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HABITAT USE, DISTRIBUTION, AND DENSITY OF DWARF SEAHORSE
(*HIPPOCAMPUS ZOSTERAE*) POPULATIONS ALONG THE
TEXAS GULF COAST

by

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Dedication

This work is dedicated to my grandmother, Eileen Leshner. Since I was young, she always did her best to expose me to different experiences and ideas. Despite the hardships she had to go through, she pursued a degree in microbiology and I have always looked up to her, being an intelligent woman in science. She has encouraged me and all of her grandchildren to follow their dreams, no matter what their interests are. She was the reason I was able to attend graduate school in Texas. Because of her, I could follow the hunch I had always had that I wanted to work in marine biology, despite having lived my entire life in the Midwest. She always told me to be safe, study hard, but have a little fun too. Without her I wouldn't be the person I am today.

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ABSTRACT

HABITAT USE, DISTRIBUTION, AND DENSITY OF DWARF SEAHORSE
(HIPPOCAMPUS ZOSTERAE) POPULATIONS ALONG THE
TEXAS GULF COAST

Story Catherine Leshner
University of Houston-Clear Lake, 2022

Thesis Chair: George Guillen, PhD

The Dwarf Seahorse (*Hippocampus zosterae*) is one of the smallest species of seahorse and resides in shallow waters throughout the Gulf of Mexico, Atlantic Coast of Florida, and Caribbean. They rely on seagrass beds for feeding, spawning, and refuge, rarely traveling far from the bed in which they were spawned. Information on density, distribution, and habitat associations in Texas are needed to address knowledge gaps and inform future management initiatives. We evaluated seagrass beds along the Texas Coast (from Galveston Bay to the Lower Laguna Madre) to determine what factors correlated with Dwarf Seahorse presence and density. Dwarf Seahorses (n=79) were captured at 30 of 80 (37.5%) sites. They were detected in all sampled bay systems except Galveston Bay. Dwarf Seahorses were found in association with all seagrass species found in Texas. Variables significantly correlated with Dwarf Seahorse presence included average

seagrass biomass, turtle grass (*Thalassia testudinum*) percent cover, and nekton species abundance, evenness, and richness. Variables significantly correlated with Dwarf Seahorse density included number of seagrass species present, the presence of turtle grass, and nekton species evenness and richness. Nekton species associated with the presence of Dwarf Seahorse included grass shrimp (*Palaemonetes* spp.), Gulf Toadfish (*Opsanus beta*), Code Goby (*Gobiosoma robustum*), and Rainwater Killifish (*Lucania parva*). There were an estimated 1,116,356 Dwarf Seahorses in Aransas Bay, 2,769,686 in Upper Laguna Madre, and 7,899,652 in Lower Laguna Madre throughout the sampling time frame. Results from this study can inform continued monitoring, development of a habitat suitability index, and management of this species and their essential habitat.

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INTRODUCTION

The Texas coast contains estuaries ranging from low salinity river dominated bays to hypersaline lagoons that contain ecologically diverse habitats including open bays, marshes, oyster reefs, intertidal islands, mangroves, and seagrass beds. These diverse habitats are home to many species of invertebrates, fish, reptiles, birds, and mammals. Anthropogenic activity such as increasing human population and creating robust petrochemical and shipping industries are prevalent within the Texas coastal zone and place stress on these coastal habitats and species (Congdon et al. 2019, Pulich and White 1991, Short and Wyllie-Echeverria 1996, Duarte et al. 2008, Thyng et al. 2013). The impacts from some of these stressors on ecosystem health along the Texas Gulf Coast (Miller 2019) and trends in seagrass habitat (Handley et al. 2007; Congdon and Dunton 2019) are documented at certain points in time, though comprehensive historical data is lacking. The association between important habitats such as seagrass beds and most non-game species remains understudied. Specifically, studies to understand how seagrass bed health impacts their ability to support Dwarf Seahorse (*Hippocampus zosterae*) populations in Texas are needed, as this species is understudied in this area. Dwarf Seahorse were recently under review for consideration under the Endangered Species Act (ESA) and in July of 2020 was determined to not warrant protection. However, all information used for the population viability of this species was from Florida, which is only one area within its range (NMFS 2020). A comprehensive analysis of this species throughout all areas of its range is needed to inform potential future listing decisions.

Seagrass Beds

The term “seagrass” describes 50 marine angiosperm species that often grow in beds or meadows (Robbins and Bell 1994). They typically grow in nearshore coastal areas composed of mono- and poly-specific beds that can be continuous or fragmented

(Robbins and Bell 1994). Bed characteristics and species composition are influenced by water depth, light and nutrient availability, sediment type, hydrodynamic regimes, and local faunal activity (Robbins and Bell 1994). All seagrass species that form seagrass beds are considered foundation species, meaning they are habitat forming species that are crucial to sustaining overall species assemblages within associated ecological communities (Hughes et al 2009).

Seagrass beds provide essential nursery grounds allowing larvae of various vertebrate and invertebrate species to successfully develop into juveniles (Jackson et al. 2001). Additionally, they act as feeding areas and provide refuge from predation for species at all stages of life (Jackson et al. 2001). Indirectly, seagrass beds maintain sea life by providing important organic matter that is incorporated into nutrient cycles within the associated water body and contributes to the total organic carbon of sediments (Jackson et al. 2001). Seagrasses' extensive shoot and root systems stabilize sediments and slow water currents, which in turn increases water clarity and reduces erosion (TPWD 2017). As primary producers, they release oxygen into their environments for surrounding organisms. Recent research also shows 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). There are multiple types of seagrass species that exist, but they all play these important roles in the habitats they are found in. Of the 50 species of seagrass worldwide, the Texas Parks and Wildlife Department (TPWD) has confirmed the existence of the five species on the Texas Gulf Coast and have outlined their most important features (TPWD 2017) (Table 1).

Table 1. Names, locations, characteristics, and facts on the five seagrass species found in Texas (TPWD 2017).

Common Name	Scientific Name	Distribution in Texas	Physical Characteristics	General Information
Shoal Grass	<i>Halodule wrightii</i>	all coastal regions except Sabine Lake	<ul style="list-style-type: none"> ▪ flat narrow leaves ▪ leaves come to three points on each blade tip 	<ul style="list-style-type: none"> ▪ most widespread due to broad range of temperature and salinity tolerance ▪ can inhabit disturbed areas ▪ establishes large colonies quickly ▪ found in water 1-3 ft deep
Star Grass	<i>Halophila engelmannii</i>	all coastal regions except Sabine Lake	<ul style="list-style-type: none"> ▪ short canopy height ▪ short elliptical serrated leaves ▪ leaves in clusters of 4-8 blades 	<ul style="list-style-type: none"> ▪ often in sheltered waters ▪ creates an understory in mixed species seagrass beds
Manatee Grass	<i>Syringodium filiforme</i>	lower coast	<ul style="list-style-type: none"> ▪ cylindrical leaves ▪ form in clusters of 2-3 	<ul style="list-style-type: none"> ▪ usually found in water 2-5 ft deep
Turtle Grass	<i>Thalassia testudinum</i>	lower coast	<ul style="list-style-type: none"> ▪ broad, flat leaves ▪ shoots can grow up to 14 inches 	<ul style="list-style-type: none"> ▪ choice food source for juvenile Green Sea Turtles ▪ present in 2-5 ft of water ▪ usually in mud-sand bottom areas
Widgeon Grass	<i>Ruppia maritima</i>	all coastal regions except Sabine Lake	<ul style="list-style-type: none"> ▪ similar to shoal grass ▪ long, branches stems ▪ flowering stalk and seed clusters present 	<ul style="list-style-type: none"> ▪ technically freshwater submerged aquatic vegetation, not true seagrass ▪ tolerate a wide range of salinities (fresh to hypersaline)

Worldwide, all species of seagrass 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 is likely due to a wide variety of causes, such as reduced water quality, overgrazing, and physical damage (Hughes et al. 2009). While there is no long-term study reflecting steady decreases in seagrass coverage along the Texas Coast, there is documentation of the impacts that shifts in water quality have on this habitat. Due to periods of drought conditions, the Upper Laguna Madre can shift to hypersaline conditions, which is well above the physiological thresholds of some species of seagrass (Congdon and Dunton 2016). Elevated suspended sediment levels can decrease light penetration and if extreme enough can physically bury seagrass beds (Congdon and Dunton 2016). Increased nitrogen enrichment due to input by wastewater treatment facilities or agricultural runoff may create plumes that impair water quality by promoting the growth of micro- and macroalgae species, which can outcompete seagrasses (Congdon and Dunton 2016). Shifts in water quality such as these may become more prevalent with increased human activity along the Gulf Coast.

Seagrasses face multiple environmental stressors in areas of high anthropogenic activity, such as Texas. Because of this, it is important to find ways to track seagrass bed health. The health of seagrass beds is tied to recruitment and abiotic factors such as light, nutrients, and disturbance levels (Hill et al. 2014). Recruitment levels can be revealed by examining seagrass bed patchiness and percent coverage levels. Degree of patchiness and connectivity of seagrass beds indicates how productive a seagrass bed is and how well it can support a marine community (Hill et al. 2014). Seagrass biomass can also be an indicator of bed health in the same way, with higher biomass indicating better health (Duarte et al. 1998). Stressors that may be reflected in these indicators of seagrass bed health will be discussed in further detail later.

Abiotic factors such as dissolved oxygen levels reflect upon seagrass bed health. Eutrophication of waterways is becoming increasingly common due to anthropogenic nutrient inputs (Honig et al. 2017). Eutrophication can trigger excessive floating and epiphytic algal growth, which limits light penetration. Reduced light penetration frequently causes underwater flora such as seagrass to die off (Chislock et al. 2013). This phenomenon, in addition to microbial decomposition of the algal blooms when they die, creates a hypoxic zone not suitable for the support of aerobic organisms (Chislock et al. 2013). Hypoxia, if persistent, can lead to massive mortality of fauna associated with seagrasses, disrupting the health and stability of the ecosystem.

Finally, similar to excessive algal growth blocking out light necessary for photosynthesis, excessive water turbidity caused by erosion or sediment input/re-suspension can reduce light penetration therefore reducing photosynthesis and the ability of marine flora such as seagrasses to persist (Longstaff & Dennison 1999). While the success of seagrasses in differing turbidity levels is dependent on other factors such as water depth, turbidity is often a good indicator of a preferable environment for seagrasses and could reflect upon seagrass bed health. Declines in seagrass as a foundation species can lead to declines in the many species that are dependent on this habitat, including Dwarf Seahorses.

Dwarf Seahorses

Seahorses (genus *Hippocampus*) have attracted substantial scientific attention due to their unusual physical characteristics and unique reproductive methods (Scales 2010). The most well-known of these is the fact that males have a brood pouch that is used to carry their offspring until they develop enough to exist outside the pouch (Lourie et al. 2004). Seahorse species are considered an iconic charismatic species and are often used to encourage marine conservation causes across the world (Harasti et al. 2014).

Charismatic, or flagship, species are defined by their ability to be easily and widely recognized in association with a certain location or habitat (Kontoleon and Swanson 2003). These charismatic species are often more frequently studied by scientists, receiving a disproportionate amount of funding relative to conservation status, and garnering media and public attention (Clark and May 2002; Tisdell and Nantha 2006; Duarte et al. 2008). While seahorses fit the definition of a charismatic species, a surprisingly large number of species are data deficient, with only eight species being fully evaluated (Scales 2010).

Seahorses are well known by the public but are still understudied and threatened by human activity and development. There are a few explanations to why seahorses continue to be an understudied group. First, seahorses are difficult to find in the wild due to their ability to camouflage, patchy distribution, and low numbers (Aylesworth 2016). Additionally, due to limited conservation resources, resource managers must make decisions on what species to prioritize. Seahorses may often take the back burner due to being a non-game, non-target species. Often fish species are prioritized in an effort to create sustainable commercial and recreational fishery management goals (Davies and Baum 2012). Seahorses are not major commercial or recreational fishery target group worldwide and their disappearance would not pose a threat to human resource needs (Aylesworth 2016).

One of the smallest species of seahorse is the Dwarf Seahorse, also known as the pygmy seahorse (*Hippocampus zosterae*). These small seahorses reside in shallow waters throughout the Gulf of Mexico, Atlantic Coast of Florida, and Caribbean (Irey 2004). They 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, Figure 1). Dwarf Seahorses stay minute in size,

typically growing to be only two centimeters, two and a half at maximum (Irey 2004). They are found in a variety of colors, ranging from yellow to black, with many shades between (Lourie et al. 2004). 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).



Figure 1. A Dwarf Seahorse captured summer of 2020 during sampling.

The typical life span of Dwarf Seahorse is up to three years (Lourie et al. 2004). Sexual maturity is reached at around three months of age (Strawn 1954). This species, along with most other seahorse species typically select seagrass, seaweed, or coral reef as their primary habitats of choice (Lourie et al. 2004; Wallis 2004). These habitats provide camouflage, anchoring structure, and protection from strong currents. Dwarf Seahorses cannot swim well and struggle to escape predators, so they typically reside where they

camouflage well and can grab onto a fixed object with their prehensile tail (Wallis 2004). Individuals will sometimes live their whole life within a few square feet of their birthplace (Wallis 2004).

In 2020, NOAA released a status report of Dwarf Seahorse stating the species was not in need of protection under the ESA. The original petition for listing states that the species should be protected due to decreasing numbers. They stated multiple reasons for seeing this decline: degradation of seagrass habitat, overutilization of Dwarf Seahorses in commercial trade and bycatch, noise pollution from anthropogenic sources, and extreme weather events. Researchers determined based on historical data that only two areas of Dwarf Seahorse range have an elevated risk of extinction, the east coast of Florida and northwest Florida (NMFS 2020). They determined that this wasn't a significant enough portion of the species' range to warrant a threat to the overall species for the foreseeable future (NMFS 2020). However, data on the Dwarf Seahorse are sparse in all other areas of their range, so consistent monitoring would be beneficial to inform future listing decisions (Table 2).

Table 2. Data from population analyses of the Dwarf Seahorse from all areas of its range (NMFS 2020).

Location	Mean Population Estimate (95% CI)
Florida	24,686,648
Cedar Key	30,516 (18,534 – 42,498)
Tampa Bay	2,023,224 (1,548,606 – 2,497,844)
Charlotte Harbor	2,527,572 (1,895,442 – 3,159,702)
Florida Bay	19,821,504 (15,613,150 – 24,029,858)
Indian River Lagoon	283,832 (103,342 – 464,322)
Alabama	Absent
Mississippi	Absent
Louisiana	Rare
Texas	Present in low abundance
Mexico	Present in unknown abundance
Cuba	Unknown
The Bahamas	Unknown
Bermuda	Possibly extirpated

Stressors

Humans have drastically changed the coastal environment of Texas, beginning with the immigration of European settlers. These settlers made changes to the natural environment such as the development of the landscape for farming and urban areas, channelizing bays for shipping, and the eventual mining of oil and natural gas. As discussed earlier, many human activities have been shown to detrimentally impact estuarine habitats such as seagrass beds and associated species (Chislock et al. 2013, Dolbeth et al. 2007, Ellison et al. 2005, Hughes et al. 2009). A reduction or loss of seagrass beds would threaten all trophic levels linked to this habitat.

Humans can directly damage seagrass beds through recreational and commercial activities. Disturbances to water quality includes discharge of toxic compounds into waterways, such as municipal and industrial permitted discharges, non-point source pollution, and oil and hazardous material spills (Short and Wyllie-Echeverria 1996). Direct mechanical damage to seagrass includes dredging, filling and propeller scarring (Short and Wyllie-Echeverria 1996). Certain commercial fishing methods such as

trawling or other dragging gears can also cause substantial harm such as leaf breakage or uprooting which can cause decreases in biomass with repetitive trawling efforts (Meyer et al. 1999).

Persistent trawling or dredging within and adjacent to seagrass beds also causes elevated turbidity which, as discussed earlier, is a threat to bed viability by decreasing the amount of light available for seagrasses to use for photosynthesis (Meyer et al. 1999; Longstaff and Dennison 1999). Longstaff and Dennison (1999) determined that after just 38 days of light deprivation, some seagrass species saw severe declines in shoot density, biomass, and canopy height. In addition to trawling, natural processes such as flooding and strong winds also contribute to elevated turbidity levels (Longstaff and Dennison 1999). Extreme weather events such as these are becoming more common due to climate change driven by anthropogenic activity (Stott 2016). The Texas coast experiences severe weather events such as strong cold fronts (blue northerners) and hurricanes regularly. Along the Gulf Coast, these storms can cause substantial declines in seagrass cover and blade length (Kowalski et al. 2009, Congdon et al. 2019). Storms can also shift sediments to be finer silt/clay dominated rather than shell/sand, which can make it easier for subsequent weather events to dislodge seagrasses (Congdon et al. 2019).

Extreme weather events increase stormwater runoff and can lead to increased sediment and nutrient loading into water bodies, resulting in eutrophication (Dokulil and Teubner 2010). Eutrophication and flooding can synergistically lead to devastation of benthic organism communities such as seagrass beds. Eutrophication acting as an initial stressor triggers the growth of dense algal communities which decreases light availability to seagrasses and causes eventual loss of species, decline in community resilience, and reduced survival of individual organisms (Dolbeth et al. 2007). A drop in salinity due to flooding creates stress in the ecosystem and slows seagrass recovery (Dolbeth et al.

2007). Both increased turbidity and eutrophication caused directly or indirectly by human activity can ultimately lead to hypoxia in seagrass beds (Chislock et al. 2013).

Declines in seagrasses may be reflected quickly in associated species, as the services they provide decline at a faster rate, far before the complete disappearance of the foundation species (Ellison et al. 2005). This relationship likely applies to seagrass beds and Dwarf Seahorse populations. When seagrass beds are threatened, Dwarf Seahorses are as well. In addition to being threatened by declining habitat, Dwarf Seahorses are also jeopardized directly by extreme weather events such as tropical storms and seasonal fronts that produce high amounts of precipitation (Wallis 2004). Flooding associated with these events can be detrimental to Dwarf Seahorses due to their limited swimming ability, making them unable to move to more favorable locations (Wallis 2004). Also, Dwarf Seahorse salinity tolerance in the wild is unknown and it is possible that prolonged reduced salinity could threaten wild populations.

Humans not only threaten seahorse population viability by destroying their habitat, but also by harming them directly through targeted harvest of this species. An average of 49,000 Dwarf Seahorse per year are harvested from Florida alone for international aquarium trade (CBD 2011). They are also caught accidentally as bycatch by shrimp trawlers operating in estuaries along the coast (Baum et al. 2003). Shrimp trawling impacts seahorse populations through direct mortality as bycatch, social disruption, and habitat damage (Baum et al. 2003). Additionally, sound pollution caused by human activity can significantly increase seahorse stress levels, which in turn reduces growth, condition, and immune status (Anderson et al. 2011). The impacts of these threats are not well documented for Dwarf Seahorses in Texas specifically, although the popularity of multiple species of seahorse for the aquarium trade, medicines, and curios is well known (USFWS 2004).

Stress on Dwarf Seahorse populations has the potential to result in extirpation because of their K-selected life history. Dwarf Seahorses exhibit a brood size much smaller than most other marine fish and are therefore more susceptible to population damage (Lourie et al. 2004). Additionally, this species exhibits genetic monogamy, meaning when partners are lost, widowed animals stop reproducing until finding a new permanent partner (Lourie et al. 2004). Low brood size, leading to low population density means lost partners are replaced more slowly, further extending the period of time that a population remains at risk (Lourie et al. 2004). As introduced, Dwarf Seahorse populations face many threats including loss of critical seagrass habitat. My research will be the first to investigate the relationship between seagrass health indicator variables and Dwarf Seahorse distribution and density in Texas.

METHODOLOGY

Site Selection

Study sites were selected from historically surveyed seagrass areas within each of the major estuaries located between Galveston Bay and the Lower Laguna Madre, Texas. The sites selected for Galveston Bay, San Antonio Bay, Aransas Bay, Corpus Christi Bay, Upper Laguna Madre, and Lower Laguna Madre were chosen from sites historically sampled by Texas Seagrass Monitoring Network run by Dr. Ken Dunton's lab at the UT Marine Science Institute (Dunton et al. 2009). Specific sites were selected based on proximity to historic Dwarf Seahorse sightings collected from multiple sources (TPWD, VertNet, Smithsonian, NOAA, iNaturalist) (Figure 2), historic seagrass type and coverage, and location within the bay (to maximize spatial coverage). Matagorda and East Matagorda Bays had not been historically monitored by the Dunton Lab, so the bays were together divided into four sections in ArcGIS Pro, with three along each of the shorelines in Matagorda Bay and one in East Matagorda Bay. One site was randomly selected in each of these sections using the ArcGIS tool "Create Random Points". One additional site was also randomly selected along the longest coast, which is composed of the south barrier islands, for a total of five sites.

The number of sites studied in each bay system was proportionate to historic seagrass coverage in each bay (Table 3) based on ArcGIS seagrass data from TPWD (TPWD, 2020). Five sites were examined in Galveston Bay and Matagorda Bay, ten in San Antonio Bay, Aransas Bay, and Corpus Christi Bay, and twenty in Upper and Lower Laguna Madre for a total of 80 along the Texas coast (Table 3, Figure 3). An entire list of sites and their geographical locations can be found in Appendix A.

