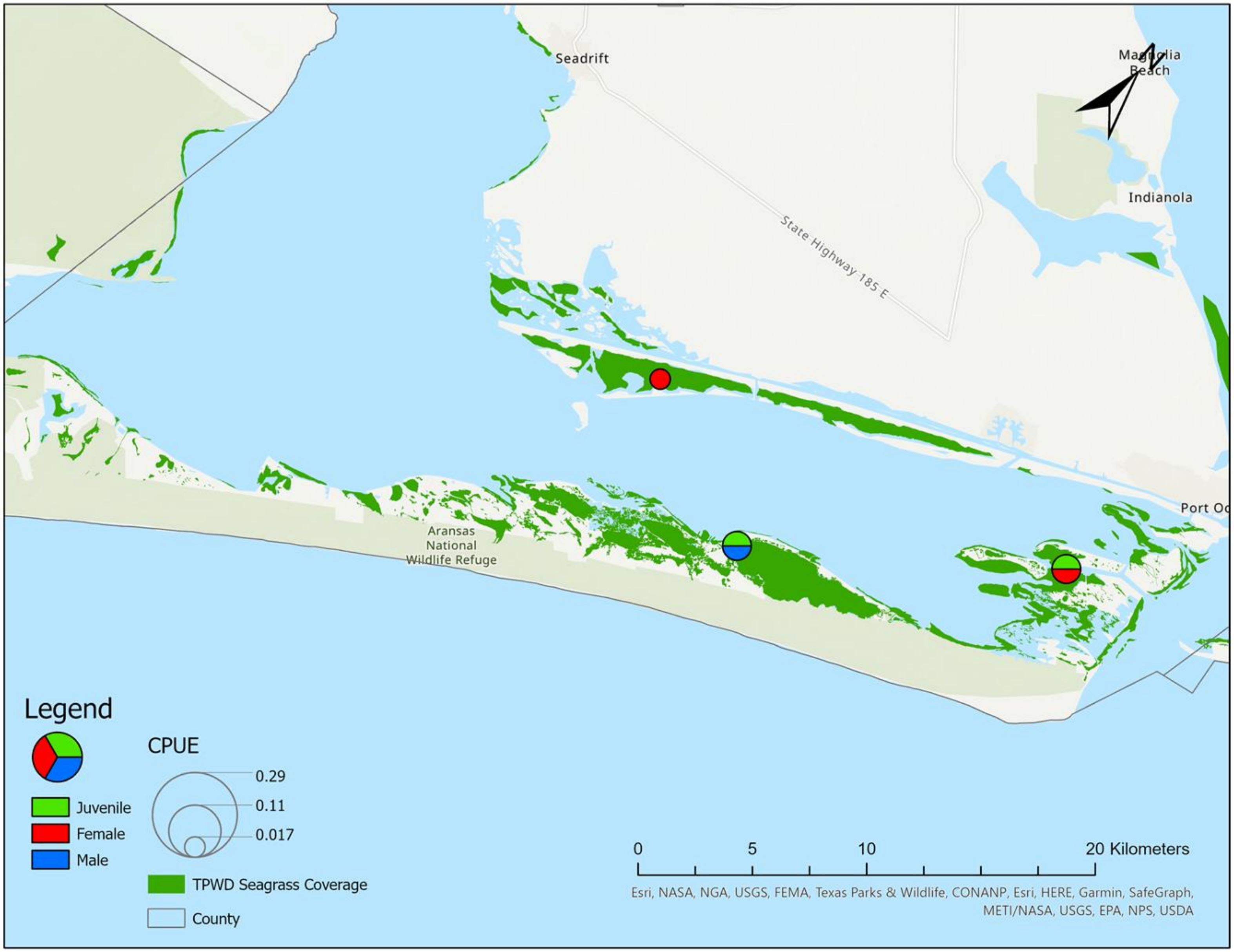


Appendix 4e. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in the Upper Laguna Madre and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter squared with pie chart showing the percent coverage of seagrass species and macroalgae.