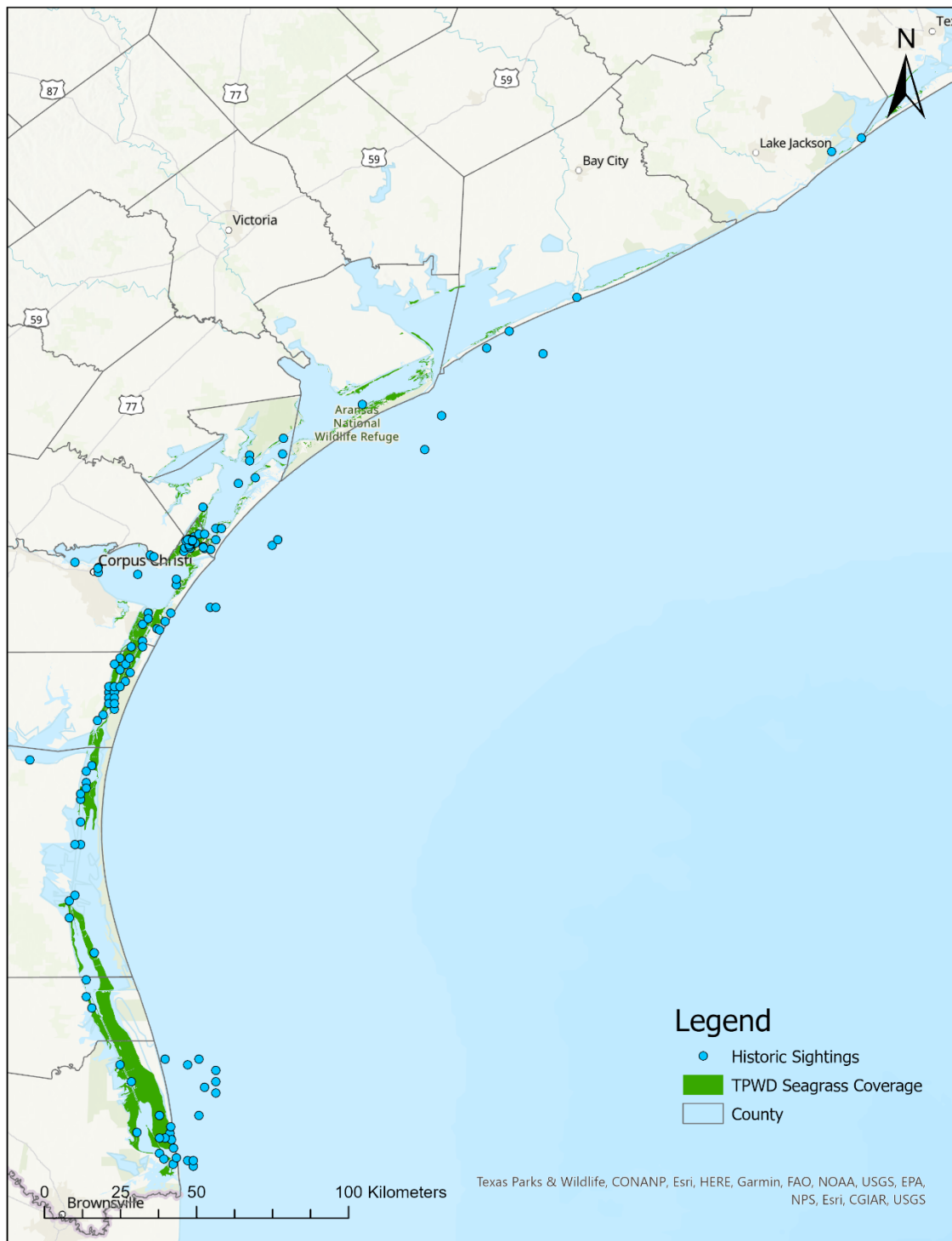


Figure 2. Map of historic Dwarf Seahorse sightings based on data from NOAA, TPWD, VertNet, Smithsonian, and iNaturalist.

Table 3. Size and seagrass coverage of estuaries included in study (TPWD, 2020), number of sites sampled, and total historic sightings of Dwarf Seahorses.

Bay System	Open Water (km²)	Seagrass Cover (km²)	Percent Cover of Seagrass	Number of sites	Historic Sightings
Galveston	1532	3.1	0.20	5	1
Matagorda	1141	25.3	2.22	5	0
San Antonio	521	71.5	13.71	10	4
Aransas	574	67.4	11.75	10	16
Corpus Christi	540	99.9	18.52	10	13
Upper Laguna	562	199.8	35.56	20	44
Lower Laguna	694	461.7	66.55	20	15

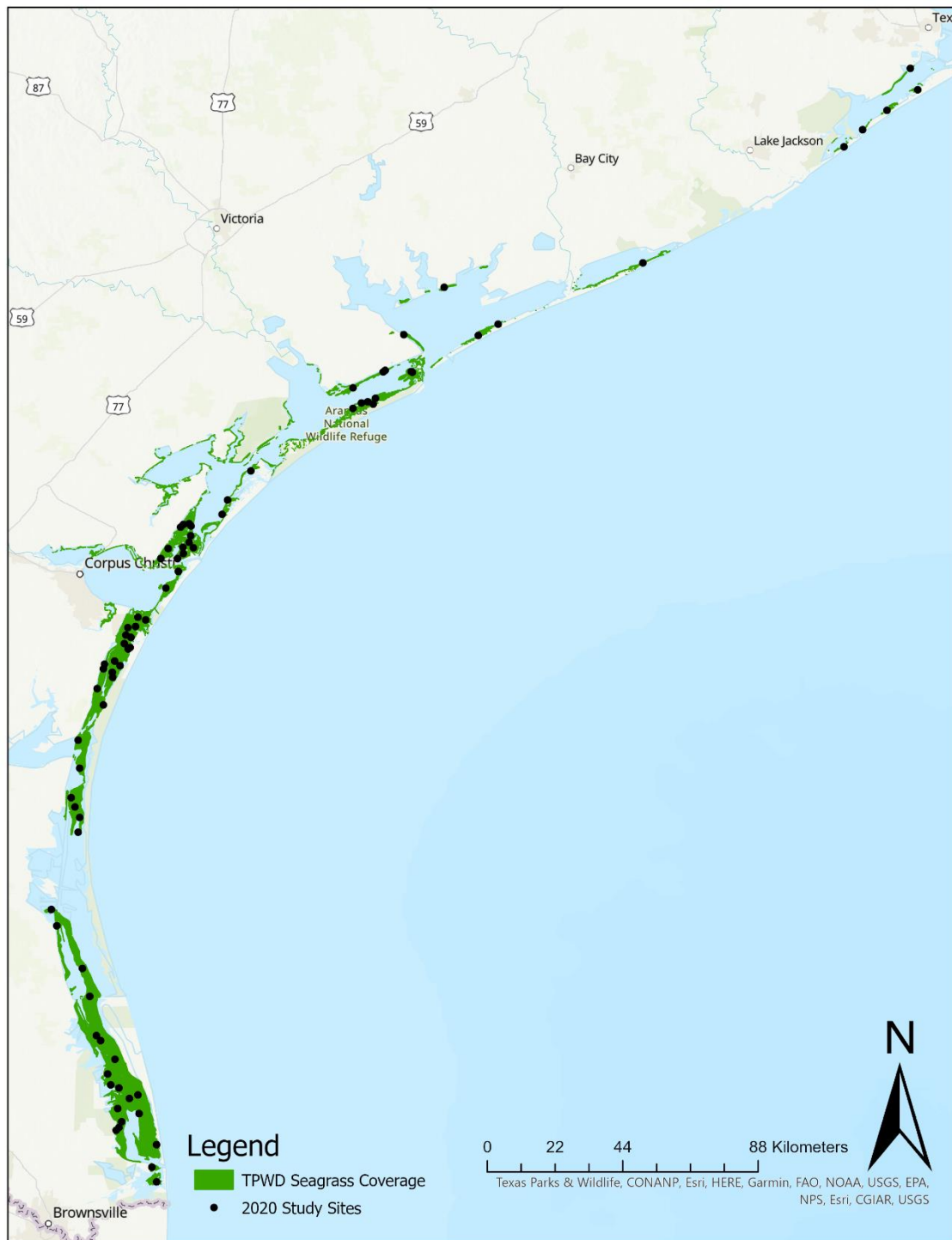


Figure 3. Distribution of 80 sites that were sampled summer of 2020

Seagrass Bed Health

Water Quality

The methodology used to measure water quality was based on procedures outlined by the Texas Commission on Environmental Quality (TCEQ) surface water quality monitoring procedures manual (TCEQ 2012). Water quality readings were taken in two locations along a vertical profile at each sample site from where the boat was anchored. Using a calibrated YSI ProDSS Handheld Multiparameter Water Quality Meter, variables including depth (m), water temperature (°C), salinity (ppt), specific conductance ($\mu\text{MHOS}/\text{cm}$ @ 25°C), dissolved oxygen both in percent saturation (%) and mg/L, pH (standard units), and turbidity (NTU) were recorded to characterize environmental conditions within the seagrass beds. Water quality vertical profile measurements were taken at both the surface (0.3m from the surface) and the bottom (0.1m from the bottom) at each site. If the total depth at the site was less than 0.5m, one reading was taken at the bottom of the water column. At this same location, a Secchi disk transparency (m) was measured at the surface using a 1.2m Secchi tube. Additionally, photosynthetically active radiation ($\mu\text{mol}/\text{m}^2\text{s}^{-1}$) was documented using a PAR meter, with an underwater reading taken at the bottom of the water column (0.1m from the bottom) and an ambient light reading taken on the boat, with the difference between these two values used for analysis. Turbidity was measured using three different metrics: NTU recorded with a data sonde, visual detection depth using a secchi tube in meters, and photosynthetically active radiation reaching the bottom of the seafloor measured in $\mu\text{mol}/\text{m}^2\text{s}^{-1}$.

Bed Biomass

The methodology used to determine seagrass bed biomass was based on procedures outlined by the Global Seagrass Monitoring Network and Texas Parks and

Wildlife Department (Dunton et al. 2009). Four locations within the seagrass bed were randomly selected to evaluate seagrass community, percent cover, biomass, and canopy height. These locations were determined by a random number generator, with the first number determining how many meters along the site boundary a transect was placed when beginning at a corner of the defined site. A second number was then randomly generated to determine how many meters into the seagrass bed would be travelled along the transect before stopping to place a sampling quadrat. A 0.25m² quadrat was then placed centrally over this position. Within this quadrat, the percent total coverage of seagrass and then percent coverage of each individual seagrass species found within the quadrat were estimated, as well as any other type of bottom coverage present including bare ground, macroalgae, oysters, or debris. Average seagrass height was also measured within the quadrat. In ideal conditions, this process was completed visually with the observer using a snorkeling mask to see within the quadrat. However, at sites where the water was too turbid to take this data visually, tactile methods were used. Finally, a 15cm diameter core was taken within the quadrat to a target depth of 20cm, placed in a plastic bag, iced, and taken back to lab. This process was repeated three more times to collect four total replicate quadrats. No core was taken if no seagrass was observed within the quadrat.

Plant biomass was isolated from the replicate cores and cleaned in the laboratory. The cleaned biomass was then placed in 60°C ovens until a consistent mass was achieved to obtain dry weight, typically after three to five days. After drying, samples were placed in a desiccator until they reached room temperature. The dried plant biomass samples were then grouped together by site, weighed, and a total biomass and average biomass weight was calculated for each site.

Dwarf Seahorse Sampling

Seahorse Catch

Seahorses were collected using a 1x1m pushnet, which is pushed through the water and rolls along the bottom, collecting organisms in its path (Strawn 1954; Figure 4). The target replicate push distance was 10 meters, but if the net was filled with macroalgae and vegetation prior to the target 10-meter distance, the push was stopped, and the distance of the push recorded. A maximum of six pushes were conducted along three transects (the two parallel edges and across the center of the square site boundaries, each 10 meters apart) (Figure 5). In the case of unsatisfactory sampling conditions (e.g., severe weather, mechanical problems, excessive macroalgae presence), a minimum of three pushnet replicates were completed. Any seahorses captured were euthanized using MS-222 and frozen to be transported to lab. All other nekton catch were identified and enumerated in the field. If individuals could not be easily identified or catch was too large for field identification, all nekton was euthanized using MS-222, preserved in 10% formalin, and transported to the lab for later identification and enumeration. All Dwarf Seahorses and voucher specimens of other species were transferred to 70% ethanol for long-term storage after identification and enumeration.



Figure 4. 1m² constructed pushnet (mesh size: 0.794 mm or 1/32 in).

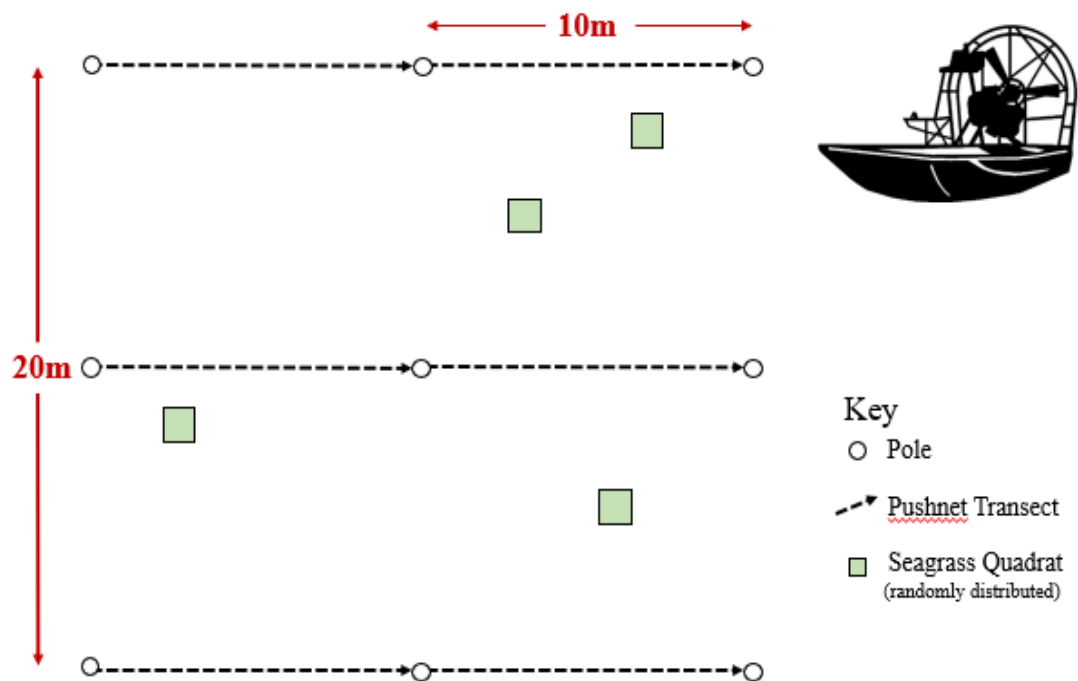


Figure 5. Site setup and pushnet sampling methodology

Determining Seahorse Sex

Basic morphometrics were recorded for all Dwarf Seahorses collected throughout the study except for one specimen from Corpus Christi Bay that was lost. All measurements were conducted in the lab after returning from sampling using a dissecting microscope and the CellSars software program. These measurements included tail length, snout length, and height in millimeters. Height was measured and is defined as the tip of the coronet to the tip of the outstretched tail with the head held at a 90° angle (Figure 6; Lourie 2003). Tail length is defined as the tip of the tail to the bottom trunk ring on the body (Lourie 2003). Snout length is typically defined in literature as the top tip of the snout to the anterior side of the pre-orbital tubercle/spine (Lourie 2003). On Dwarf Seahorses, this spine is very small and difficult to identify, even with a microscope. For the purpose of this study, snout length was defined as the top tip of the snout to the center of the eye (Figure 6). Sex was determined by the presence of a brood pouch. Specimens with a height of 16mm or smaller were defined as juvenile unless the presence or absence of a brood pouch was obvious (Masonjones and Lewis 1996). Tail and snout length were recorded to aid in the sex determination process if the presence of a pouch was unclear, as males on average have longer snouts and tails (Lourie, 2003). Measurements and sex of each individual Dwarf Seahorse captured can be found in Appendix C.

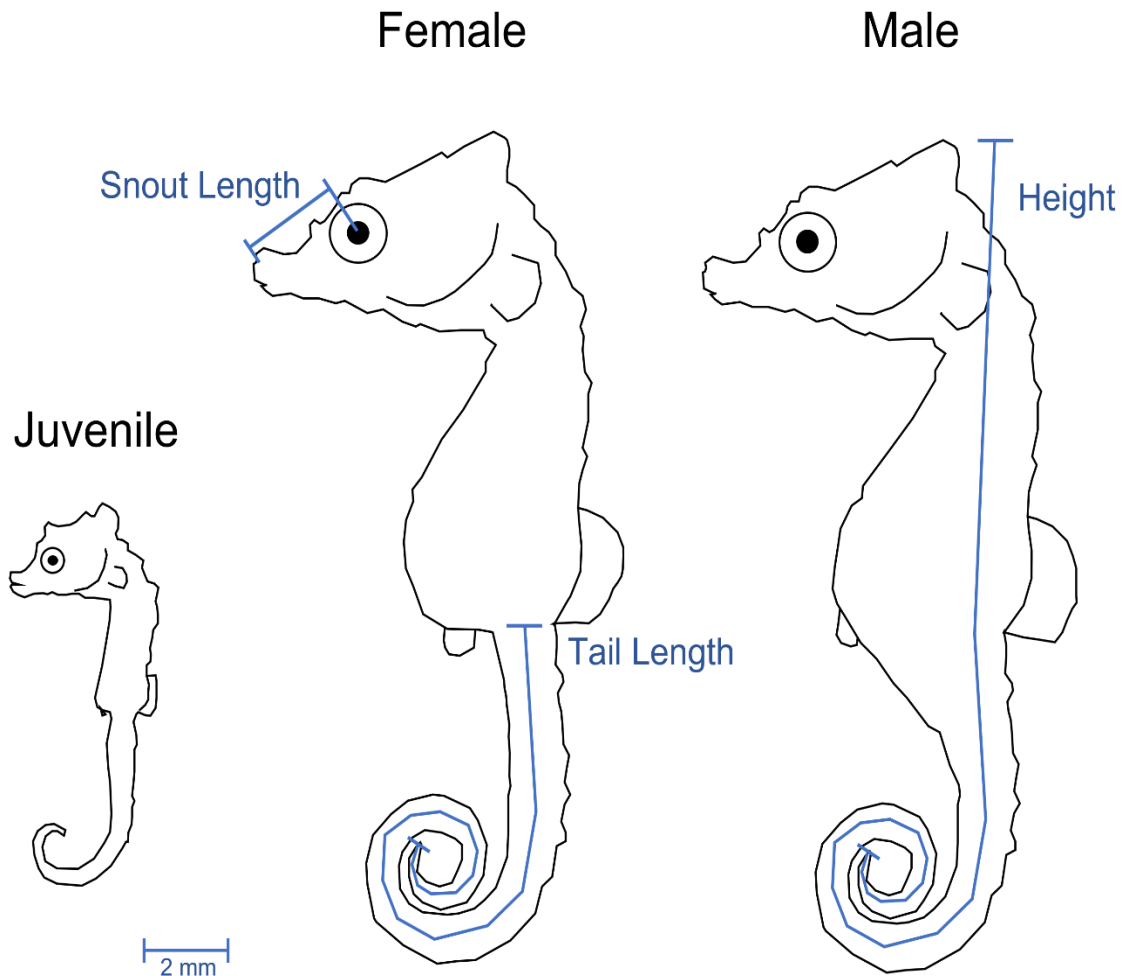


Figure 6. Seahorse measurement diagram from Oakley et al. 2022

Data Analysis

Community diversity indices (abundance (N), relative abundance (%), taxa richness (S), and Shannon Diversity (H)) were calculated for both nekton and seagrass community structure. Richness refers to the number of species captured. Abundance in catch per unit effort (CPUE) refers to the number of individuals captured per unit effort, which is each m^2 for the purpose of this study. Evenness refers to the distribution of abundance across all species captured at a site. The Shannon H diversity index takes into account species richness and evenness. R Studio (2021.09.0) software was used to analyze the relationship between water quality variables (temperature ($^{\circ}\text{C}$), salinity (ppt),

specific conductance ($\mu\text{MHOS}/\text{cm}$ @ 25°C), pH, dissolved oxygen saturation (% and mg/L), turbidity (NTU), secchi depth (m), PAR difference ($\mu\text{mol}/\text{m}^2/\text{s}$), seagrass biomass and percent cover, and seagrass and nekton community diversity and seahorse presence and density. An $\alpha = 0.05$ was used to indicate significance. The relationship between Dwarf Seahorse CPUE and water quality variables were assessed using zero-inflated binomial or Poisson linear models. The relationship between Dwarf Seahorse presence/absence and categorical environmental variables was assessed using Kruskal-Wallis one-way analysis of variance. RStudio was also used to create figures for data visualization. ArcGIS Pro was used to generate maps of seahorse catch, seagrass distribution, and seahorse sex distribution.

Nekton community data was used to conduct MDS (multi-dimensional scaling) resulting in diagrams to compare the similarity among sites using Primer7 software. Similarities between the nekton community present at sites where Dwarf Seahorses were detected to those sites where Dwarf Seahorses were not detected was conducted using the ANOSIM function in Primer7. Using this program's Pearson's Correlation function, we also identified other marine species associated with the presence of Dwarf Seahorse.

A preliminary Dwarf Seahorse population estimate was conducted in the three bay systems with the highest Dwarf Seahorse CPUE. Ninth arc-second resolution bathymetric tiles were used from NOAA's Continuously Updated Digital Elevation Model. Tiles were selected to cover the entire Aransas Bay, Upper Laguna Madre, and Lower Laguna Madre areas. These tiles were then uploaded into ArcGIS Pro and merged to create three separate raster layers, one for each bay system. Each layer was then clipped in ArcGIS Pro to the area covered by the TPWD documented seagrass layer. It was confirmed that these layers only included the sampled seagrass areas and were confined into the determined outlines of the separate bay systems. If some of the layer

extended outside of this area, it was clipped. Finally, the Reclassify tool was used to distinguish areas that had a water depth between zero and four feet (1.22 meters). Figure 7, Figure 8, and Figure 9 show the areas within sampled seagrass beds with a depth between 0 and 1.22 meters in orange in Aransas Bay, Upper Laguna Madre, and Lower Laguna Madre. The cell count from the attribute table of these maps and the known cell size was used to calculate the area covered by seagrass that had water depths of 1.22 meters maximum. This number was then used along with total CPUE for the entire bay system to calculate an initial population estimate in these three bay systems.