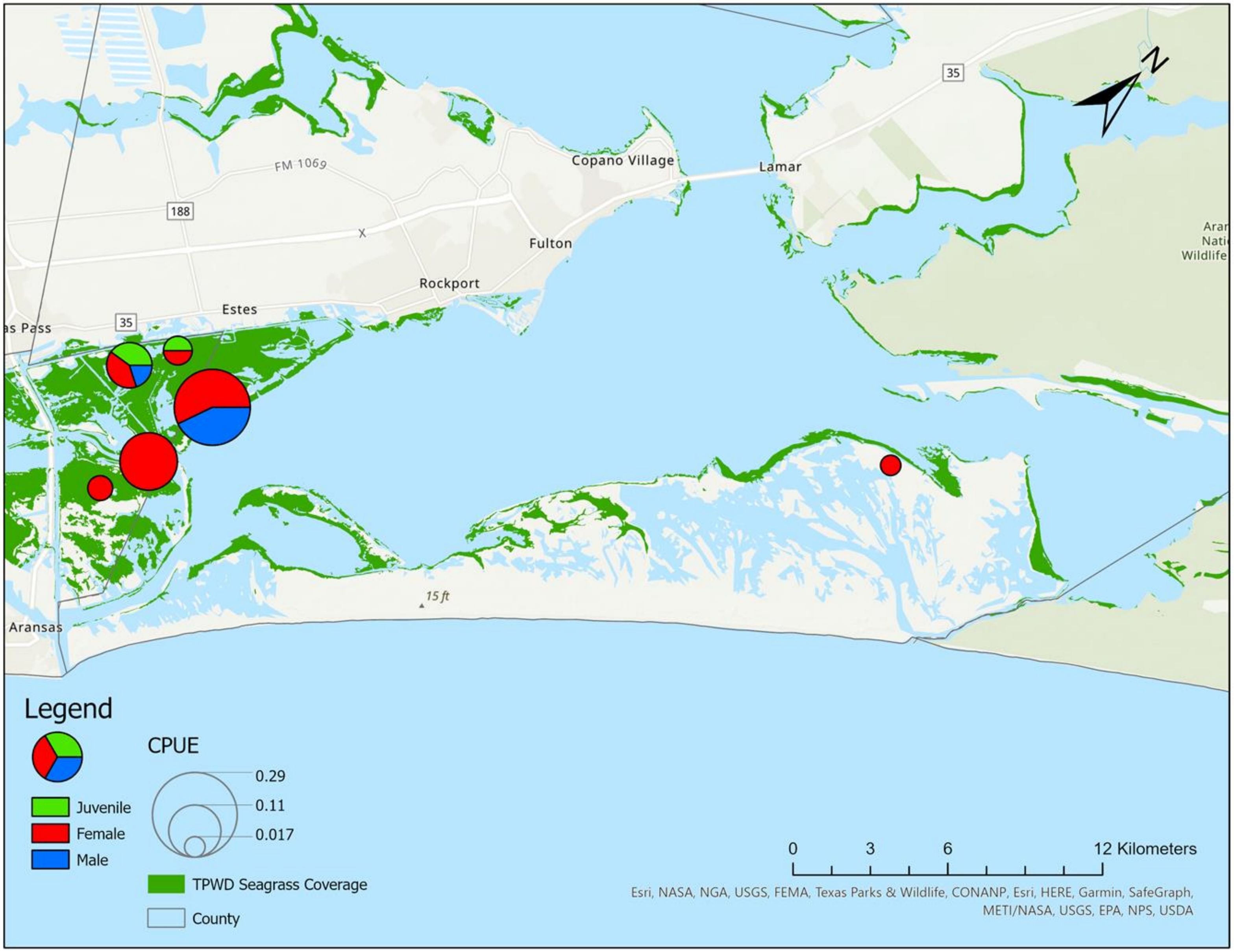


Appendix 4f. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in the Upper Laguna Madre and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter squared with pie chart showing the percent coverage of seagrass species and macroalgae.

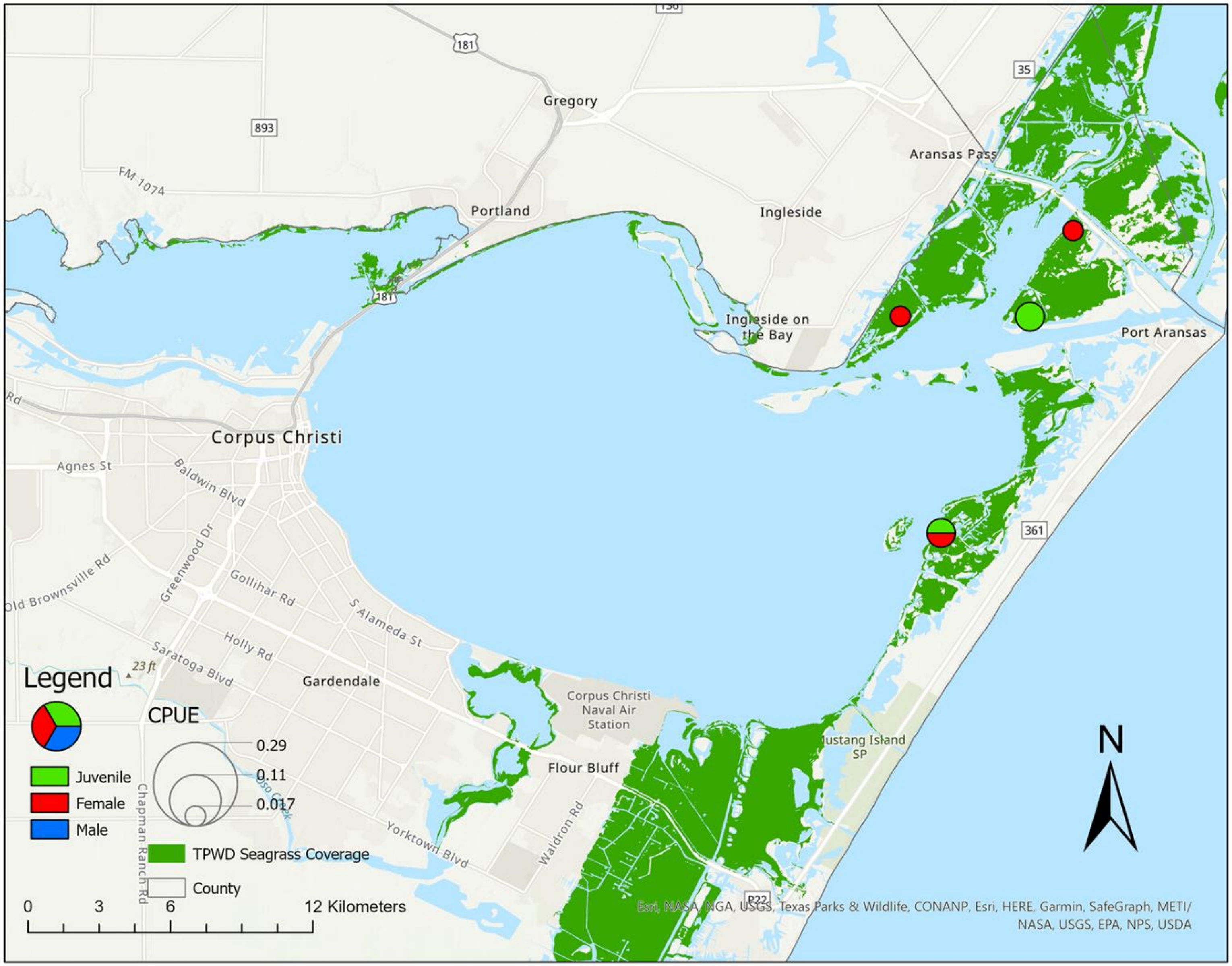
Appendix 5a. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in Matagorda Bay and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter squared with pie chart showing the percent of Dwarf Seahorse by maturity and sex.