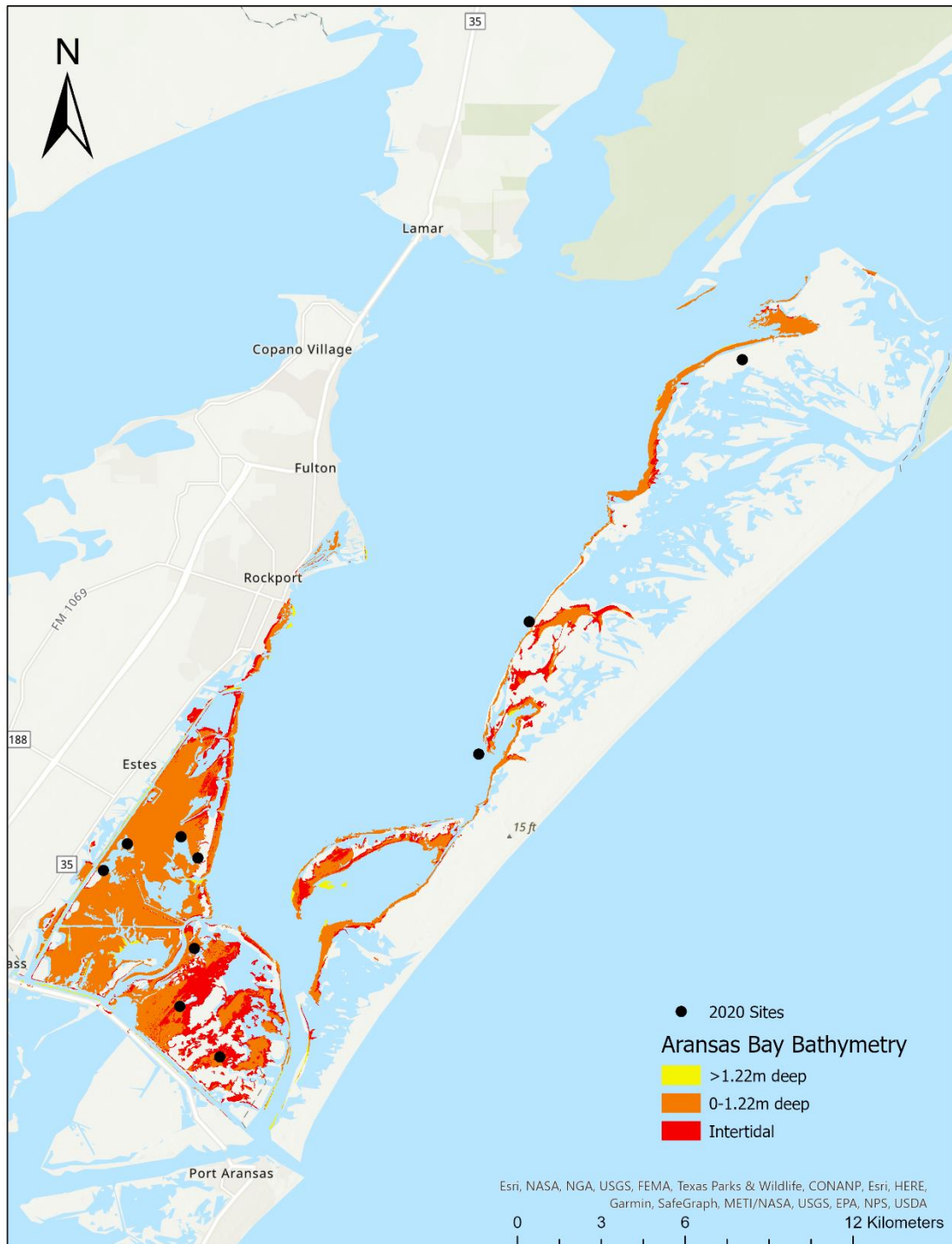


Figure 7. Area in Aransas Bay covered in seagrass with legend showing depths. Areas used in analysis are orange (0 – 1.22m in depth).

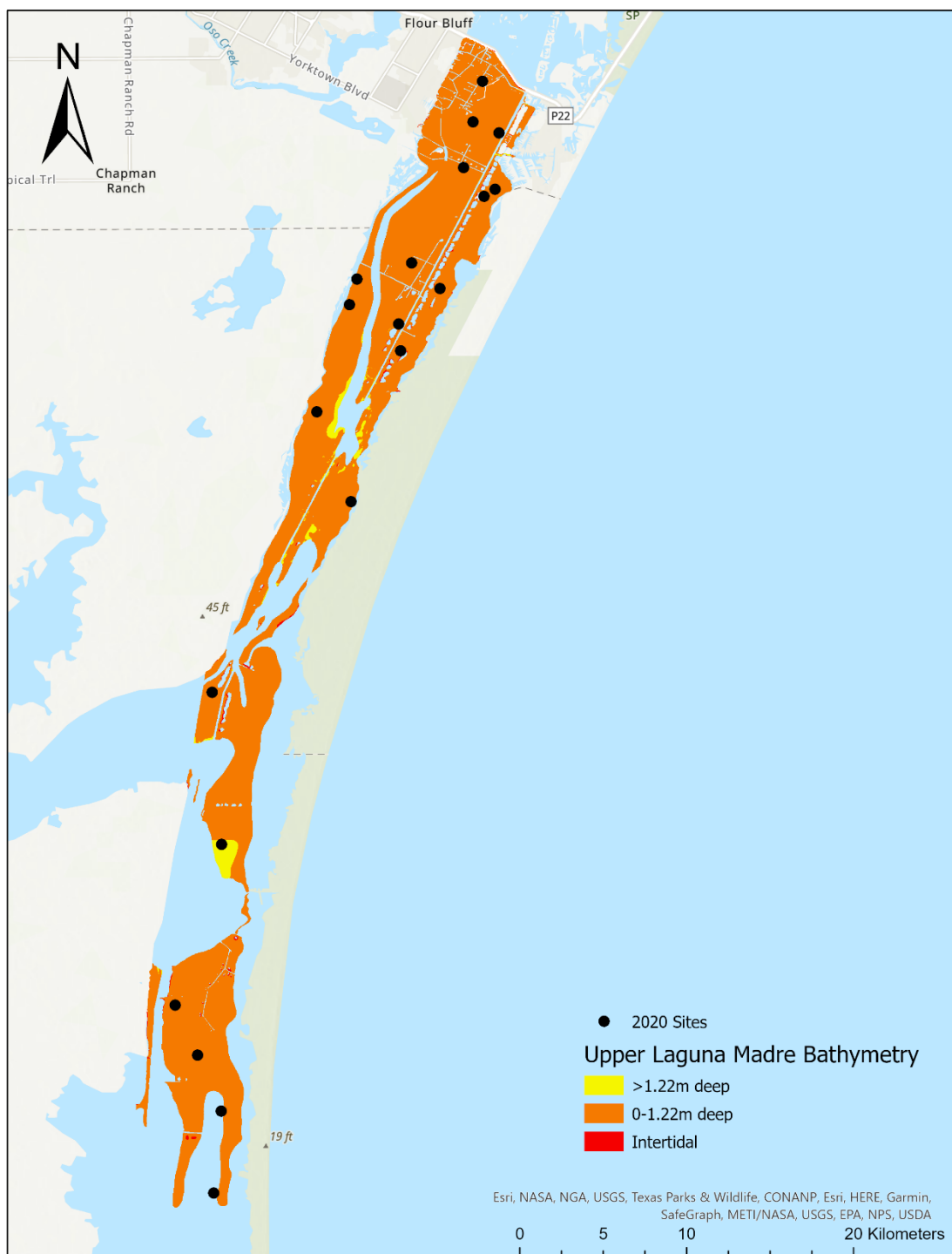


Figure 8. Area in Upper Lower Laguna Madre covered in seagrass with legend showing depths. Areas used in analysis are orange (0 – 1.22m in depth).

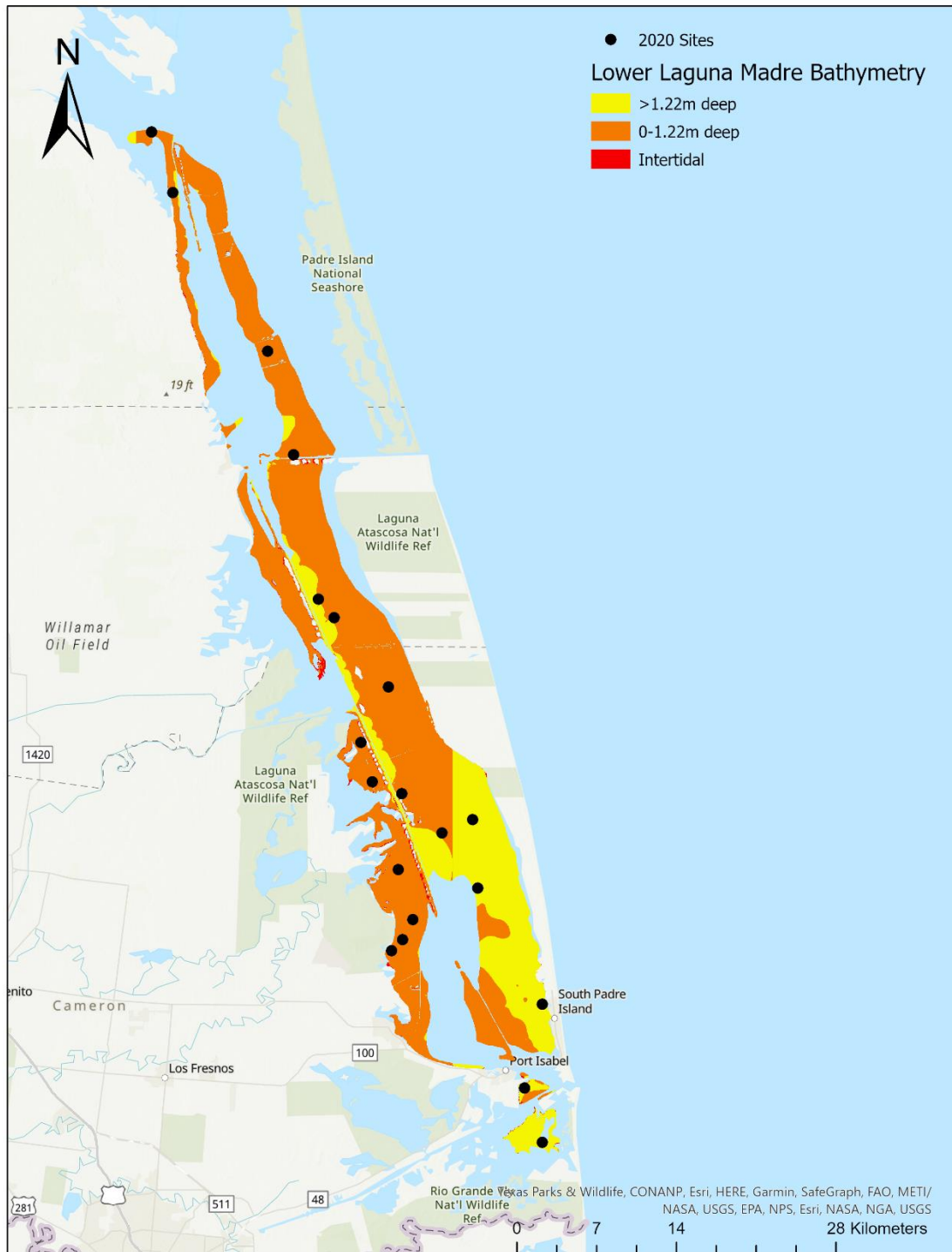


Figure 9. Area in Lower Laguna Madre covered in seagrass with legend showing depths. Areas used in analysis are orange (0 – 1.22m in depth).

RESULTS

Between June 2 and August 20, 2020, 80 sites were sampled between Galveston Bay and the Lower Laguna Madre in Texas. Average measured water quality values were calculated for each bay system and general trends were detected moving from the upper Texas coast to the lower coast. All five seagrass species known to exist in Texas were detected throughout this study. Differences were seen in seagrass coverage and community between the upper and lower Texas coasts. 79 Dwarf Seahorse were captured at 30 of our 80 sites (37.5% of sites). Catch per unit effort of Dwarf Seahorse increased as we moved down the coast, with highest CPUE in Aransas Bay.

Water Quality

Water quality metrics were collected at all 80 sites, except for PAR difference, which had a sample size of 75 due to equipment malfunction or missed readings (Table 4). Temperatures ranged from 26.2 to 36.0°C with highest average temperatures in the Lower Laguna Madre and lowest in Galveston Bay. Specific conductivity ranged from 26,421 to 65,064 μ S/cm with highest average specific conductivity occurring in the Upper Laguna Madre and lowest average in Galveston Bay. The highest average salinity was also in the Upper Laguna Madre and lowest was in Galveston Bay, with a range from 16.03 to 43.65ppt. Dissolved oxygen at sites ranged from 0.60mg/L to 15.96mg/L with San Antonio Bay having the highest average and Aransas Bay, the lowest. Average recorded pH across the coast was fairly consistent (Table 4) with the minimum detected pH recorded as 7.89 and maximum as 8.94.

Lowest recorded NTU was 0.28 and highest was 106.01. The bay system with the highest average turbidity was Matagorda Bay and the system with the lowest was Corpus Christi Bay. Deepest average Secchi depth (indicating low turbidity) was also in Corpus Christi Bay, but shallowest (indicating high turbidity) was in Galveston Bay. Minimum

Secchi depth was 0.1m and maximum was greater than 1.2m, meaning that visual depth was greater than the Secchi tube could detect. Finally, lowest PAR difference (indicating lower turbidity) was $18.9 \mu\text{mol}/\text{m}^2\text{s}^{-1}$ and highest was 1949.9 (high turbidity). The greatest average difference between ambient and underwater PAR value was in the Matagorda Bay system. With this metric, unlike the other two, Corpus Christi Bay was second lowest, with the lowest difference value recorded in the Aransas Bay system, indicating the most light attenuation was reaching seagrass beds in this bay system.

Some trends in water quality were observed moving from north to south along the Texas Coast. Water temperature increased while moving from Galveston Bay to Lower Laguna Madre in respective order. Specific conductivity and salinity also followed a similar trend, with the exception of the Lower Laguna Madre, where both decreased from the Upper Laguna Madre (Table 5). Dissolved oxygen appears to be slightly higher in the upper bays (Galveston Bay, Matagorda Bay, and San Antonio Bay) than in the lower bays (Aransas Bay, Corpus Christi Bay, Upper and Lower Laguna Madre). pH did not appear to follow a consistent pattern along the coast. While there is some variation, in general there was a trend of higher turbidity in the upper bays and lower turbidity in the lower bays. This higher turbidity is reflected in higher NTU numbers, higher PAR differences, and lower or shallower Secchi reading.

Table 4. The number of samples, minimum, quartile 1, median, mean, quartile 3, and maximum values of each water quality metric recorded during the study.

Variable	N	Minimum	Q1	Median	Mean	Q3	Maximum
Temperature	80	26.2	28.7	29.8	30.0	31.1	36.0
Specific Conductivity	80	26,421	38,849	50,043	45,907	52,969	65,064
Salinity	80	16.03	24.50	32.62	29.68	34.76	43.65
DO (mg/L)	80	0.60	4.50	6.50	6.36	8.19	15.96
pH	80	7.8	8.1	8.2	8.2	8.3	8.9
NTU	80	0.28	3.51	6.76	9.60	11.60	106.01
Secchi	80	0.10	0.33	0.47	0.59	0.82	1.20
PAR Difference	75	18.9	391.3	558.7	692.1	1797.0	1949.9
Total Depth	80	0.24	0.56	0.74	0.76	0.97	1.38

Table 5. Average of all recorded water quality metrics in each studied bay system.

Bay System	Temp (°C)	Sp. Cond (µS/cm)	Sal (ppt)	DO (%sat)	DO (mg/L)	pH	NTU	PAR Diff (µmol/m²s⁻¹)	Secchi (m)	Total Depth (m)
Galveston	28.30	32312	19.75	111.58	7.77	8.112	13.092	865.0	0.23	0.82
Matagorda	28.56	37731	23.85	104.70	7.11	8.176	30.190	1086.3	0.38	0.68
San Antonio	28.44	42922	27.54	114.96	7.64	8.233	12.875	530.5	0.37	0.64
Aransas	29.14	49341	31.93	89.64	5.73	8.173	9.540	434.3	0.63	0.77
Corpus Christi	30.55	53246	35.03	98.86	6.01	8.179	3.717	478.6	0.93	0.65
Upper Laguna	30.70	54054	35.57	90.75	5.50	8.116	7.223	564.2	0.64	0.72
Lower Laguna	30.99	39313	24.99	100.87	6.56	8.387	7.299	809.1	0.62	0.90

Seagrass

All five seagrass species known to inhabit Texas waters were detected throughout this study. The highest average seagrass percent coverage was observed in the Lower Laguna Madre (63.4%), followed by the Upper Laguna Madre (57.5%), Aransas Bay (50.8%), Corpus Christi Bay (49.1%), San Antonio Bay (45.4%), Matagorda Bay (21.0%), and Galveston Bay (18.7%) respectively (Table 6). The percentage of bare ground recorded in each bay system reflects almost the opposite of the seagrass coverage, but not quite, with a difference seen in Corpus Christi Bay. The bay systems with the most bare ground coverage to least were Galveston Bay, Matagorda Bay, San Antonio Bay, Aransas Bay, Lower Laguna Madre, Upper Laguna Madre, then Corpus Christi Bay (Table 6). Macroalgae was also documented in all bay systems along the Texas Gulf Coast except for Galveston Bay. The highest percentage of macroalgae coverage was recorded in Corpus Christi Bay at 27.5% with the lowest in Matagorda Bay (0.1%). The high macroalgae cover in Corpus Christi Bay explains why it has the least bare ground coverage across the coast.

Geographic trends were documented throughout the study, with changes in average percent cover, canopy height, and biomass across the coast, as well as shifts in seagrass bed community structure. There was a clear difference between average canopy height and percent coverage between the upper bay systems (Galveston Bay, Matagorda Bay, San Antonio Bay) and the lower bays (Aransas Bay, Corpus Christi Bay, Upper Laguna Madre, Lower Laguna Madre) (Table 6). The three upper bay systems had lower seagrass percent cover and canopy height in comparison to the four lower bay systems. Biomass seems to follow a similar pattern, although the Upper Laguna Madre had average biomass values more similar to the upper bays.

Seagrass community structure shifts from shoal grass (*Halodule wrightii*), widgeon grass (*Ruppia maritima*), and star grass (*Halophila englemanni*) dominated in the upper bays to shoal grass, turtle grass (*Thalassia testudinum*), and manatee grass (*Syringodium filiforme*) dominated lower along the coast. Shoal grass was detected in all bay systems and star grass was detected in all except for Matagorda Bay. Turtle grass was first detected in Aransas Bay and was then found in all following lower bay systems. Manatee grass was detected in Corpus Christi Bay and all following lower bay systems. Widgeon grass was detected inconsistently along the study range, being found in Galveston, Aransas, and Corpus Christi Bays.

Table 6. The number of sites, average percent cover of all seagrass species and macroalgae, average biomass, average canopy height, and total average percent cover of seagrass and bare in each bay system. “Cover” indicates average percent coverage. Shoal = shoal grass percent coverage, Turtle = turtle grass, Star = star grass, Manatee = manatee grass, and Widgeon = widgeon grass.

Major Bay System	n	Shoal Cover	Turtle Cover	Star Cover	Manatee Cover	Widgeon Cover	Biomass (g)	Canopy Height (cm)	Seagrass Cover	MACRO Cover	Bare Cover
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

Seahorse Presence and Density

A total of 79 Dwarf Seahorses were caught at 30 of the 80 sites (Table 7). They were detected in all sampled bay systems except for Galveston Bay. Dwarf Seahorses were the only species of seahorse captured throughout this study. CPUE at individual sites ranged from 0.017/m² to 0.136/m². Average CPUE across all sites was 0.017/m². CPUE of 0.017 is equivalent to catching one Dwarf Seahorse in 60m² of effort and 0.136 equivalent to 8.16 Dwarf Seahorses in 60m² of effort, but in this study is reflected in a site where 3 Dwarf Seahorses were caught in 22m² of effort. The highest density in CPUE of Dwarf Seahorses were detected in Aransas Bay (0.038), with Lower Laguna Madre (0.023), Upper Laguna Madre (0.017), Corpus Christi Bay (0.011), San Antonio Bay (0.008), and Matagorda Bay (0.003) following. There was no significant difference in CPUE among bay systems (Figure 10).

Table 7. Dwarf Seahorse capture throughout study including number captured, percent of sampled sites with Dwarf Seahorse presence detected, and CPUE at each bay system with grand totals of number of sites and Dwarf Seahorse captured average percentage sites with Dwarf Seahorse detection and CPUE.

Major Bay System	Number of sites	Number of Dwarf Seahorses Captured	% 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

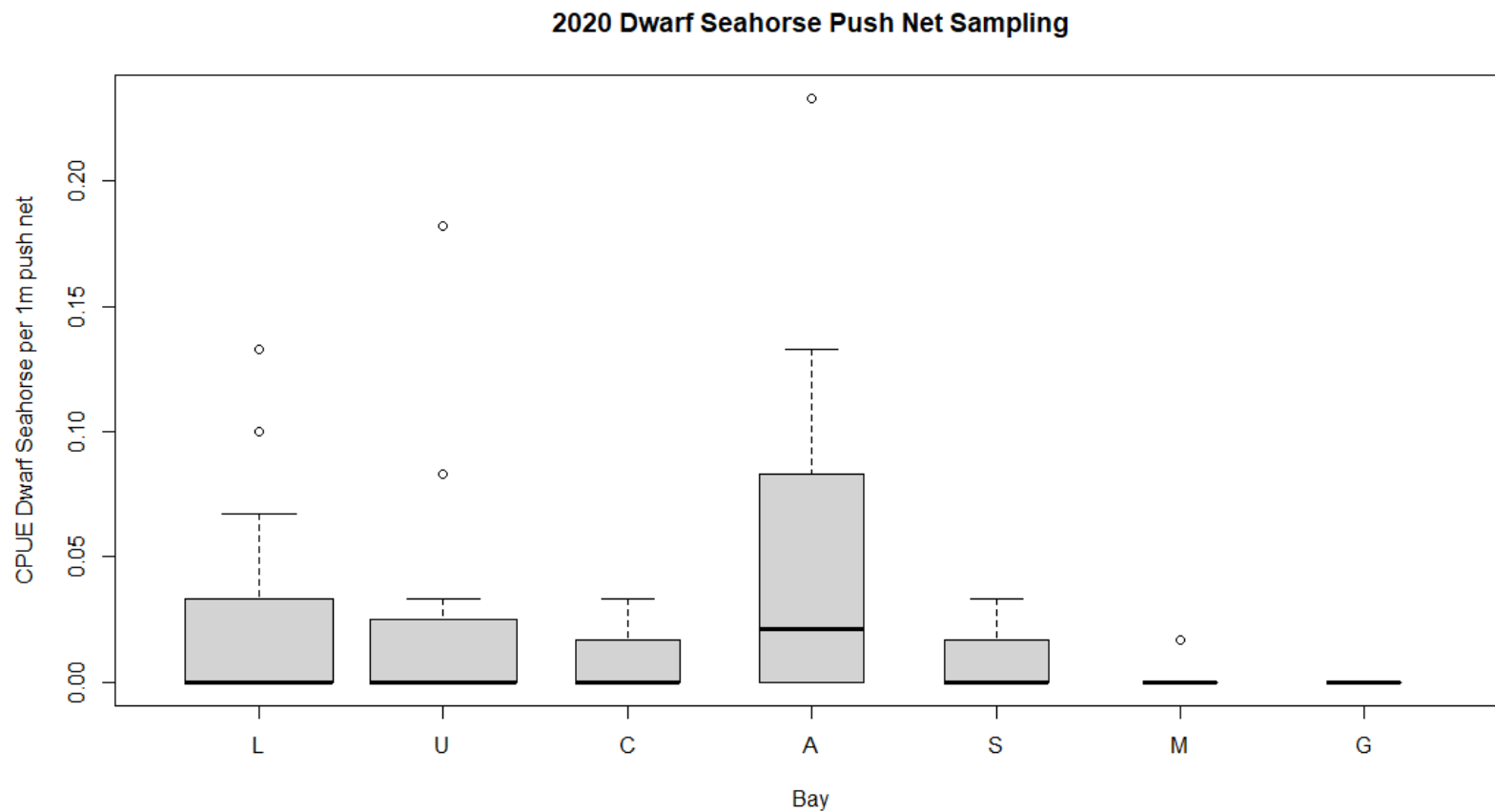


Figure 10. CPUE of Dwarf Seahorse in each bay system (L = Lower Laguna Madre, U = Upper Laguna Madre, C = Corpus Christi Bay, A = Aransas Bay, S = San Antonio Bay, M = Matagorda Bay, G = Galveston Bay).