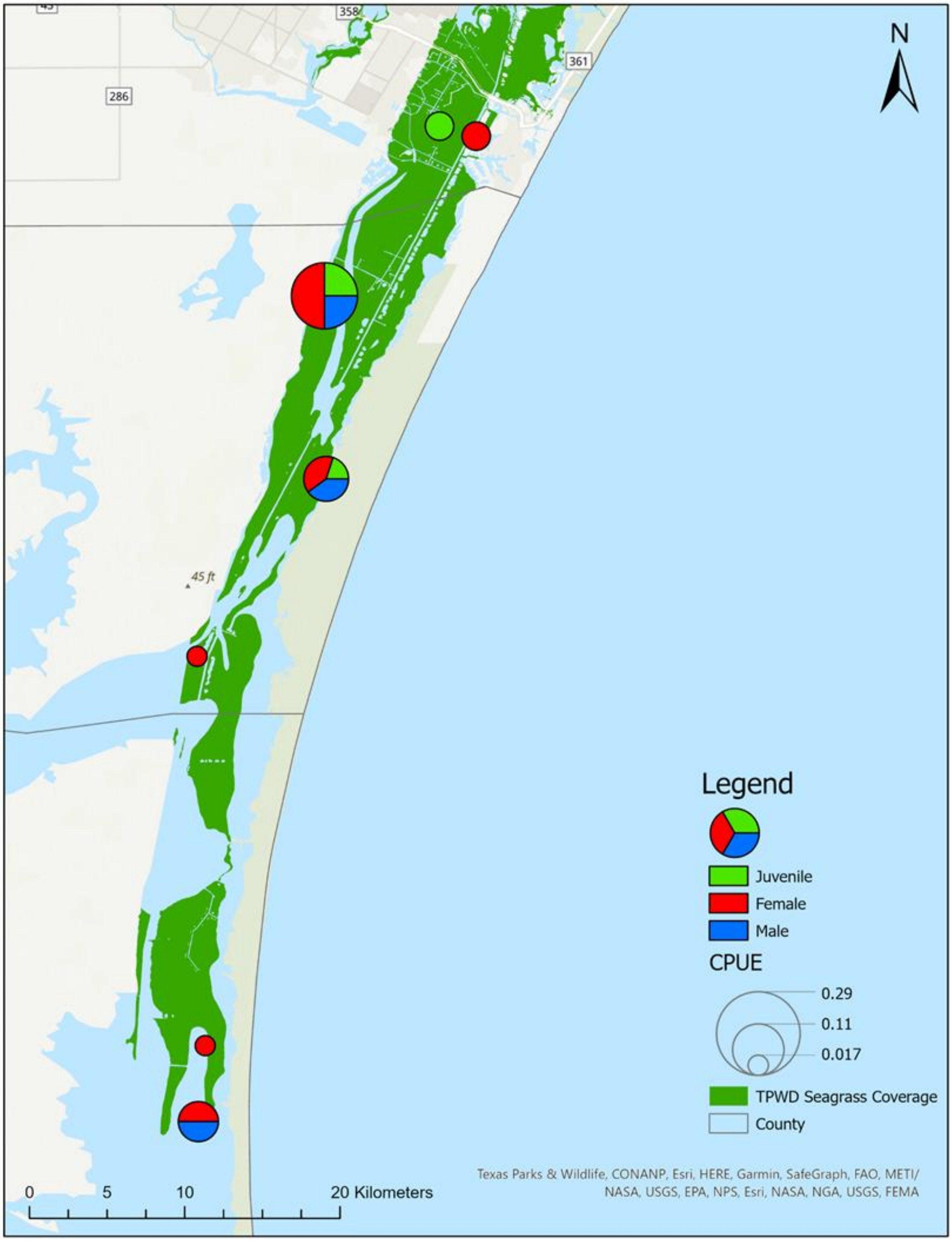
Appendix 5b. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in San Antonio Bay and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter squared with pie chart showing the percent of Dwarf Seahorse by maturity and sex.