One Dwarf Seahorse was captured in Matagorda Bay at one of the five sites with a CPUE of $0.003/\text{m}^2$ throughout the whole bay system (Figure 11). In San Antonio Bay, five Dwarf Seahorse were caught at three sites, distributed across Espiritu Santo Bay, where most sites were located (Figure 12). The bay system with the highest CPUE was Aransas Bay, where 20 Dwarf Seahorse were caught at six out of 10 total sites. Most were captured in the southwestern area of the bay, near Aransas Pass (Figure 13). Capture numbers dropped in Corpus Christi Bay, with 6 Dwarf Seahorse captured at 4 sites. Most of these captures were near where most Dwarf Seahorses were captured in Aransas Bay, just on the other side of Aransas Pass (Figure 14). Dwarf Seahorse were distributed fairly evenly throughout both Upper and Lower Laguna Madre though CPUE was higher in Lower Laguna Madre in comparison to Upper Laguna Madre. In Upper Laguna Madre, 19 Dwarf Seahorses were captured at 7 sites with a bay CPUE of $0.017/\text{m}^2$ (Figure 15). In Lower Laguna Madre, 28 individuals were captured at 9 sites with a CPUE of 0.023, second highest along the Texas coast (Figure 16).

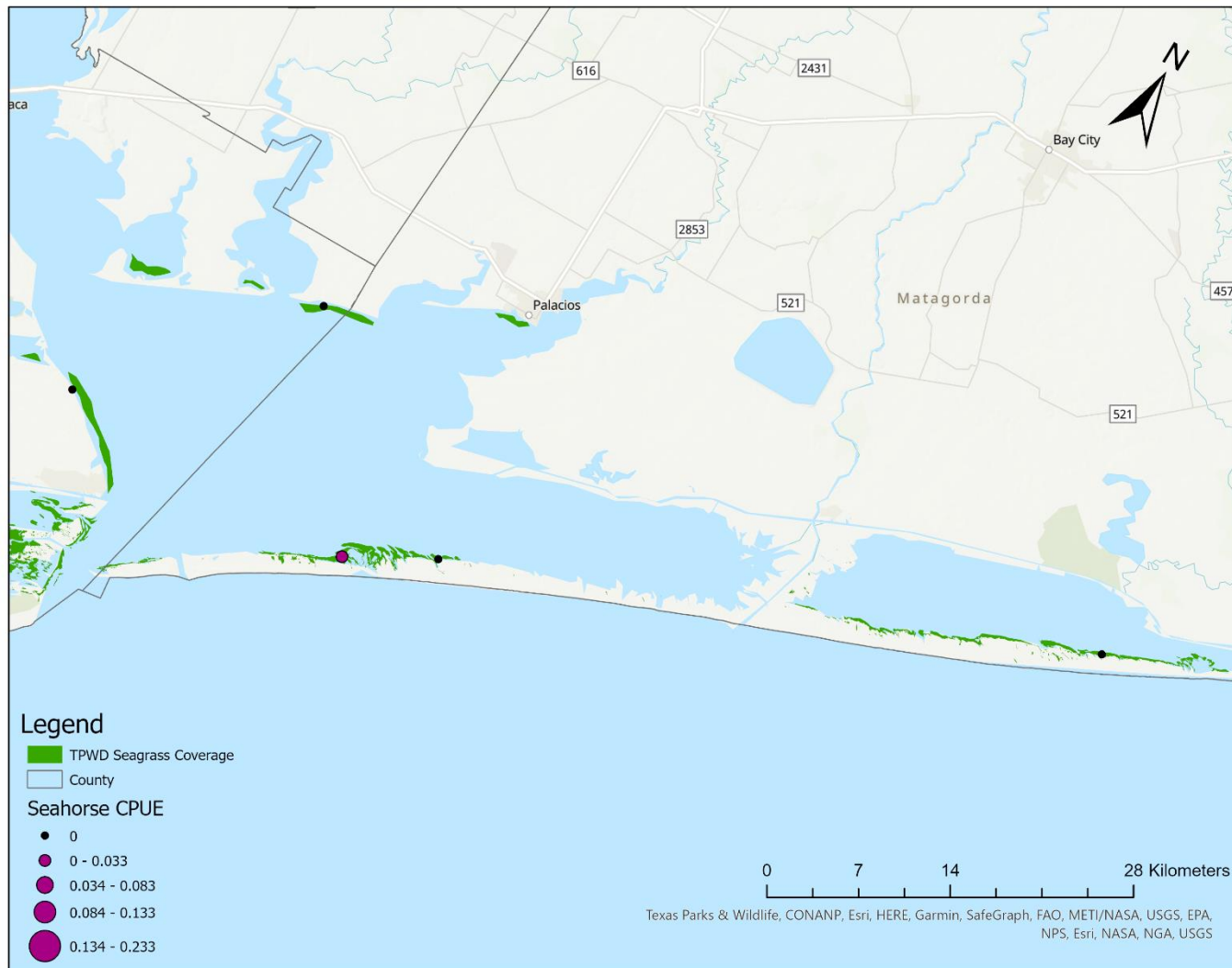


Figure 11. Documented seagrass, historic Dwarf Seahorse sightings, and CPUE of Dwarf Seahorse in Matagorda Bay. CPUE is in purple with circle size based on CPUE value.

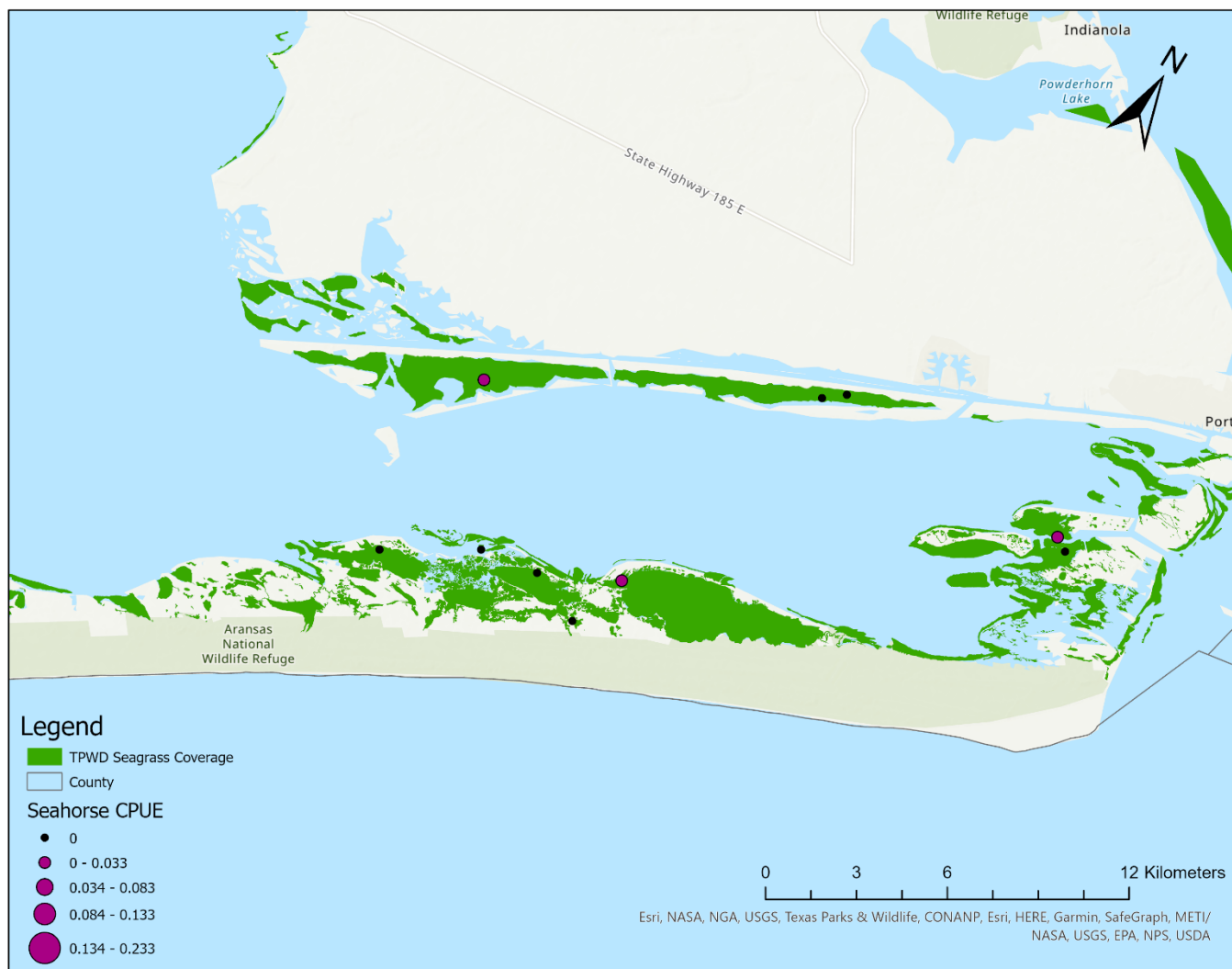


Figure 12. Documented seagrass, historic Dwarf Seahorse sightings, and CPUE of Dwarf Seahorse in San Antonio Bay. CPUE is in purple with circle size based on CPUE value.

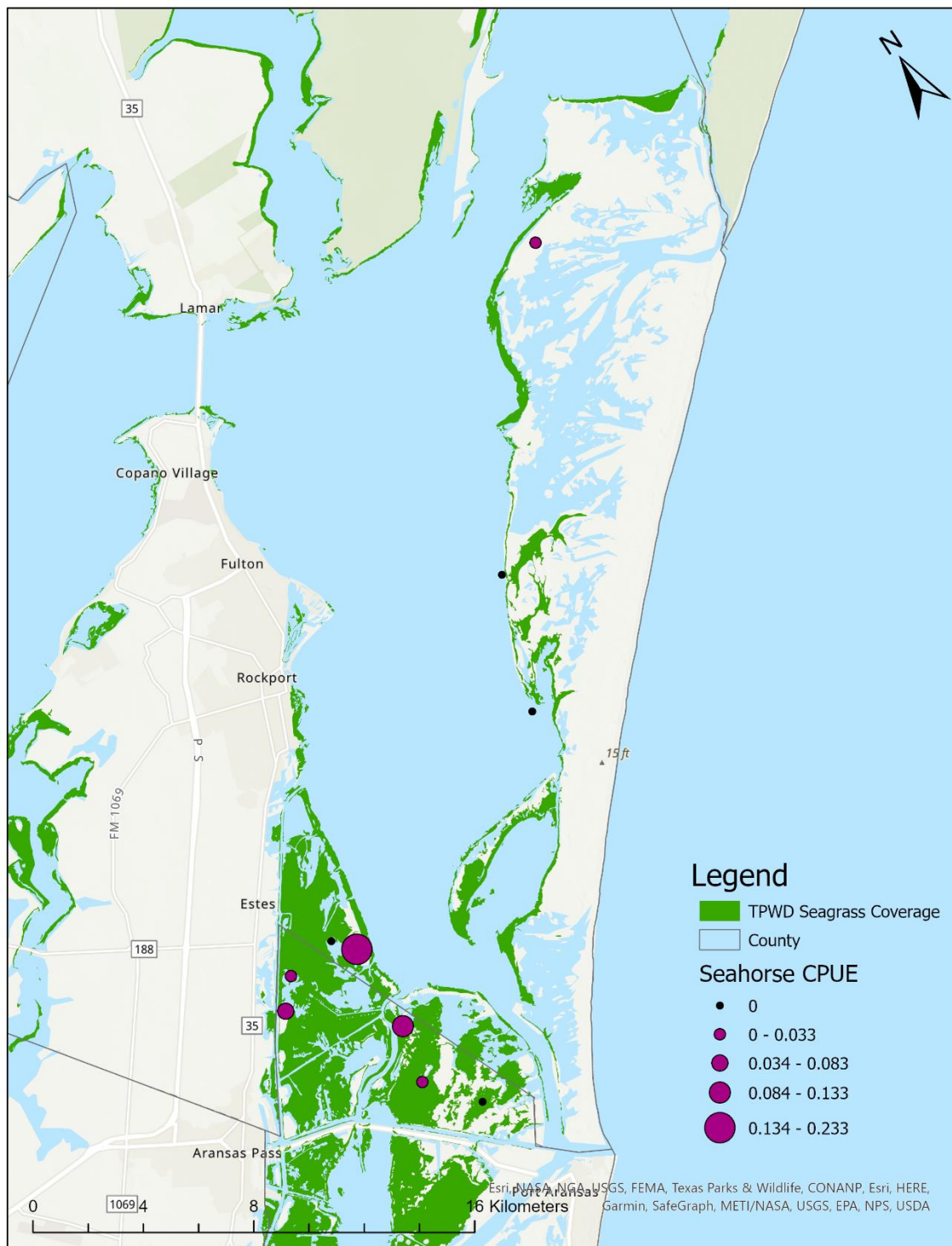


Figure 13. Documented seagrass, historic Dwarf Seahorse sightings, and CPUE of Dwarf Seahorse in Aransas Bay. CPUE is in purple with circle size based on CPUE value.

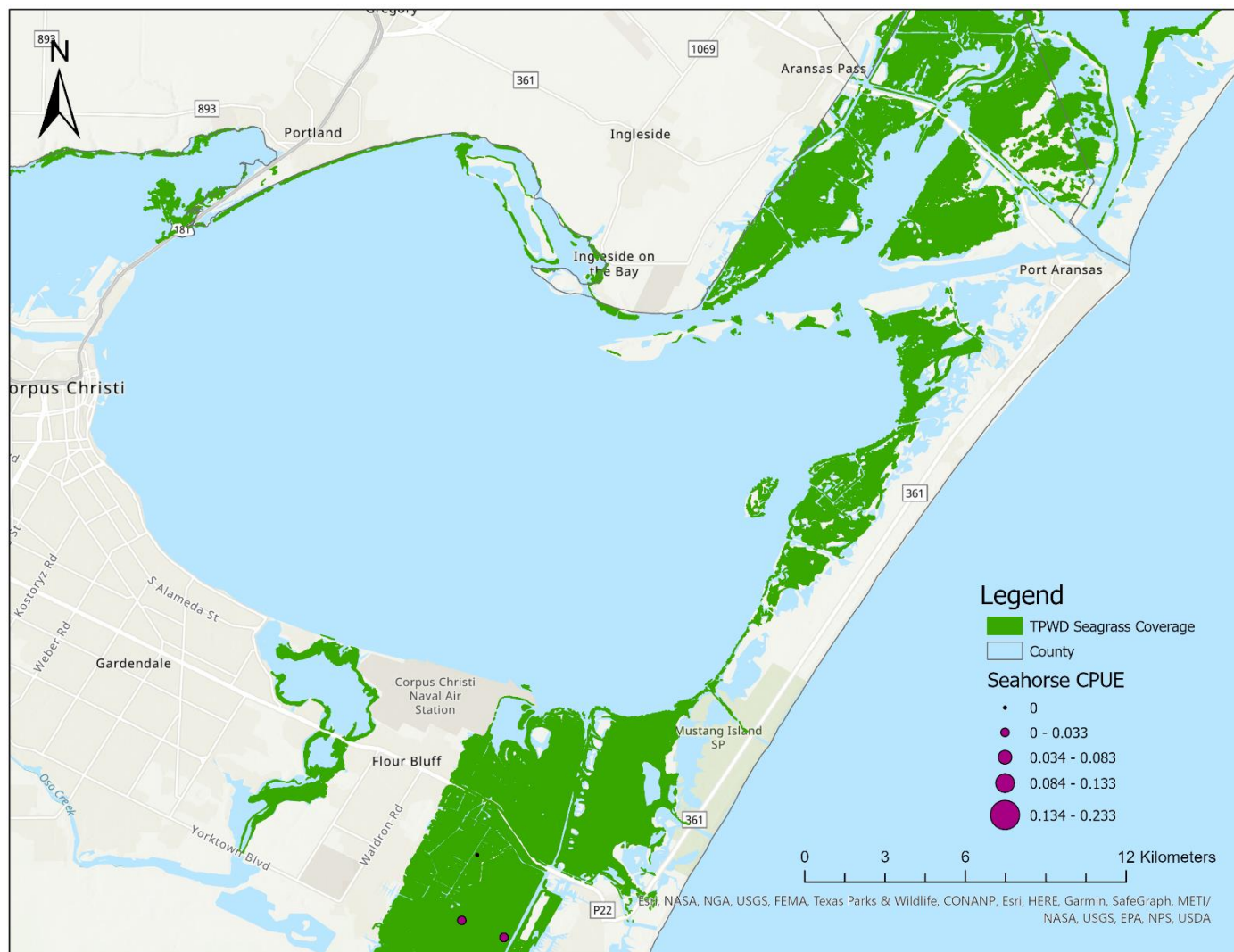


Figure 14. Documented seagrass, historic Dwarf Seahorse sightings, and CPUE of Dwarf Seahorse in Corpus Christi Bay. CPUE is in purple with circle size based on CPUE value.

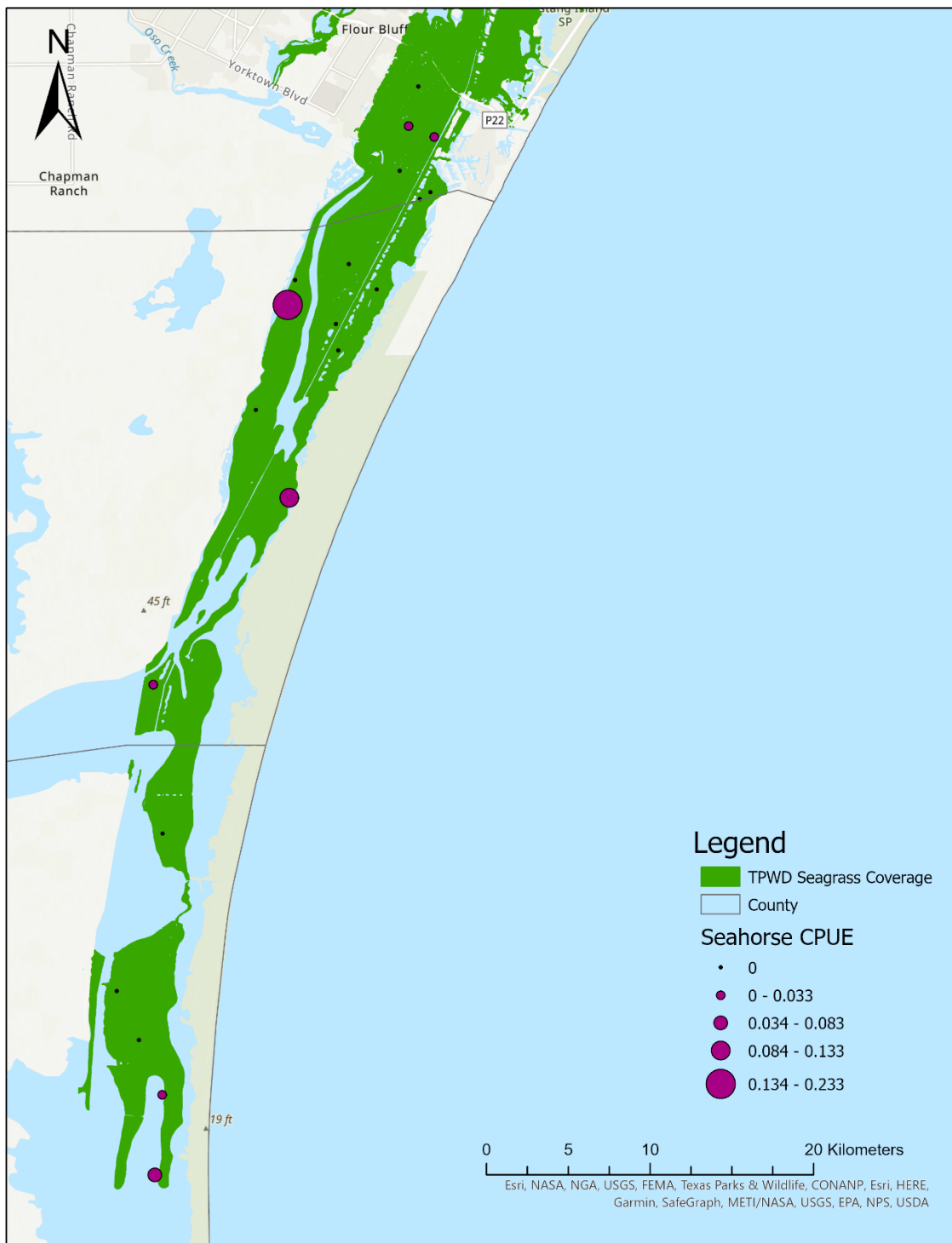


Figure 15. Documented seagrass, historic Dwarf Seahorse sightings, and CPUE of Dwarf Seahorse in Upper Laguna Madre. CPUE is in purple with circle size based on CPUE value.

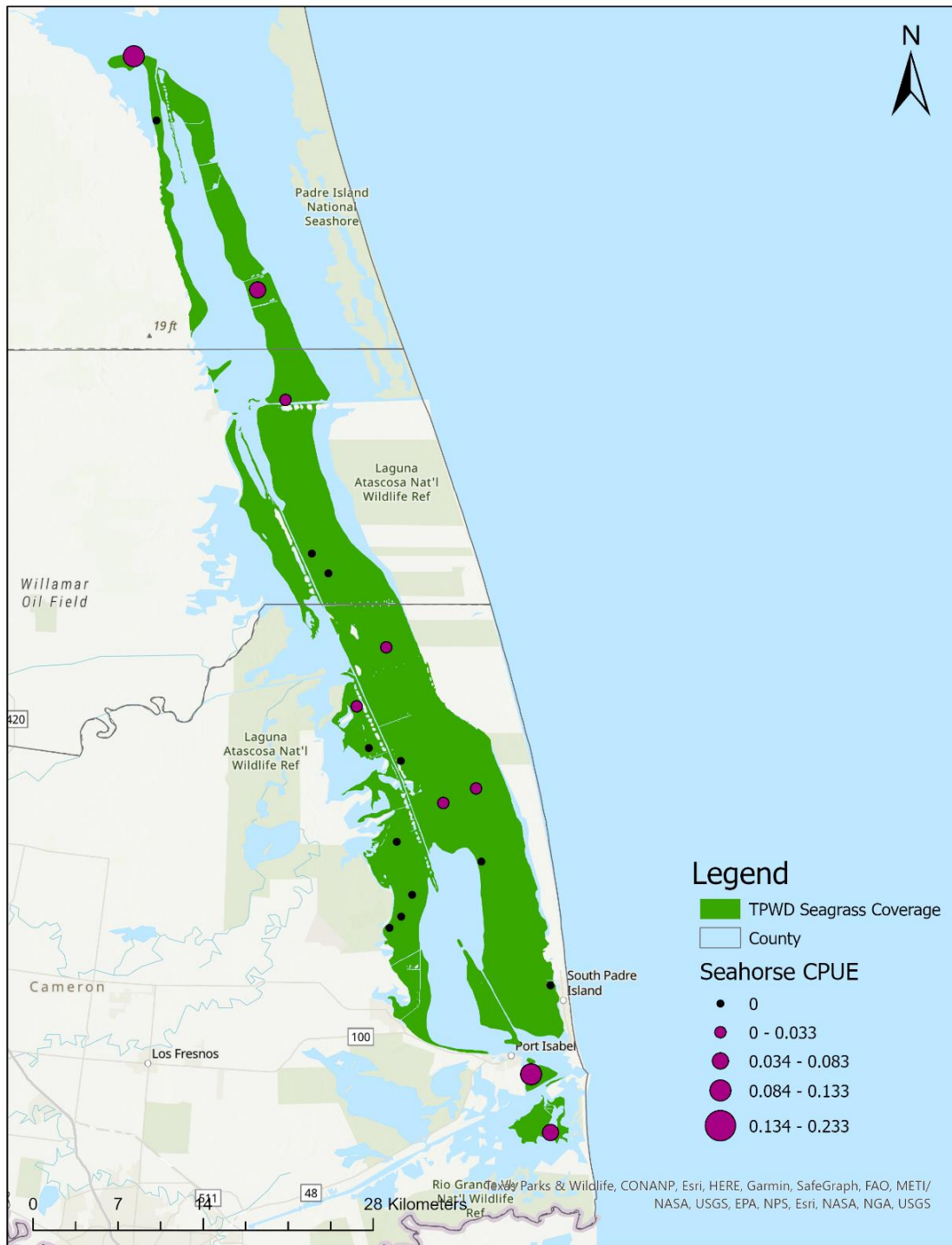


Figure 16. Documented seagrass, historic Dwarf Seahorse sightings, and CPUE of Dwarf Seahorse in Lower Laguna Madre. CPUE is in purple with circle size based on CPUE value.

Habitat Associations

Each water quality and physical habitat metric was analyzed for correlation with Dwarf Seahorse presence and density in CPUE. No significant correlations were found with a confidence of 95% between each water quality metrics and Dwarf Seahorse presence or CPUE.

When analyzing seagrass bed data with Dwarf Seahorse presence, a couple variables were found to be significant. First, the presence and percent cover of each seagrass species and macroalgae was evaluated with the CPUE and presence of Dwarf Seahorses. The percent cover of turtle grass at a site had a significantly positive association with the presence of Dwarf Seahorses and was the only significant association with an individual seagrass species found in relation to species presence (p-value = 0.0095; Figure 17). Average seagrass biomass was also significantly associated with the presence of Dwarf Seahorse. The higher the average biomass at a site, the more likely it was that an individual would be captured (p-value = 0.0372; Figure 18). Seagrass Shannon H diversity was significantly higher at sites where Dwarf Seahorse were captured in comparison to sites where they were not (p-value = 0.0007, Figure 19). Though not significant at 95% confidence, there does seem to be a potential relationship between average canopy height (cm) of the seagrass sampled and the likelihood a Dwarf Seahorse would be captured there (p-value = 0.0777).

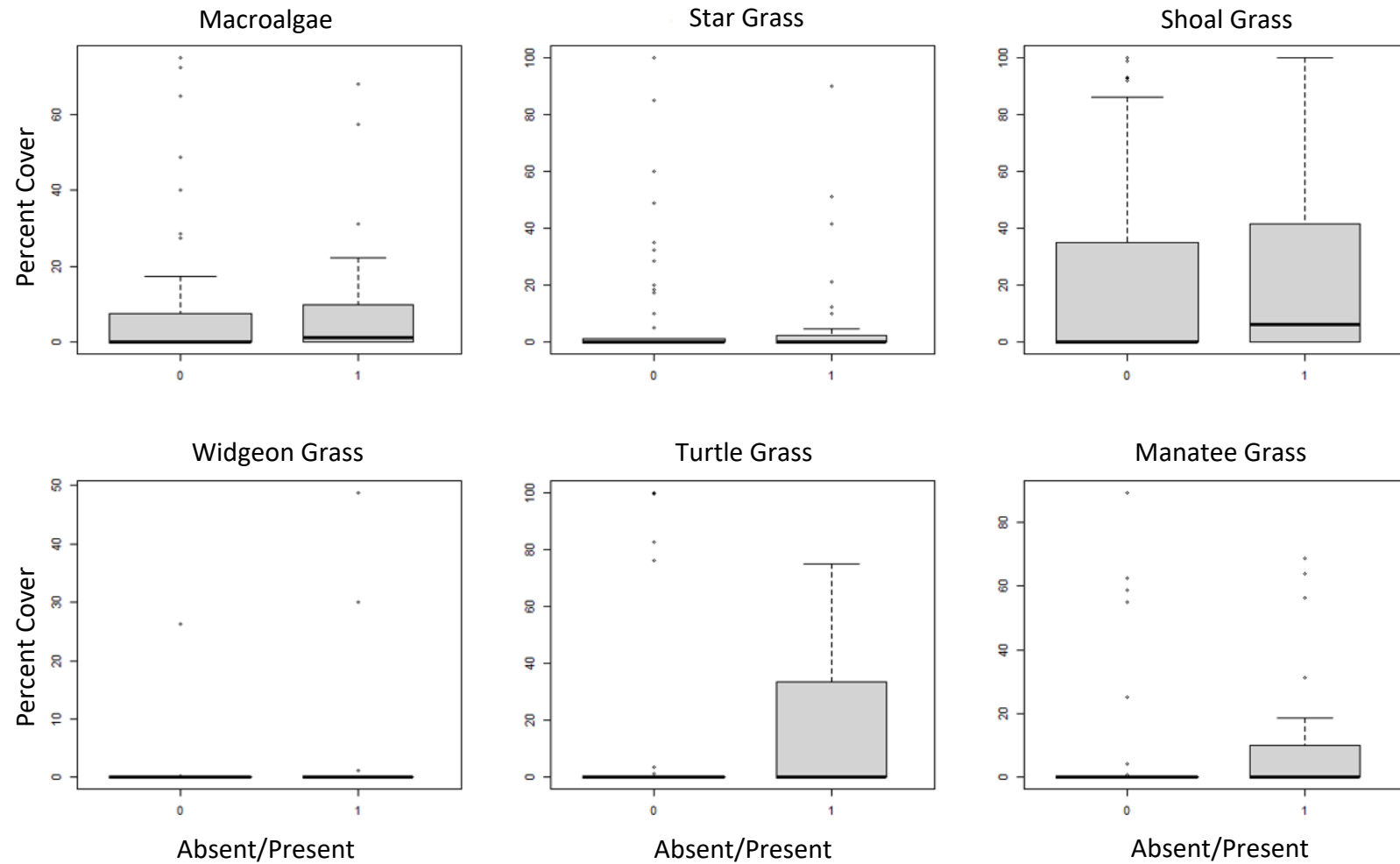


Figure 17. Absence/presence of Dwarf Seahorse vs percent cover of vegetation for each seagrass species and macroalgae. Only significant relationship is with turtle grass (p-value = 0.0095).

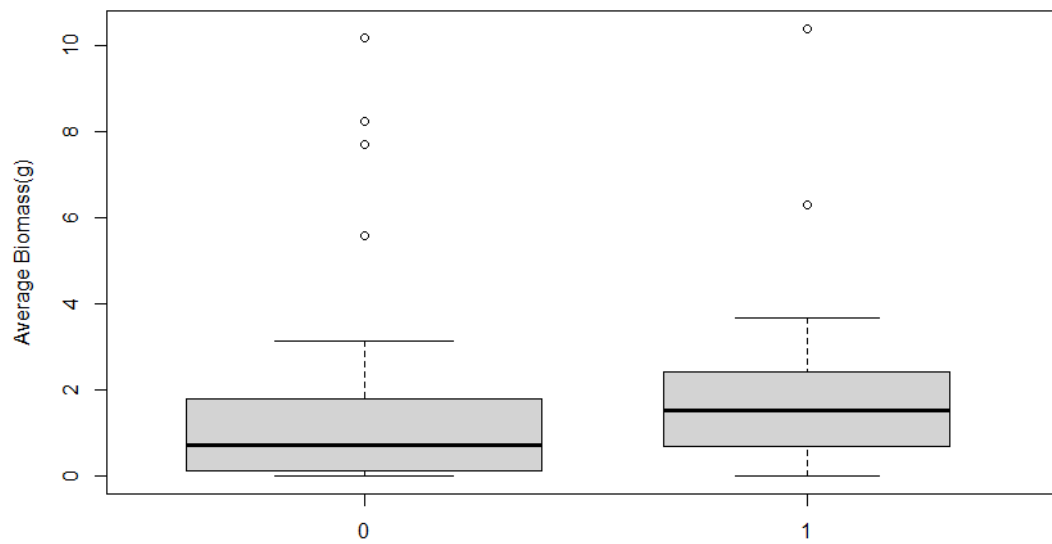


Figure 18. Absence/presence of Dwarf Seahorse vs average seagrass biomass in grams (p-value = 0.0372).

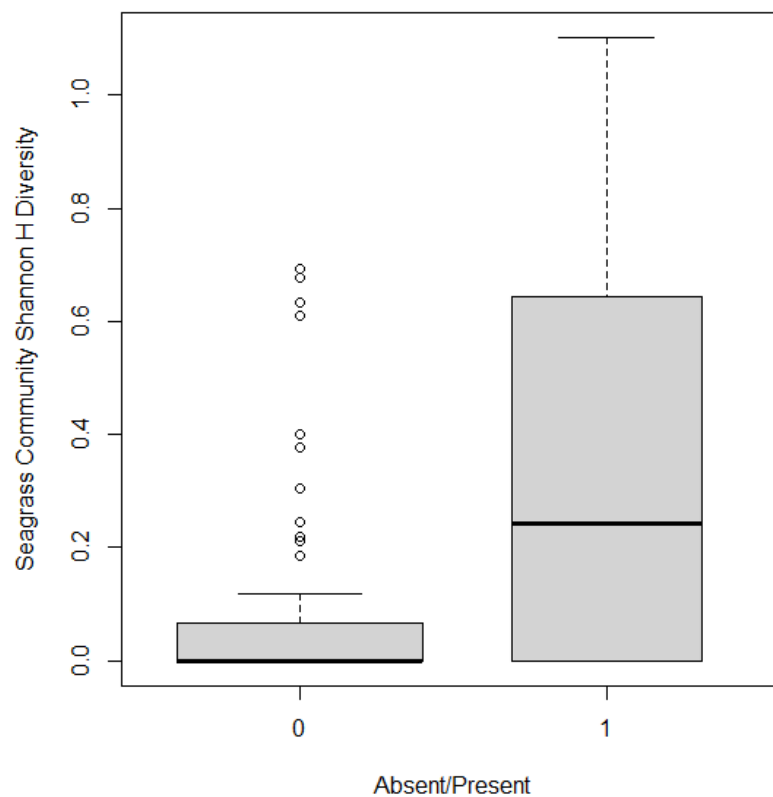


Figure 19. Absence/presence of Dwarf Seahorse vs seagrass Shannon H Diversity (p-value = 0.0007).

Similar analyses were conducted with Dwarf Seahorse CPUE. As with presence/absence, the percent coverage and presence of each species of seagrass and macroalgae were evaluated in relationship to Dwarf Seahorse CPUE. Similar to seahorse presence/absence, the only species presence significantly associated with Dwarf Seahorse CPUE was turtle grass (p-value = 0.0031; Figure 20). While not significant, there seemed to be a trend of increased CPUE of Dwarf Seahorses at sites where manatee grass was present (p-value = 0.0559). A statistically significant relationship was also detected between the richness of the seagrass community at a site and the CPUE of Dwarf Seahorse captured (p-value = 0.0466; Figure 21), but post-hoc analysis showed no significant differences between sites with differing seagrass species richness categories.

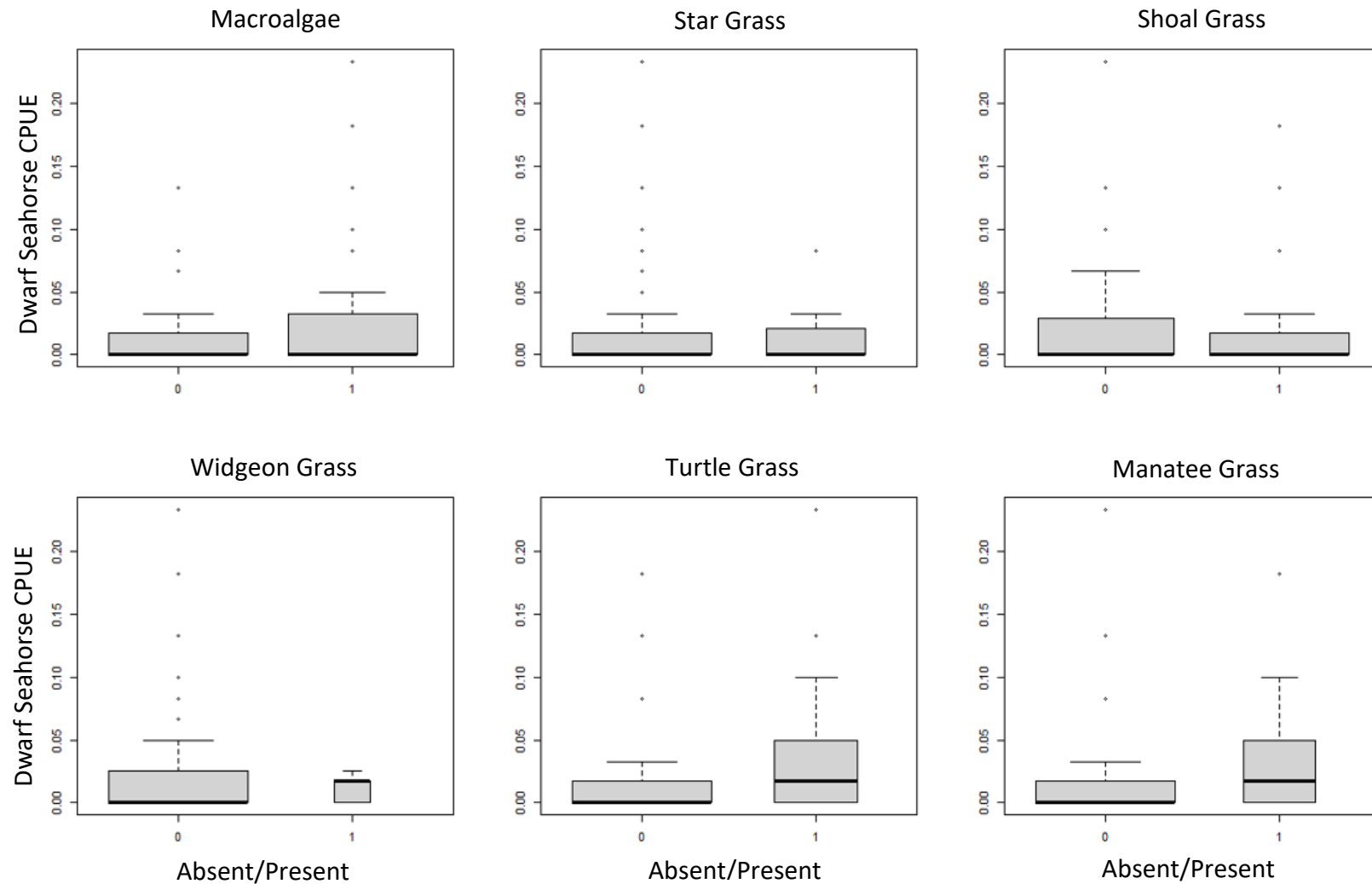


Figure 20. Presence/absence of each seagrass species and macroalgae vs Dwarf Seahorse CPUE. The only significant relationship was with turtle grass (p-value = 0.0031).

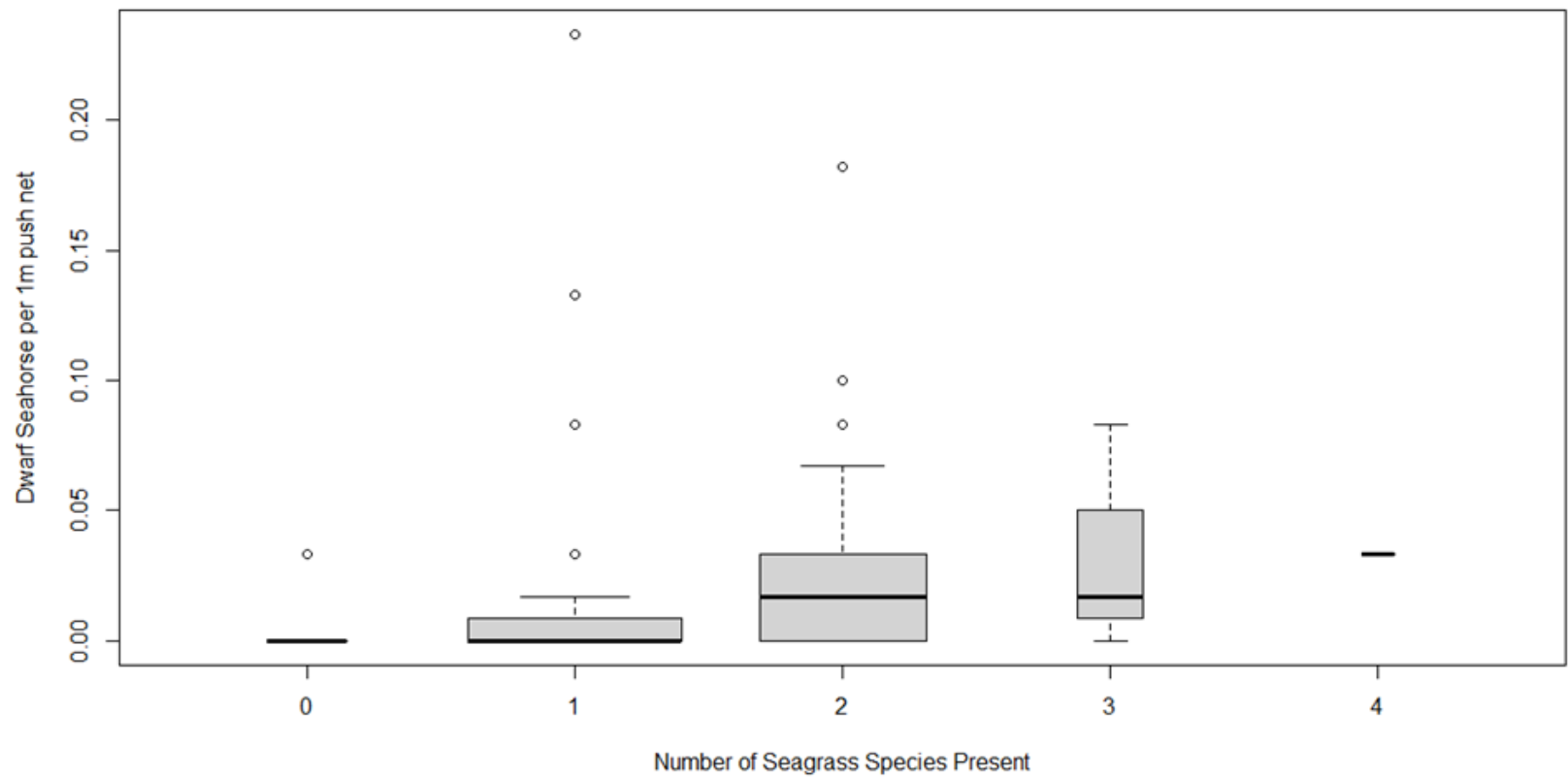


Figure 21. Relationship between species richness of a site vs Dwarf Seahorse CPUE (p-value = 0.0466).