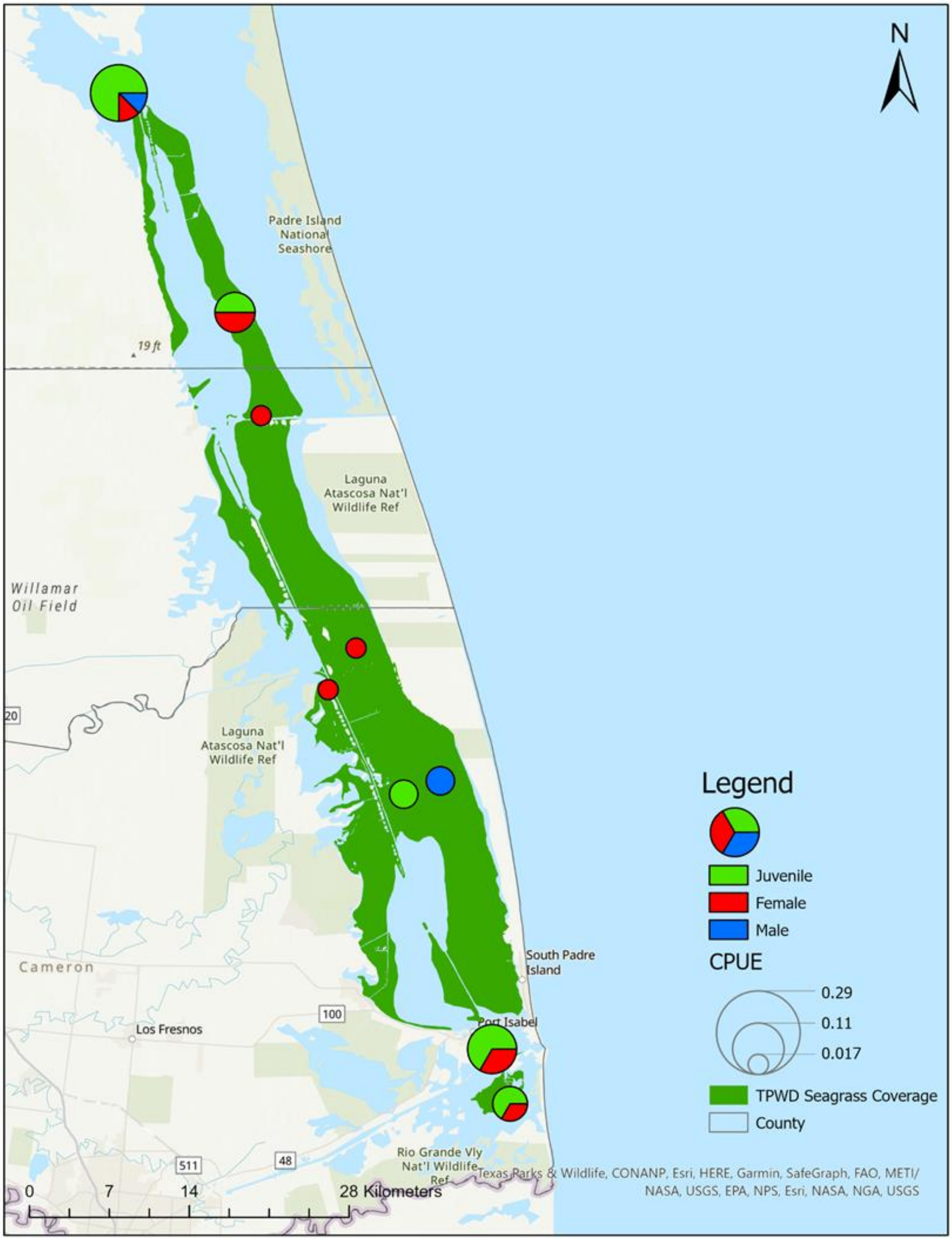
Appendix 5c. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in Aransas Bay and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter squared with pie chart showing the percent of Dwarf Seahorse by maturity and sex.



Appendix 5d. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in Corpus Christi Bay and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter squared with pie chart showing the percent of Dwarf Seahorse by maturity and sex..



Appendix 5e. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in the Upper Laguna Madre and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter squared with pie chart showing the percent of Dwarf Seahorse by maturity and sex.



Appendix 5f. Map of seagrass coverage and year-1 (2020) distribution and abundance study sites in the Lower Laguna Madre and the catch per unit effort (CPUE) of Dwarf Seahorses captured per meter squared with pie chart showing the percent of Dwarf Seahorse by maturity and sex..

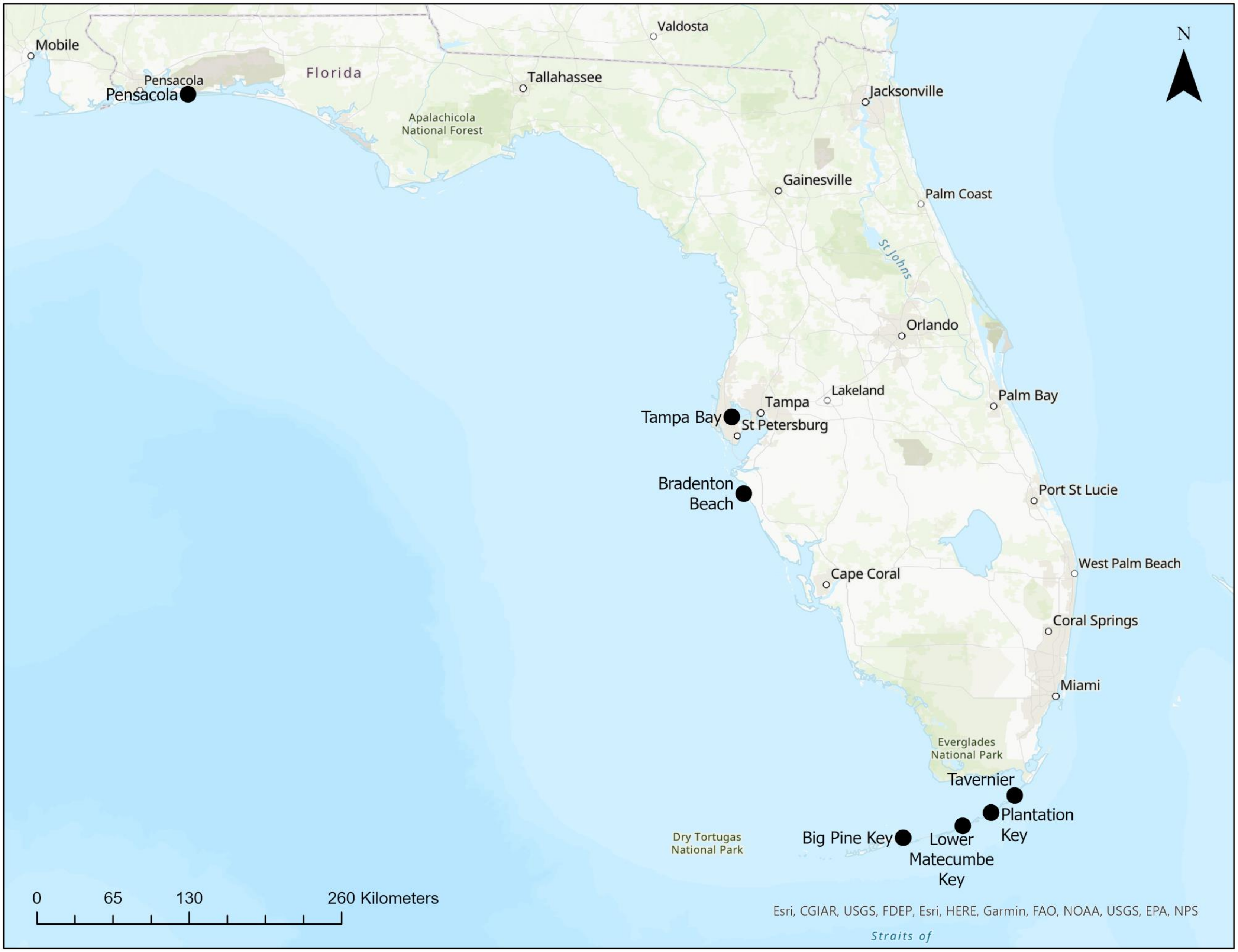
Appendix 6. Summary of all catch from year-1 (2020) presented in catch per unit effort as number of individuals per meter^2 by bay system in order of greatest catch. *Lower Laguna Madre (L), Upper Laguna Madre (U), Corpus Christi Bay (C), Aransas Bay (A), San Antonio Bay (S), Matagorda Bay (M), and Galveston Bay (G).*