Figures 22 through 27 display the seagrass community in each bay system at sites Dwarf Seahorses were captured. Galveston Bay is excluded because no Dwarf Seahorses were detected there. In Matagorda and San Antonio Bays, sites where Dwarf Seahorses were present were shoal grass dominated with some star grass present (Figure 22 and Figure 23). In Aransas and Corpus Christi Bays, turtle grass and macroalgae were the major sources of plant cover, with some star grass, widgeon grass, and our first detection of manatee grass in Corpus Christi Bay (Figure 24 and Figure 25). In both Upper and Lower Laguna Madre, both macroalgae and all seagrass species were present except for widgeon grass, with turtle grass dominating (Figure 26 and Figure 27).

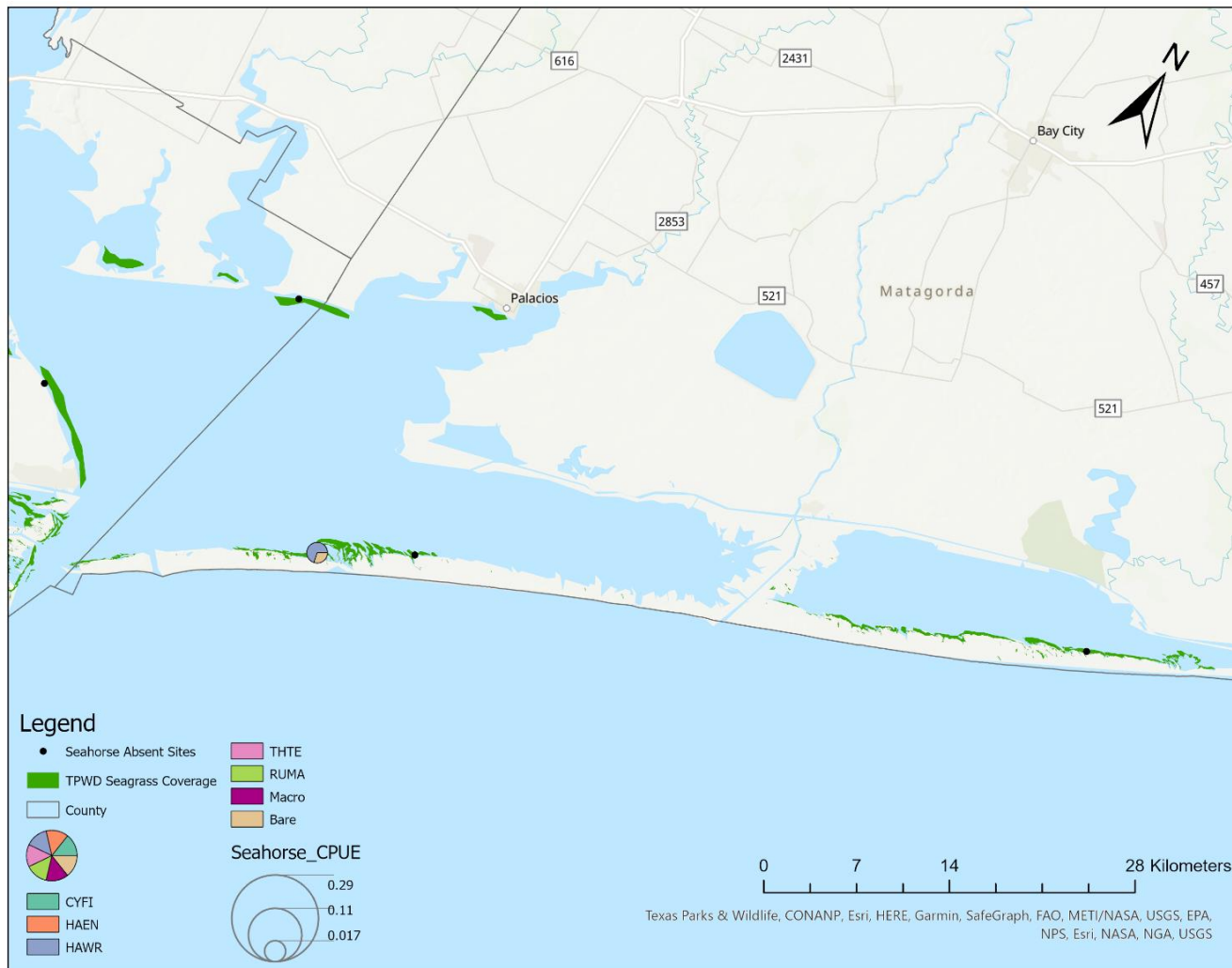


Figure 22. Seagrass species presence with pie piece size reflecting percent cover at each site in Matagorda Bay. CYFI = manatee grass, HAEN = star grass, HAWR = shoal grass, THTE = turtle grass, RUMA = widgeon grass, MACRO = macroalgae.

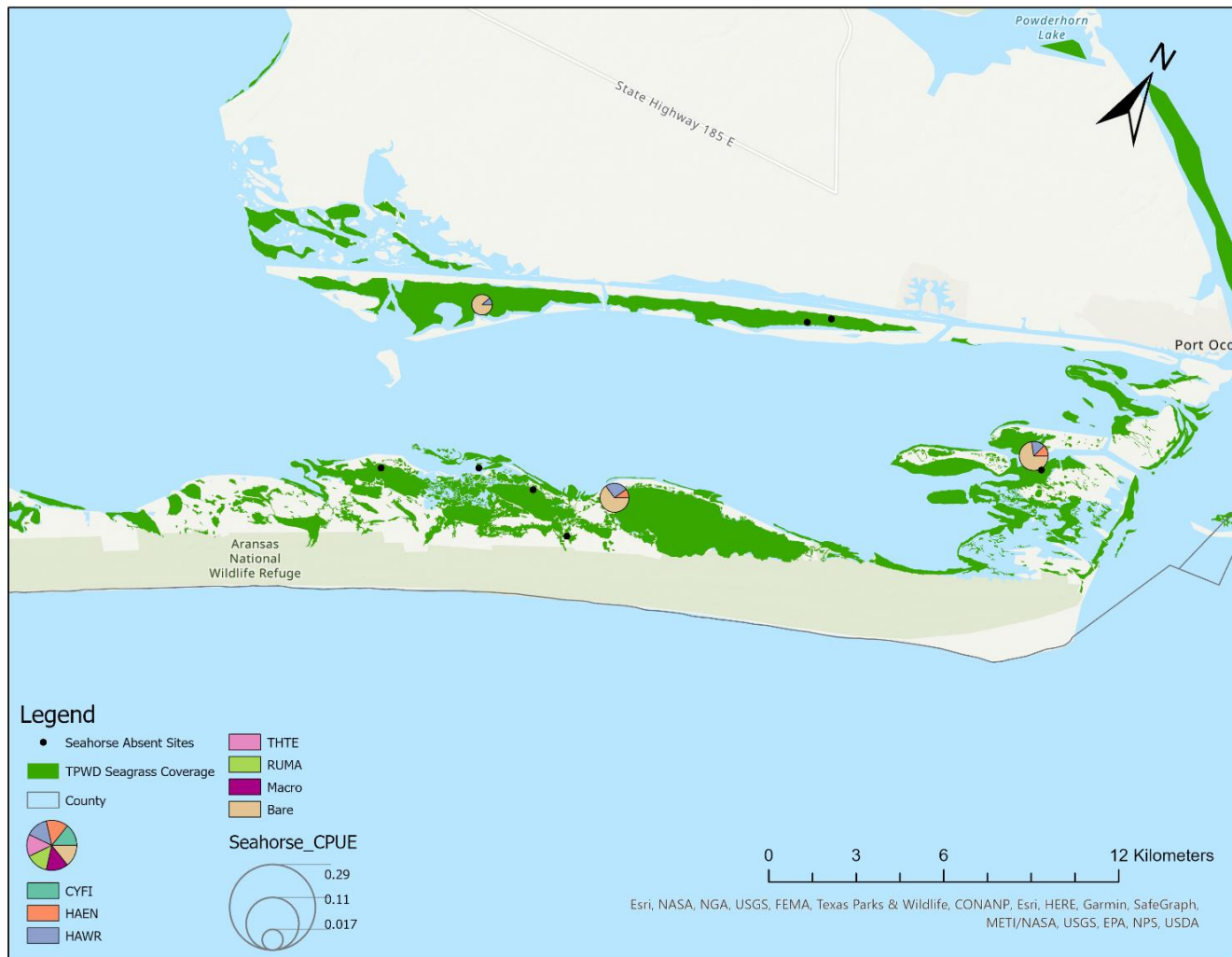


Figure 23. Seagrass species presence with pie piece size reflecting percent cover at each site in San Antonio Bay, specifically Espiritu Santo. CYFI = manatee grass, HAEN = star grass, HAWR = shoal grass, THTE = turtle grass, RUMA = widgeon grass, MACRO = macroalgae.

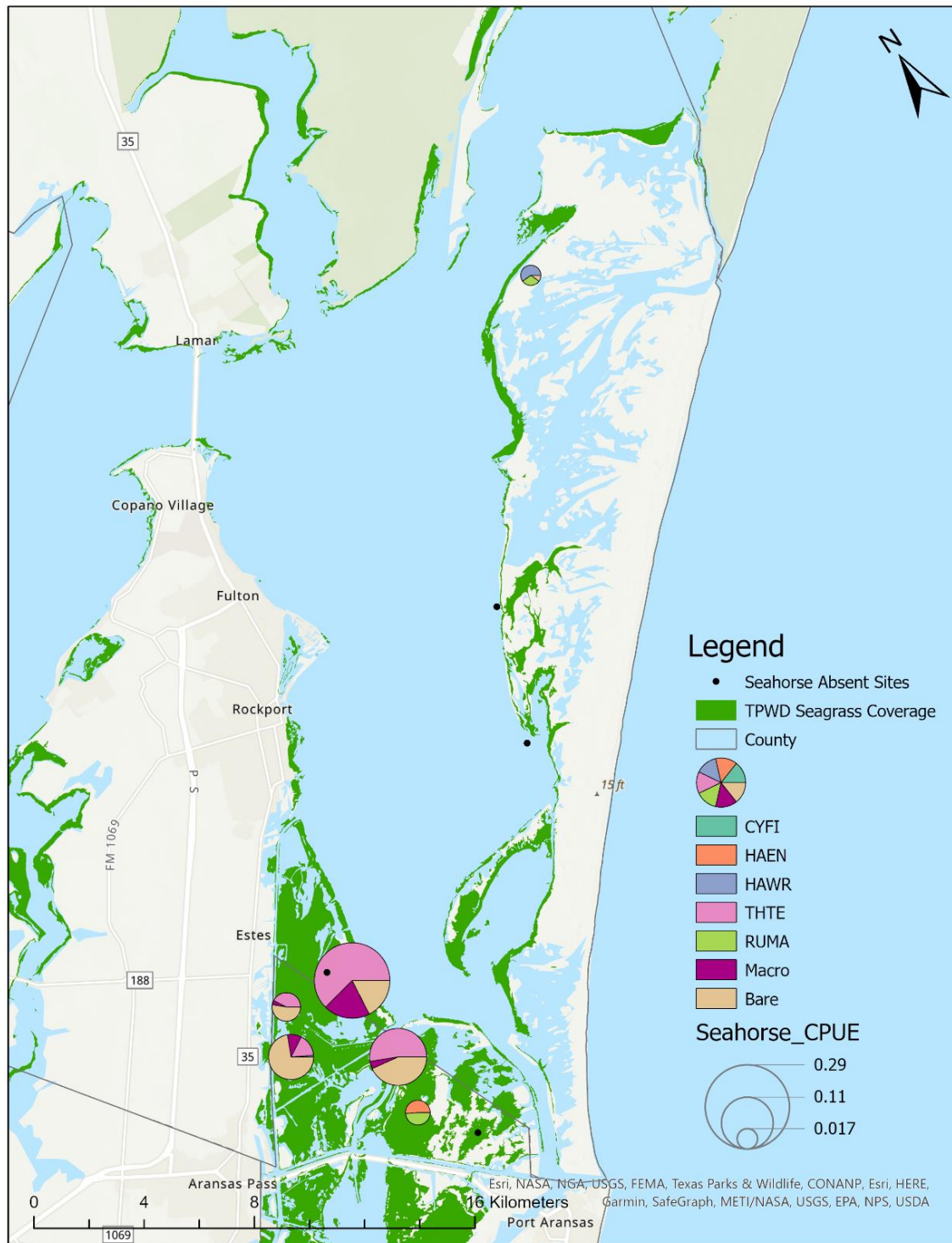


Figure 24. Seagrass species presence with pie piece size reflecting percent cover at each site in Aransas Bay. CYFI = manatee grass, HAEN = star grass, HAWR = shoal grass, THTE = turtle grass, RUMA = widgeon grass, MACRO = macroalgae.

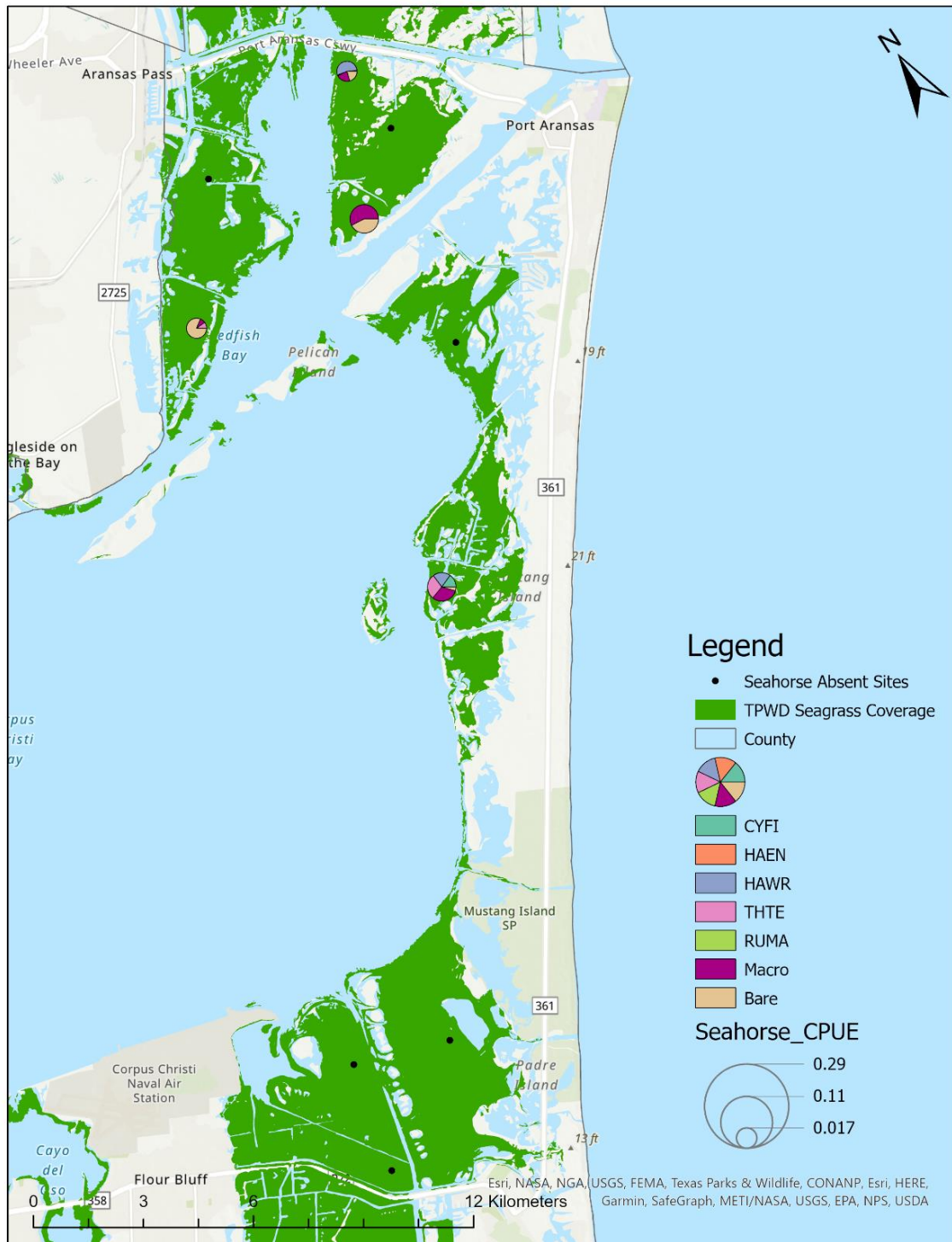


Figure 25. Seagrass species presence with pie piece size reflecting percent cover at each site in Corpus Christi Bay. CYFI = manatee grass, HAEN = star grass, HAWR = shoal grass, THTE = turtle grass, RUMA = widgeon grass, MACRO = macroalgae.

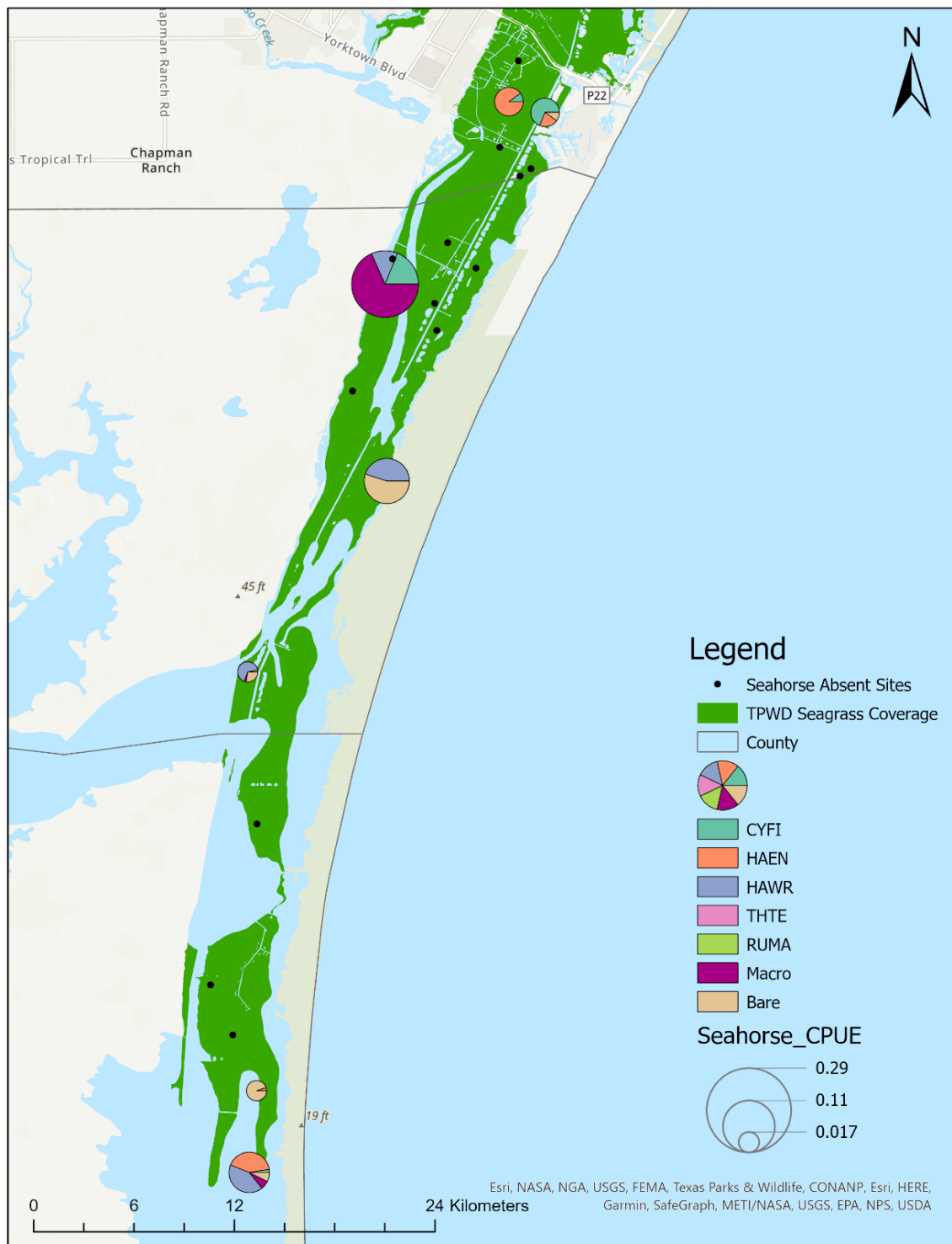


Figure 26. Seagrass species presence with pie piece size reflecting percent cover at each site in Upper Laguna Madre. CYFI = manatee grass, HAEN = star grass, HAWR = shoal grass, THTE = turtle grass, RUMA = widgeon grass, MACRO = macroalgae.