Scientific Name	**Common Name**	**G**	**M**	**S**	**A**	**C**	**U**	**L**	**Grand Total**
Palaemonetes	Palaemonetes	0.220	4.460	0.232	14.046	6.552	4.390	3.734	18.238
Taphromysis louisianae	Mysid Shrimp	2.183	3.303	10.618	19.740	0.298	0.076	2.271	17.717
Ctenophora	Comb Jellyfish	0.523	5.847	0.120	0.402	0.016	5.052	2.075	8.528
Tozeuma carolinense	Arrow Shrimp	0.007	0.007	0.003	0.960	0.708	0.237	2.103	3.077
Panopeid	Mud Crab	-	0.007	0.073	0.683	1.408	0.665	0.182	1.794
Penaeid	Penaeid sp	0.657	0.503	0.002	0.252	0.434	0.110	0.776	1.483
Syngnathus scovelli	Gulf Pipefish	0.037	0.020	0.158	0.115	0.077	0.251	0.501	0.910
Ctenogobius boleosoma	Darter Goby	1.033	0.053	0.040	0.046	0.200	0.001	0.020	0.428
Gobiosoma robustrum	Code Goby	-	0.010	0.042	0.113	0.193	0.205	0.061	0.413
Callinectes sapidus	Blue Crab	0.550	0.047	0.205	0.031	0.021	0.009	0.014	0.298
Gobiidae	Gobiidae	-	0.273	0.335	0.015	0.002	0.009	-	0.252
Lucania parva	Rainwater Killifish	-	-	-	0.288	0.186	0.006	0.025	0.244
Lagodon rhomboides	Pinfish	-	-	0.158	0.048	0.163	0.021	0.024	0.221
Opsanus beta	Gulf Toadfish	-	0.003	0.005	0.075	0.095	0.047	0.025	0.149
Syngnathus louisianae	Chain Pipefish	0.017	0.010	0.090	0.092	0.016	0.018	0.029	0.145
Eucinostomus melanopterus	Flagfin Mojarra	-	-	-	0.004	0.028	-	0.088	0.103
Hippocampus zosterae	**Dwarf Seahorse**	-	**0.003**	**0.008**	**0.038**	**0.011**	**0.017**	**0.023**	**0.065**
Alpheus heterochaelis	Bigclaw Snapping Shrimp	-	-	0.020	0.019	0.039	0.008	0.014	0.058
Anchoa mitchilli	Bay Anchovy	0.063	0.077	0.005	-	-	0.005	0.006	0.048
Cynoscion nebulosus	Spotted Seatrout	0.003	0.003	0.007	0.004	0.004	0.010	0.017	0.034
Bairdiella chysoura	Silver Perch	0.003	0.007	0.002	0.008	0.011	0.001	0.011	0.023
Salpidae	Salp	-	-	0.035	-	-	-	0.003	0.020
Sciaenidae spp.	Sciaenid	0.020	0.040	0.002	-	-	-	-	0.016
Eucinostomus spp.	Eucinostomus	-	-	-	0.002	-	-	0.010	0.011
Brevoortia spp.	Brevoortia	-	-	-	-	-	0.002	0.008	0.010
Gobiosoma bosc	Naked Goby	-	0.007	0.007	0.004	0.002	-	0.003	0.010
Clibanarius vittatus	Thinstripe Hermit Crab	0.007	-	0.003	0.006	0.002	-	0.002	0.008
Clupeidae	Clupeidae	-	-	-	-	-	-	0.008	0.008
Myrophis punctatus	Speckled Worm Eel	0.003	-	0.002	-	0.007	-	0.003	0.008
Menidia beryllina	Inland Silverside	0.007	-	-	0.002	-	0.002	0.002	0.006
Symphurus plagiusa	Blackcheek tonguefish	0.013	-	0.002	-	0.004	-	-	0.006
Libinia spp.	Spider Crab	-	-	0.002	-	0.002	0.003	0.001	0.005
Microgobius gulosus	Clown Goby	-	-	0.010	-	-	-	-	0.005
Achirus lineatus	Lined Sole	-	-	0.002	-	0.002	-	0.001	0.003
Syngnathus sp.	Pipefish	-	-	-	0.002	-	0.001	0.001	0.003
Paralichthys lethostigma	Southern Flounder	-	-	-	-	-	0.001	0.001	0.002
Ascidian	Sea Squirt	0.003	0.003	-	-	-	-	-	0.002
Brevoortia patronus	Gulf Menhaden	-	-	-	-	-	-	0.002	0.002
Microgobius thalassinus	Green Goby	-	-	0.002	-	-	0.001	-	0.002
Parablennius marmoreus	Seaweed Blenny	-	-	-	-	-	0.001	-	0.001
Cyprinodon variegatus	Sheepshead Minnow	-	-	-	0.002	-	-	-	0.001
Eucinostomus spp.	Eucinostomus	-	0.003	-	-	-	-	-	0.001
Orthopristis chrysoptera	Pigfish	-	-	-	-	0.002	-	-	0.001
Lactophyrs triqueter	Smooth Trunkfish	-	-	-	-	-	-	0.001	0.001
Porcellanidae spp	Porcelain Crab	-	-	-	0.002	-	-	-	0.001
Unidentifiable	Unidentifiable	0.003	0.003	12.200	0.025	0.004	0.014	0.026	6.153
	Grand Total	5.357	14.690	24.395	37.044	10.483	11.167	12.067	60.525

Appendix 7. Summary of all catch from year-2 (2021) presented in catch per unit effort as number of individuals per meter^2 by gear type in order of greatest catch. Throw trap (TT), push net (PN), beam trawl (BT), 15' straight seine (SN-S), and 60' bag seine (SN-B).

Scientific Name	**Common Name**	**TT**	**PN**	**BT**	**SN-S**	**SN-B**	**Grand Total**
Palaemonetes spp.	Grass Shrimp	29.222	0.526	0.519	0.241	0.005	0.321
Panopeid	Mud Crab	22.481	0.089	0.162	0.064	0.005	0.100
Acetes americanus	Sergestid Shrimp	18.556	0.018	0.006	0.299	-	0.080
Lagodon rhomboides	Pinfish	4.704	0.013	0.046	0.365	0.052	0.079
Tozeuma carolinense	Arrow Shrimp	2.222	0.101	0.088	0.136	-	0.062
Penaeid spp.	Penaeid Shrimp	2.926	0.048	0.083	0.078	0.036	0.053
Anchoa mitchilli	Bay Anchovy	0.074	-	0.040	0.215	-	0.024
Leostomus xanthurus	Spot	-	-	-	-	0.047	0.022
Brevoortia patronus	Gulf Menhaden	4.333	0.018	-	0.020	0.003	0.021
Squilla spp.	Mantis Shrimp	0.519	0.002	0.006	0.113	-	0.014
Syngnathus scovelli	Gulf Pipefish	0.815	0.009	0.003	0.002	-	0.006
Ctenogobius boleosoma	Darter Goby	0.630	0.005	-	-	-	0.004
Taphromysis louisianae	Mysid Shrimp	0.037	0.007	-	-	-	0.003
Lucania parva	Rainwater Killifish	0.185	0.001	0.003	0.019	-	0.003
Gobiidae	Larval Gobie	0.407	0.005	-	-	-	0.003
Callinectes sapidus	Blue Crab	0.481	0.002	0.003	0.001	0.001	0.003
Bairdiella chysoura	Silver Perch	0.037	-	0.006	0.018	-	0.002
Cyprinodon variegatus	Sheepshead Minnow	-	-	-	-	0.005	0.002
Opsanus beta	Gulf Toadfish	0.407	0.001	0.003	0.002	0.001	0.002
Mugil curema	White Mullet	-	-	-	-	0.004	0.002
Alpheus heterochaelis	Bigclaw Snapping Shrimp	0.630	-	-	-	0.001	0.002
Mugil cephalus	Striped Mullet	-	-	-	-	0.004	0.002
Gobiosoma robustrum	Code Goby	0.333	0.001	0.006	0.002	-	0.002
Fundulus grandis	Gulf Killifish	-	-	-	-	0.003	0.001
Menidia beryllina	Inland silverside	-	-	-	0.008	0.001	0.001
Hippocampus zosterae	**Dwarf Seahorse**	**0.222**	**0.001**	**0.003**	**-**	**-**	**0.001**
Ariopsis felis	Hardhead Catfish	0.185	-	-	0.003	-	0.001
Cynoscion arenarius	Sand Seatrout	-	-	-	0.007	-	0.001
Fundulus similis	Longnose Killifish	-	-	-	-	0.002	0.001
Syngnathus louisianae	Chain Pipefish	-	0.001	0.003	0.001	-	0.001
Chloroscombrus chrysurus	Atlantic Bumper	-	-	-	0.005	-	0.001
Elops saurus	Ladyfish	-	-	-	-	0.001	0.000
Synodus foetens	Inshore Lizardfish	0.037	-	-	0.002	-	0.000
Caranx hippos	Crevalle Jack	-	-	-	0.002	-	0.000
Cynoscion nebulosus	Spotted Seatrout	0.037	-	-	0.002	-	0.000
Archosargus probatocephalus	Sheepshead	-	-	-	0.002	-	0.000
Micropogonias undulatus	Atlantic Croaker	-	-	-	0.002	-	0.000
Pogonias cromis	Black Drum	-	-	-	-	-	0.000
Symphurus plagiusa	Blackcheek Tonguefish	0.037	-	-	-	-	0.000
Chasmodes longimaxilla	Stretchjaw Blenny	-	-	-	-	-	0.000
Citharichthys spilopterus	Bay Whiff	-	-	-	-	-	0.000
Gobiosoma bosc	Naked Goby	0.037	-	-	-	-	0.000
Libinia sp.	Spider Crab	0.037	-	-	-	-	0.000
Lolliguncula brevis	Atlantic Brief Squid	-	-	-	0.001	-	0.000
Myrophis punctatus	Speckled Worm Eel	0.037	-	-	-	-	0.000
Selene setapinnis	Atlantic Moonfish	-	-	-	0.001	-	0.000
Oligoplites saurus	Leatherjack	-	-	-	0.001	-	0.000
Unidentifiable	Unidentifiable	0.074	0.002	-	0.006	-	0.002
	Grand Total	89.704	0.852	0.977	1.615	0.170	0.820

Appendix 8. Map of sites where Florida genetic samples originated, modified from Fedrizzi et al. 2015.



Reviewed by:

________________________________ Date: ______________________

Mark Fisher, Project Coordinator

Approved by:

________________________________ Date: ______________________

Chelsea Acres, Rare and Listed Species Coordinator