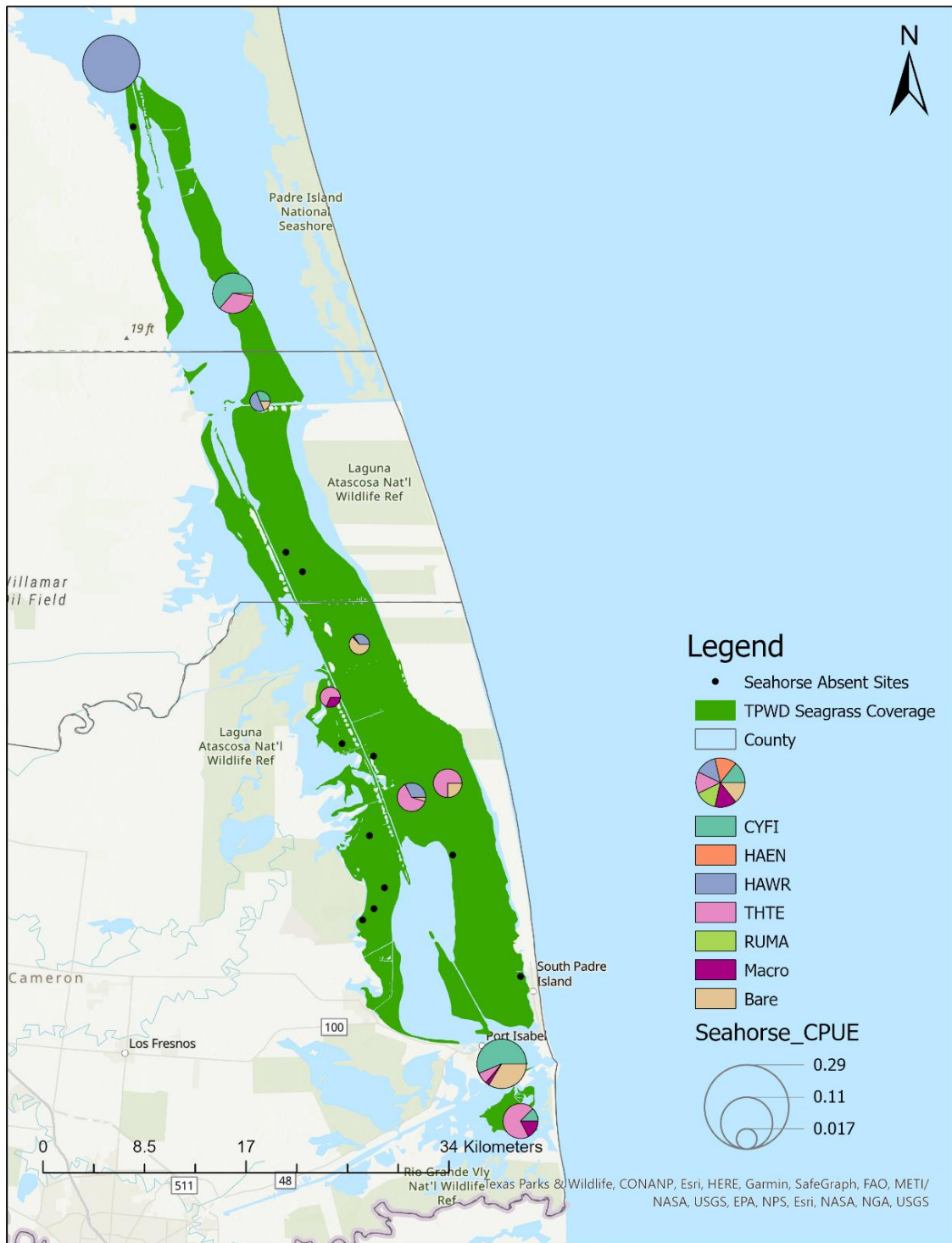


Figure 27. Seagrass species presence with pie piece size reflecting percent cover at each site in Lower Laguna Madre. CYFI = manatee grass, HAEN = star grass, HAWR = shoal grass, THTE = turtle grass, RUMA = widgeon grass, MACRO = macroalgae.

Nekton Community Structure

Throughout the course of the study, 72,630 organisms including smaller fishes and invertebrates were captured. Of these organisms, 3,753 were defined as fish, 68,792 as marine invertebrates (e.g. shrimp, crabs, and jellies), 70 were benthic invertebrates, 3 unidentifiable due to poor preservation, and 12 were of unconfirmed species due to their early larval state.

Of the fish catch, 34.52 % of individuals captured belonged to the Synathid family, including Gulf Pipefish (*Syngnathus scovelli*), Chain Pipefish (*Syngnathus louisianae*), and Dwarf Seahorse (*H. zosterae*) (5.69% of fish catch) (Table 8). The family with the second highest capture rate was Gobiidae (29.14%), consisting of multiple goby species as well as some unidentified larval gobies that made up about 5% of fish catch. The next most prevalent catch was the Pinfish (*Lagodon rhomboides*, family Sparidae, 9.85%) followed by and Gulf Toadfish (*Opsanus beta*, family Batrachoididae, 8.12%). The Scianidae family made up 5.48% of catch, including Spotted Seatrout (*Cynoscion nebulosus*), Silver Perch (*Bairdiella chysoura*), and some unidentified larval Scianidae species. Finally, Rainwater Killifish (*Lucania parva*, family Fundulidae) made up just over 5% of the fish catch throughout the study. CPUE of all of these species in all seven bay systems can be seen in Table 9. The remaining 7.91% of catch consisted of 15 species of fish from 12 families (Appendix B).

Of marine invertebrates, 42.81% captured were shrimp from the families Palaemonidae, Penaeidae, or were unidentified during the study (Table 10). The second most captured invertebrate, Ctenophores, were found at a much lower relative abundance (14.09%). Following are Mysid shrimp (*Taphromysis louisianae*, 12.69%), mud crabs (Panopeid sp., 10.88%), arrow shrimp (*Tozeuma carolinense*, 9.00%), and finally blue crabs (*Callinectes sapidus*, 6.14%). The CPUE of all of these species throughout the

sampled bays are found in Table 11. The remaining invertebrates captured belonged to seven families and seven species (Appendix B).

Table 8. The top six fish families, 13 species, and three genus' where individuals could not be identified to species that were captured and what percentage they made up of the overall fish catch (relative abundance).

Family	Relative Abundance %	Species	Relative Abundance%
Syngnathidae	34.52	<i>Syngnathus scovelli</i>	20.81
		<i>Syngnathus louisianae</i>	7.72
		<i>Hippocampus zosterae</i>	5.69
		<i>Syngnathus sp.</i>	0.30
Gobiidae	29.14	<i>Gobiosoma robustum</i>	15.94
		<i>Ctenogobius boleosoma</i>	6.19
		<i>Gobiidae sp.</i>	5.48
		<i>Gobiosoma bosc</i>	0.81
		<i>Microgobius gulosus</i>	0.51
		<i>Microgobius thalassinus</i>	0.20
Sparidae	9.85	<i>Lagodon rhomboides</i>	9.85
Batrachoididae	8.12	<i>Opsanus beta</i>	8.12
Scianidae	5.38	<i>Cynoscion nebulosus</i>	3.05
		<i>Bairdiella chysoura</i>	1.93
		<i>Sciaenidae sp.</i>	0.41
Fundulidae	5.08	<i>Lucania parva</i>	5.08
All other families	7.91	All other species	7.91

Table 9. The top 10 fish species captured in terms of CPUE, their common name, total CPUE, and CPUE in each bay system. G = Galveston Bay, M = Matagorda Bay, S = San Antonio Bay, A = Aransas Bay, C = Corpus Christi Bay, U = Upper Laguna Madre, L = Lower Laguna Madre.

Scientific Name	Common Name	G	M	S	A	C	U	L	Grand Total
<i>Syngnathus scovelli</i>	Gulf Pipefish	0.037	0.020	0.158	0.115	0.077	0.251	0.501	0.910
<i>Ctenogobius boleosoma</i>	Darter Goby	1.033	0.053	0.040	0.046	0.200	0.001	0.020	0.428
<i>Gobiosoma robustum</i>	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
<i>Lucania parva</i>	Rainwater Killifish	-	-	-	0.288	0.186	0.006	0.025	0.244
<i>Lagodon rhomboides</i>	Pinfish	-	-	0.158	0.048	0.163	0.021	0.024	0.221
<i>Opsanus beta</i>	Gulf Toadfish	-	0.003	0.005	0.075	0.095	0.047	0.025	0.149
<i>Syngnathus louisianae</i>	Chain Pipefish	0.017	0.010	0.090	0.092	0.016	0.018	0.029	0.145
<i>Eucinostomus melanopterus</i>	Flagfin Mojarra	-	-	-	0.004	0.028	-	0.088	0.103
<i>Hippocampus zosterae</i>	Dwarf Seahorse	-	0.003	0.008	0.038	0.011	0.017	0.023	0.065

Table 10. The top six invertebrate families, 7 species or groups, and unidentified individuals that were captured and what percentage they made up of the overall invertebrate catch (relative abundance).

Family	Species	Relative Abundance %
Palaemonidae	<i>Palaemonetes sp.</i>	23.42
Penaeidae	<i>Penaeid sp</i>	16.74
N/A	UID Shrimp <i>sp.</i>	2.65
Ctenophora	<i>Ctenophora</i>	14.09
Mysidae	<i>Taphromysis louisianae</i>	12.69
Panopeidae	<i>Panopeid sp</i>	10.88
Hippolytidae	<i>Tozeuma carolinense</i>	9.00
Portunidae	<i>Callinectes sapidus</i>	6.14
All other families	All other species	4.53

Table 11. The top 10 invertebrate species or groups captured in terms of CPUE, their common name, total CPUE, and CPUE in each bay system. G = Galveston Bay, M = Matagorda Bay, S = San Antonio Bay, A = Aransas Bay, C = Corpus Christi Bay, U = Upper Laguna Madre, L = Lower Laguna Madre

Scientific Name	Common Name	G	M	S	A	C	U	L	Grand Total
<i>Palaemonetes</i>	Palaemonetes	0.220	4.460	0.232	14.046	6.552	4.390	3.734	18.238
<i>Taphromysis louisianae</i>	Mysid Shrimp	2.183	3.303	10.618	19.740	0.298	0.076	2.271	17.717
<i>Ctenophora</i>	Comb Jellyfish	0.523	5.847	0.120	0.402	0.016	5.052	2.075	8.528
<i>Tozeuma carolinense</i>	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
<i>Callinectes sapidus</i>	Blue Crab	0.550	0.047	0.205	0.031	0.021	0.009	0.014	0.298
<i>Alpheus heterochaelis</i>	Bigclaw Snapping Shrimp	-	-	0.020	0.019	0.039	0.008	0.014	0.058
Salpidae	Salp	-	-	0.035	-	-	-	0.003	0.020
<i>Clibanarius vittatus</i>	Thinstripe Hermit Crab	0.007	-	0.003	0.006	0.002	-	0.002	0.008

Nekton catch Shannon H diversity, evenness, richness, and abundance in CPUE were analyzed to investigate significant relationships to Dwarf Seahorse presence/absence and CPUE. In terms of Dwarf Seahorse presence/absence, community evenness (p-value = 0.0004), richness (p-value < 0.0001), and abundance in CPUE (p-value = 0.0085) all had statistically significant relationships (Figure 28, Figure 29, and Figure 30). When analyzing these same metrics for Dwarf Seahorse CPUE, only community species evenness (p-value = 0.0017) and richness (p-value < 0.0001; Figure 31) had significant relationships with CPUE.

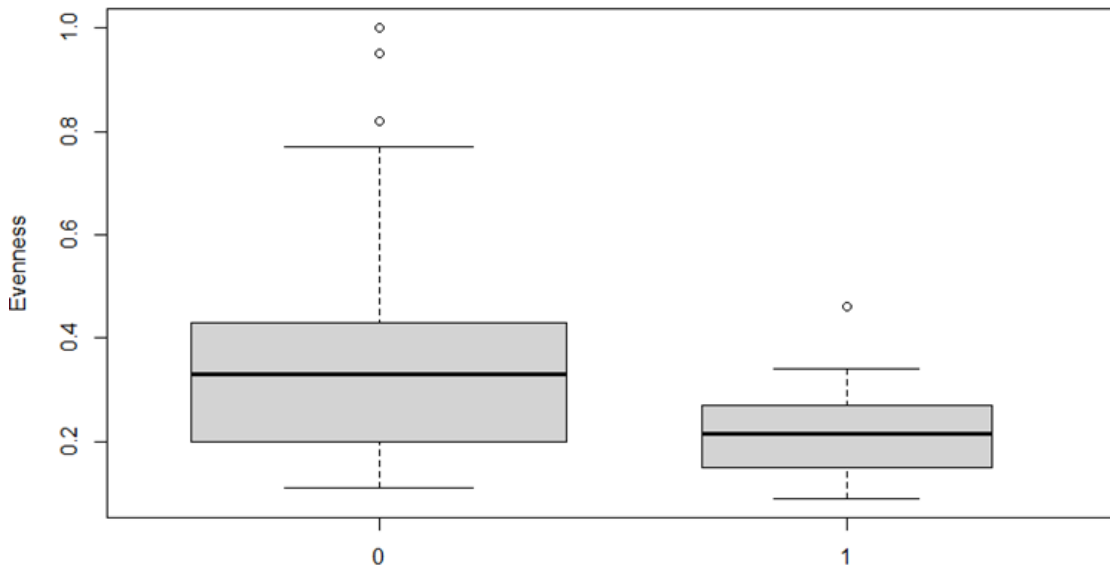


Figure 28. Absence presence of Dwarf Seahorse vs catch community evenness (p-value = 0.0004).

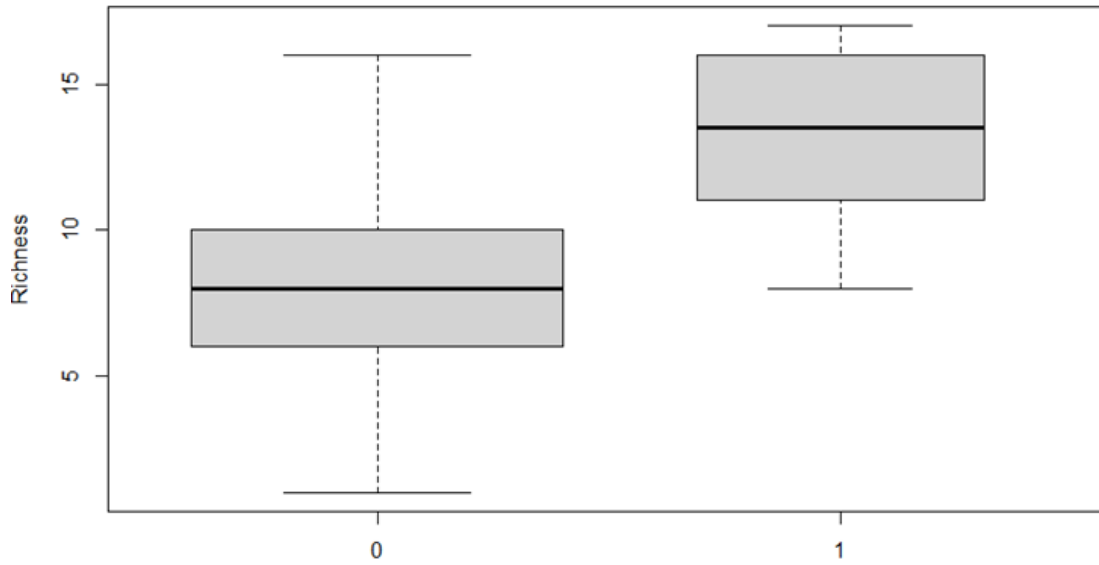


Figure 29. Absence/presence of Dwarf Seahorse vs catch community richness (p-value < 0.0001).

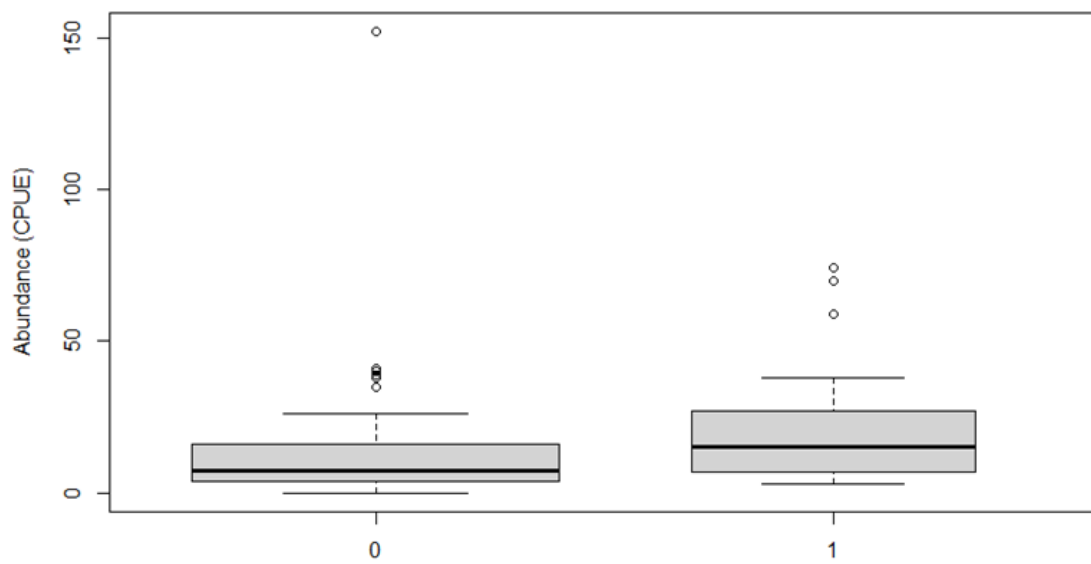


Figure 30. Absence/presence of Dwarf Seahorse vs catch community abundance per unit effort (CPUE) (p-value = 0.0085).

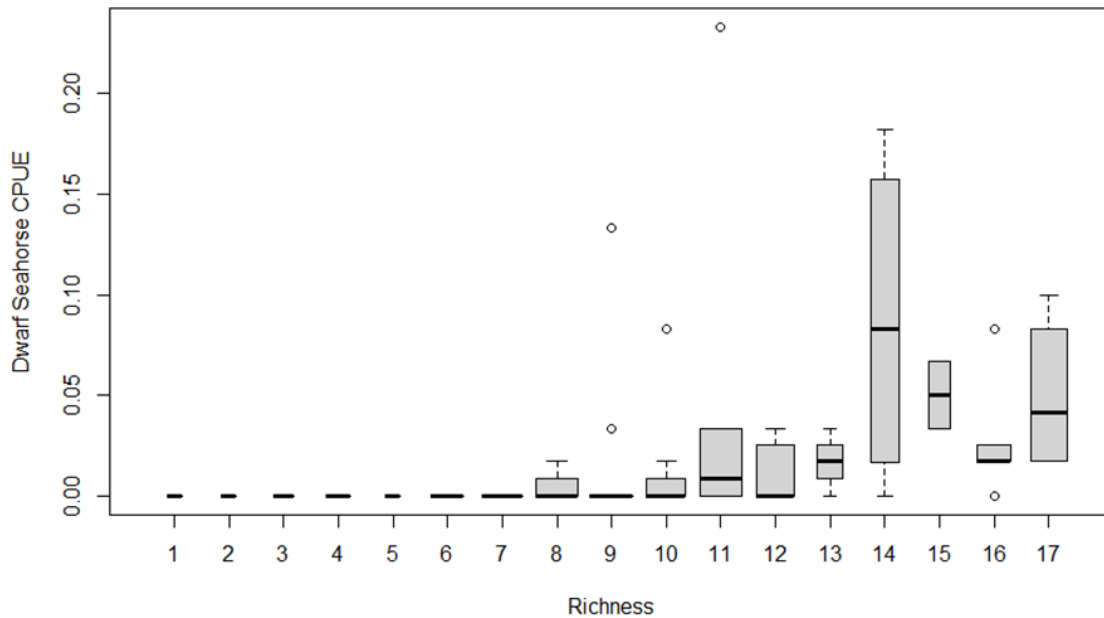


Figure 31. Catch community richness categories vs Dwarf Seahorse CPUE (p-value < 0.0001)

A non-metric MDS was also conducted in Primer7 to compare the similarities in the community at sites. This analysis was done using all catch, just nekton catch, and just fish catch data. The significance of this data is best visualized in Figure 32, comparing sites with Dwarf Seahorse present vs sites where Dwarf Seahorse were absent using the nekton community data. Based on an ANOSIM analysis (p – value = 0.0170) and as appears visually, there is a significant difference in community structure between sites with Dwarf Seahorses present and those without. Sites where Dwarf Seahorse were not detected are indicated with red triangles and sites where Dwarf Seahorse were detected are blue circles.

Figure 32 also shows the distribution of sites based on similarity of nekton community and six nekton species that had a Pearson’s correlation value of 25% or greater, significantly contributing to site placement on the nMDS plot. Sites with Dwarf Seahorse present plot towards the bottom center area of the figure. Other species that contribute to positioning a site in a similar downward direction include gulf toadfish,

rainwater killifish, and code goby (*Gobiosoma robustum*). Shrimp belonging to the family Palaemonidae are also in this same general downward direction. This suggest that these species could often be found in association with Dwarf Seahorse. Oppositely, the presence of blue crabs (*Callinectes sapidus*) in an area may indicate that it is not a place we would find Dwarf Seahorse, as this species places sites in the opposite direction on the plot.

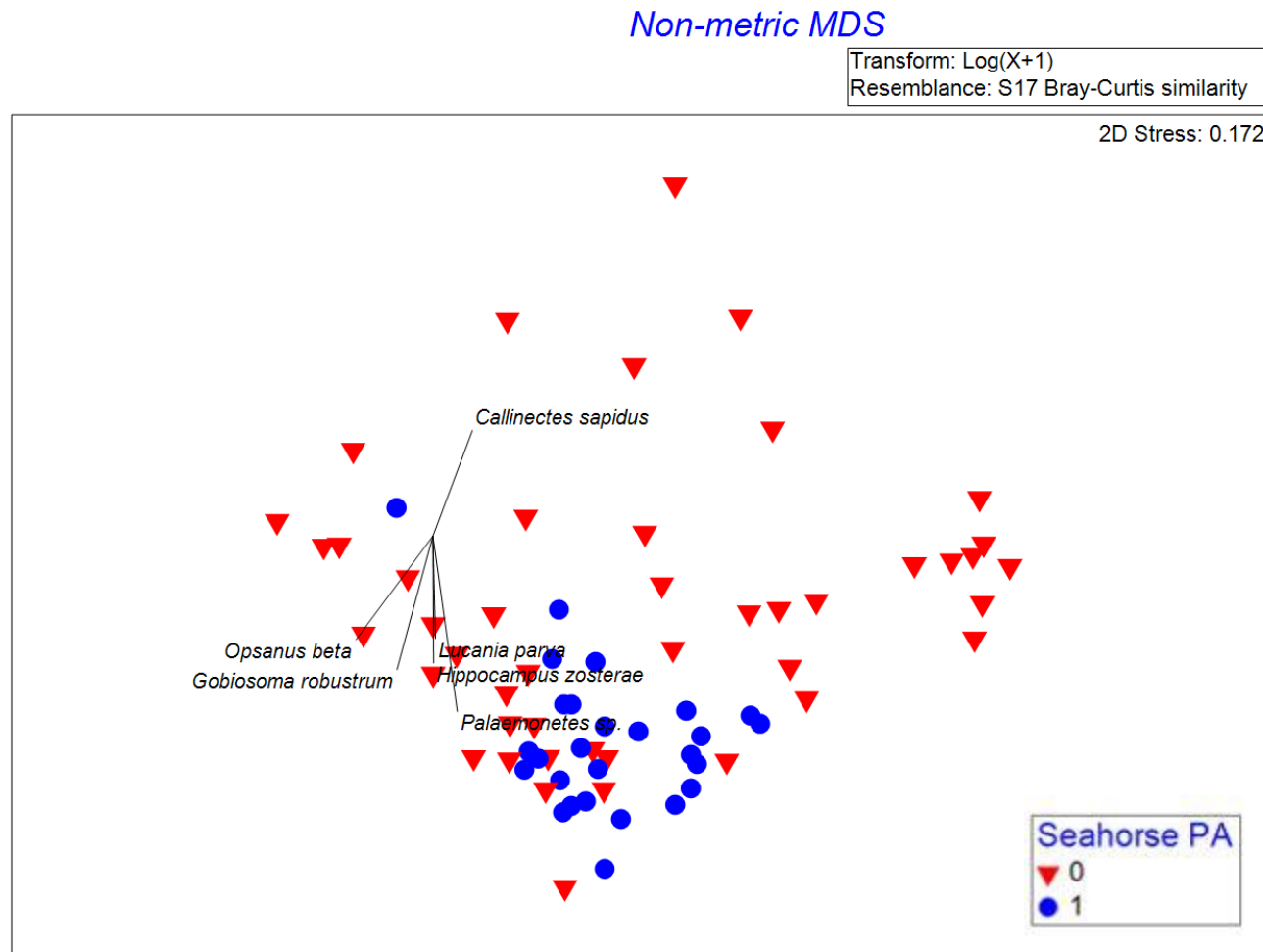


Figure 32. nMDS created in Primer of all Summer 2020 sample sites based on nekton community with blue circles representing sites with Dwarf Seahorse present and red triangles representing sites with Dwarf Seahorse absent. Species on figure have Pearson's correlation of over 0.25. From Oakley et al. 2022.

Preliminary Population Estimate

Preliminary population estimates were calculated based on the data acquired during this study for the Dwarf Seahorse populations in the three bay systems with the highest effort and number of sites per coverage of seagrass: Aransas Bay, Lower Laguna Madre, and Upper Laguna Madre. This analysis estimates a total of 11,785,694 Dwarf Seahorses in areas that have historical seagrass coverage based on data from TPWD and live at a depth of 1.22m or less. These two factors determined the area considered to be under sample parameters. Aransas, the bay system found to have the highest CPUE during the study has the lowest amount of seagrass coverage, 94.93km², out of the three bays based on TPWD seagrass layers (Table 12). Only 29.38km² of this area was seagrass covered area and had a depth between 0 and 1.22m. Using the covered area in meters and our CPUE, an estimate of 1,116,356 Dwarf Seahorse is yielded in Aransas Bay.

Despite having lower CPUE, Dwarf Seahorse population estimates are higher in both the Upper Laguna and Lower Laguna Madre due to their higher coverage of seagrass area and corresponding higher sample parameter area. The Upper Laguna Madre has 162.92km² of area that meets both of our sampling parameters. The Lower Laguna Madre has an even higher area that meets both our depth parameters at 343.46km². The population estimate of Dwarf Seahorse for the Upper Laguna Madre is over 2.5 times higher than that in Aransas Bay, at 2,769,686 Dwarf Seahorses. The estimated population in the Lower Laguna Madre based on data from this study is 7,899,652 Dwarf Seahorses, more than both of the other bays combined. This rough estimate projects almost 12 million Dwarf Seahorses in these three bay systems along the Texas coast at the time of sampling.

Table 12. Open water, seagrass coverage, and seagrass coverage with average depths of 0-1.22m within seagrass beds in kilometers squared (km²), CPUE of Dwarf Seahorse, and estimated Dwarf Seahorse population size by bay system

Bay system	Open Water (km²)	Coverage of Seagrass (km²)	Seagrass coverage with average depth < 1.22m (km²)	Percent Cover of Seagrass included in Population Estimate	Dwarf Seahorse CPUE	Estimated Population
Aransas	574.18	94.93	29.38	30.9	0.038	1,116,356
Upper Laguna	561.81	201.08	162.92	81.0	0.017	2,769,686
Lower Laguna	693.81	393.20	343.46	87.3	0.023	7,899,652

DISCUSSION

This novel study of the Dwarf Seahorse along the Texas coast identified significant associations between Dwarf Seahorse presence and the seagrass and nekton communities they were found among. It also provides an initial idea of the density and distribution of Dwarf Seahorse throughout 7 major bay systems along the Texas Coast. Finally, it includes a preliminary population estimate for Dwarf Seahorses that utilize seagrass beds in waters less than 1.22m in depth in the three bay systems with the highest CPUE.

No significant associations were found between recorded water quality metrics and Dwarf Seahorse presence or density in CPUE. There is not much known about what water conditions Dwarf Seahorse prefer in the wild. Literature suggests that Dwarf Seahorse persist in larger numbers in areas of lower turbidity (Erftemeijer and Lewis 2006). This aligns with our results, seeing an increase in Dwarf Seahorse CPUE in lower bay systems with lower average turbidity levels. It is not surprising that no significant associations were found between Dwarf Seahorse catch and water quality metrics. Each site was only sampled once for this study and all sampling took place during one summer season, meaning water quality conditions were relatively consistent.

These consistent conditions were disturbed during some of our sampling efforts. Some sites in the Upper Laguna Madre were sampled just three days after category 1 Hurricane Hanna hit the Padre Island area (Brown et al. 2021). The area was still experiencing higher water levels than usual, which may have influenced Dwarf Seahorse catch, vegetation analysis, and water quality. The depth of the water may have decreased the efficacy of sampling with the pushnet. The high winds and storm surge also could have displaced vegetation (both detached macroalgae and floating mats of seagrass) that contained Dwarf Seahorses. Alternatively, the storm also could have moved individuals

into our sampling area that are not typically residents there. We acknowledge that the Dwarf Seahorse catch in these areas sampled immediately after Hurricane Hanna may not be representative of typical densities and additional sampling is recommended for this bay system.

The reliance of Dwarf Seahorse on seagrass beds for protective habitat and the relationships between seagrasses and water quality are well known, as discussed in the introduction. Our results reinforce existing knowledge on the associations between Dwarf Seahorses and seagrass. Data in this study detected significant relationships between increased canopy height and biomass and Dwarf Seahorse presence. Due to the Dwarf Seahorse's small size, it may be thought that they would prefer environments with seagrass species with smaller blade sizes. However, we saw quite the opposite, with the only significant relationships between an individual seagrass species and Dwarf Seahorse being with turtle grass, the most robust seagrass species encountered throughout this study. Both the presence and percent cover of this seagrass species significantly influenced Dwarf Seahorse CPUE and presence, respectively.

While we cannot be certain why this relationship may exist, there are a few potential explanations. First, as mentioned, turtle grass has the highest biomass of all Texas seagrass species and a tall canopy height. These features have the potential to slow water velocity and currents in areas where the species densely populates, protecting slow moving organisms such as Dwarf Seahorse. Secondly, turtle grass is a climax species, with its presence meaning the ecosystem is stable and has been established in the area for substantial time (Congdon et al. 2019). Turtle grass colonizes an area slower than a pioneer species such as shoal grass would (Congdon et al. 2019). The longevity of the ecosystem suggests that the nekton community in this area is stable which is particularly important for small, inert organisms that are largely at the whim of their environment.

Finally, turtle grass may indicate the environmental health of the area. Low leaf density and biomass can indicate a seagrass bed highly disturbed by human activity, such as anchoring boats and prop scarring (Williams 1988). Low shoot density, leaf area, and biomass often suggest elevated water column nutrients such as soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen (DIN) (Tomasko and Lapointe 1991). This nutrient loading can originate from anthropogenic sources such as septic tanks or natural sources such as large bird rookeries. Overall, higher turtle grass percent cover suggests a preferred environment for Dwarf Seahorses to persist.

As mentioned, turtle grass is the most robust seagrass species in Texas in terms of height and biomass, but manatee grass is the second tallest and biomass heavy. The relationship between manatee grass presence and Dwarf Seahorse CPUE was not significant at a 95% confidence level but was close. The hypothesized explanations for the relationship between Dwarf Seahorse and turtle grass could also apply to manatee grass. The heartiness of these two species and their significant or almost significant relationships with Dwarf Seahorse presence and CPUE could also be the explanation for the associations between Dwarf Seahorse presence and seagrass average biomass and canopy height. Other seagrass species have a lower biomass (shoal grass, widgeon grass, star grass), so they often had little influence on the average biomass of site when heavier seagrass species were present. These same species also tend to have a shorter canopy height so their presence in areas where there were also taller seagrass species decreased the average canopy height in these sampled areas. It is possible that this influence of smaller species on average canopy height explains why it did not have a significant influence at 95% confidence.

Results from this study also indicate a potential relationship between the biodiversity observed at a site and Dwarf Seahorse presence and CPUE based on a couple

indices. First, Shannon H diversity of both the seagrass community and the associated nekton community were positively correlated with Dwarf Seahorse presence. It is possible we saw this association because sites with the presence of seagrass species that had significant influence on the Dwarf Seahorse population (turtle grass and maybe manatee grass) tended to be present when the highest number of seagrass species were detected. Interestingly, a Dwarf Seahorse was captured at a site with no detected seagrass coverage but with macroalgae only. Further study of the relationships between this species and seagrass is needed for more confidence in these detected relationships.

The relationship between Dwarf Seahorses and seagrass beds could be different in different areas along the Texas coast. As discussed earlier, environmental variables such as turbidity, salinity, and human activity levels influence the success and recovery of seagrass beds (Meyer et al. 1999, Longstaff and Dennison 1999, Short and Wyllie-Echevarria 1996, Dolbeth et al. 2007, Hill et al. 2014, Chislock et al. 2013). There are known salinity and turbidity gradients along the Texas coast (Bugica et al. 2020). Higher turbidity levels are seen in upper bays due to channelization, shoreline development, and wastewater and pollutant discharge, while there is lower salinity due to multiple freshwater inflows into these bay systems (Pulich and White 1991, Bugica et al. 2020). These factors contribute to losses of seagrass in these areas (Pulich and White 1991). The lower bays have lower coastal human influence levels and less freshwater inflow, potentially creating a better environment for seagrass proliferation. We saw the potential impacts of the differences in habitat quality reflected in our Dwarf Seahorse catch. Upper bays had both less species presence and percent cover, so Dwarf Seahorses may only be able to select for a more diverse seagrass bed habitat in lower bay areas where there are both more species present and higher coverage. It is also important to note that throughout sampling, we observed that the four randomly distributed quadrats used for

the seagrass quantification data did not always summarize all seagrass species present in the area. Due to this, we could have missed potential associations between other species and Dwarf Seahorses. This should be taken into account when planning future studies and further analysis is needed to better confirm if seagrass richness has a significant influence on the Dwarf Seahorse population.

The diversity of the residing nekton community also had positive correlation with Dwarf Seahorse presence along the Texas Coast. The nekton community assemblages at sites examined throughout this study were variable. At some sites, only ctenophores and other invertebrates were detected using the pushnet while at others there were hundreds of individuals from multiple species captured, while a few others had no catch at all. Pushnet sampling is a slow-moving gear type and was developed to capture smaller, slower moving species, such as the Dwarf Seahorse. Sites where Dwarf Seahorse were present had fairly similar community assemblages. Species that seemed to contribute to site placement in the same ordination as Dwarf Seahorse were pinfish, gulf toadfish, rainwater killifish, code goby, as well as *Palaemonetes* or grass shrimp species.

Nekton community evenness, richness, and abundance in CPUE were all positively correlated with Dwarf Seahorse presence, and community evenness and richness were also positively correlated with Dwarf Seahorse CPUE. There are many benefits to a diverse community assemblage. A robust abundance and variety of individuals from all trophic levels in an ecosystem is a necessary balance for all to thrive (Sala and Knowlton 2006). Areas of lower diversity are more susceptible to stress during disturbance and experience fewer ecosystem services (Sala and Knowlton 2006).

Some habitat and community associations and information on Dwarf Seahorse presence and density were discovered during the course of this study, but much is still not known about this species. Most studies on this species to date have been conducted in

Florida, but typically on captive populations in laboratory settings. Recently, a population viability analysis for the Dwarf Seahorse was conducted in Florida (Carlson et al. 2019). In the initial population size estimates, a conservative model was used and only the male population size was evaluated. Five areas were evaluated: Cedar Key (male initial population estimate: 15,388), Tampa Bay (128,457), Charlotte Harbor (359,703), Florida Bay (2,081,036), and North Indian River Lagoon (111,019). Many factors were taken into consideration while creating this population estimate equation, such as adult survival rates, natural mortality rates, fecundity, growth information, seagrass density, and multiple Dwarf Seahorse density estimations from varying sources. They used 5 and 10% quantile density estimates to yield population estimates, reflecting very conservative numbers.

My Texas estimates in Aransas Bay (1,116,356 individuals), Upper Laguna Madre (2,769,686) and Lower Laguna Madre (7,899,652) are much larger than Florida numbers. They include all capture of males, females, and juveniles however. Interestingly, males made up the smallest portion of our catch. We captured 14 males, 36 females, and 28 juveniles, meaning males made up just 17.94% of Dwarf Seahorse catch. Males comprised of 20% (4 of 20) of catch in Aransas Bay, 26.32% (5 of 19) in Upper Laguna Madre, and 12% (3 of 25) in Lower Laguna Madre. Sex ratios of Dwarf Seahorse catch in all bay systems can be seen in Appendix D. If we were to apply these percentages to our population estimates, we could project 223,271 males in Aransas Bay, 728,981 in Upper Laguna Madre, and 947,958 males in Lower Laguna Madre. These numbers are more similar to what is seen in the Florida estimations, but these analyses are difficult to compare due to the major differences in calculation methodologies.

It is likely that these Texas numbers are an underestimation of the actual population of Dwarf Seahorse. This data only included sampled seagrass beds in water

depths of 1.22m or less. It is very likely that this species exists in areas deeper than this. In addition, this depth data was taken from NOAA sources, not from the Dunton lab or our sampling. Because of this, there are some inconsistencies between the depths and locations of our sampled sites and the depths of areas used in this analysis. The NOAA data indicates that some of the areas we sampled are deeper than 1.22m. These inconsistencies can be seen in Figure 7, Figure 8, and Figure 9 in the Data Analysis section of the Methodology. In order to use our own depth data in this type of analysis, we would need to collect depths in many more locations across the bay systems. While there currently is not a better alternative due to data limitations, this does indicate some flaws in our estimates by excluding sampled areas. Also, in another study we conducted in the summer of 2021, we compared Dwarf Seahorse capture using different gear types (Oakley et al. 2022). We found that the pushnet was not the most effective way of capturing these species, but a throw trap was more efficient yielding a significantly higher CPUE. This means the CPUE values used to calculate these population estimates are likely lower than what is accurate.

Carlson et al. (2019) discuss the heavy reliance Dwarf Seahorses have on vulnerable nearshore seagrass habitats. This reliance means the success of Dwarf Seahorse populations are directly dependent on seagrass bed health. Unfortunately, these nearshore seagrass beds can be highly influenced by human activity. One of the primary causes of seagrass loss in Florida is harmful algal blooms (HABs) (Carlson et al. 2019). These blooms decrease water quality and can decrease photosynthesis levels due to shading, causing hypoxia and releasing toxins deadly to fish (Thyng et al. 2013). While HABs are not as prevalent in Texas, depending on coastal circulation trends for the year, HABs do appear along the Texas coast (Thyng et al. 2013). HABs can also be exacerbated by nutrient runoff from anthropogenic activity (Carlson et al. 2019). These

algal blooms, along with other human influence such as sediment suspension blocking light for photosynthesis and burying plants, physical damage from recreational boats and trawlers, and the perpetual threat of anthropogenic climate change and sea level rise pose additional threats to Dwarf Seahorses in Texas.

Though the population estimate values between Carleson et al. (2019) in Florida and ours are difficult to compare, it is a first step in evaluating the viability of this species throughout its range. While currently Dwarf Seahorse are not a candidate species for listing under the Endangered Species Act, this decision is based on data from only one location (Florida) throughout their historical range. This study was just the first to analyze this species and its habitat associations in Texas, much more information is needed to determine the status of the Dwarf Seahorse in this area. If we continue to consistently monitor this species throughout all areas of its range, a more comprehensive listing decision can be made in the future.

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APPENDIX A:

LOCATIONS OF ALL SAMPLED SITES

Appendix A. List of all sites in each bay system, their geographical location, Dwarf Seahorse catch, sampling effort, and CPUE

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

Appendix A. List of all sites in each bay system, their geographical location, Dwarf Seahorse catch, sampling effort, and CPUE

Bay System	Site ID	Latitude	Longitude	Dwarf Seahorse Catch	Effort (m ²)	Dwarf Seahorse CPUE
Corpus Christi	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
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

Appendix A. List of all sites in each bay system, their geographical location, Dwarf Seahorse catch, sampling effort, and CPUE

Bay System	Site ID	Latitude	Longitude	Dwarf Seahorse Catch	Effort (m ²)	Dwarf Seahorse CPUE
Lower Laguna Madre	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 B:

ALL SPECIES CPUE

Appendix B. Scientific and common names of all species captured throughout the study and their CPUE in each bay system. G = Galveston Bay, M = Matagorda Bay, S = San Antonio Bay, A = Aransas Bay, C = Corpus Christi Bay, U = Upper Laguna Madre, L = Lower Laguna Madre.

Scientific Name	Common Name	G	M	S	A	C	U	L	Grand Total
<i>Palaemonetes</i>	Palaemonetes	0.220	4.460	0.232	14.046	6.552	4.390	3.734	18.238
<i>Taphromysis louisianae</i>	Mysid Shrimp	2.183	3.303	10.618	19.740	0.298	0.076	2.271	17.717
<i>Ctenophora</i>	Comb Jellyfish	0.523	5.847	0.120	0.402	0.016	5.052	2.075	8.528
<i>Tozeuma carolinense</i>	Arrow Shrimp	0.007	0.007	0.003	0.960	0.708	0.237	2.103	3.077
<i>Panopeid</i>	Mud Crab	-	0.007	0.073	0.683	1.408	0.665	0.182	1.794
<i>Penaeid</i>	Penaeid sp	0.657	0.503	0.002	0.252	0.434	0.110	0.776	1.483
<i>Syngnathus scovelli</i>	Gulf Pipefish	0.037	0.020	0.158	0.115	0.077	0.251	0.501	0.910
<i>Ctenogobius boleosoma</i>	Darter Goby	1.033	0.053	0.040	0.046	0.200	0.001	0.020	0.428
<i>Gobiosoma robustum</i>	Code Goby	-	0.010	0.042	0.113	0.193	0.205	0.061	0.413
<i>Callinectes sapidus</i>	Blue Crab	0.550	0.047	0.205	0.031	0.021	0.009	0.014	0.298
<i>Gobiidae</i>	Gobiidae	-	0.273	0.335	0.015	0.002	0.009	-	0.252
<i>Lucania parva</i>	Rainwater Killifish	-	-	-	0.288	0.186	0.006	0.025	0.244
<i>Lagodon rhomboides</i>	Pinfish	-	-	0.158	0.048	0.163	0.021	0.024	0.221
<i>Opsanus beta</i>	Gulf Toadfish	-	0.003	0.005	0.075	0.095	0.047	0.025	0.149
<i>Syngnathus louisianae</i>	Chain Pipefish	0.017	0.010	0.090	0.092	0.016	0.018	0.029	0.145
<i>Eucinostomus melanopterus</i>	Flagfin Mojarra	-	-	-	0.004	0.028	-	0.088	0.103
<i>Hippocampus zosterae</i>	Dwarf Seahorse	-	0.003	0.008	0.038	0.011	0.017	0.023	0.065
<i>Alpheus heterochaelis</i>	Bigclaw Snapping Shrimp	-	-	0.020	0.019	0.039	0.008	0.014	0.058

Appendix B. Scientific and common names of all species captured throughout the study and their CPUE in each bay system. G = Galveston Bay, M = Matagorda Bay, S = San Antonio Bay, A = Aransas Bay, C = Corpus Christi Bay, U = Upper Laguna Madre, L = Lower Laguna Madre.

Scientific Name	Common Name	G	M	S	A	C	U	L	Grand Total
<i>Anchoa mitchilli</i>	Bay Anchovy	0.063	0.077	0.005	-	-	0.005	0.006	0.048
<i>Cynoscion nebulosus</i>	Spotted Seatrout	0.003	0.003	0.007	0.004	0.004	0.010	0.017	0.034
<i>Bairdiella chysoura</i>	Silver Perch	0.003	0.007	0.002	0.008	0.011	0.001	0.011	0.023
<i>Salpidae</i>	Salp	-	-	0.035	-	-	-	0.003	0.020
<i>Sciaenidae spp.</i>	Sciaenid	0.020	0.040	0.002	-	-	-	-	0.016
<i>Eucinostomus spp.</i>	Eucinostomus	-	-	-	0.002	-	-	0.010	0.011
<i>Brevoortia spp.</i>	Brevoortia	-	-	-	-	-	0.002	0.008	0.010
<i>Gobiosoma bosc</i>	Naked Goby	-	0.007	0.007	0.004	0.002	-	0.003	0.010
<i>Clibanarius vittatus</i>	Thinstripe Hermit Crab	0.007	-	0.003	0.006	0.002	-	0.002	0.008
<i>Clupeidae</i>	Clupeidae	-	-	-	-	-	-	0.008	0.008
<i>Myrophis punctatus</i>	Speckled Worm Eel	0.003	-	0.002	-	0.007	-	0.003	0.008
<i>Menidia beryllina</i>	Inland Silverside	0.007	-	-	0.002	-	0.002	0.002	0.006
<i>Symphurus plagiura</i>	Blackcheek tonguefish	0.013	-	0.002	-	0.004	-	-	0.006
<i>Libinia spp.</i>	Spider Crab	-	-	0.002	-	0.002	0.003	0.001	0.005
<i>Microgobius gulosus</i>	Clown Goby	-	-	0.010	-	-	-	-	0.005
<i>Achirus lineatus</i>	Lined Sole	-	-	0.002	-	0.002	-	0.001	0.003
<i>Syngnathus sp.</i>	Pipefish	-	-	-	0.002	-	0.001	0.001	0.003
<i>Paralichthys lethostigma</i>	Southern Flounder	-	-	-	-	-	0.001	0.001	0.002
<i>Ascidian</i>	Sea Squirt	0.003	0.003	-	-	-	-	-	0.002
<i>Brevoortia patronus</i>	Gulf Menhaden	-	-	-	-	-	-	0.002	0.002
<i>Microgobius thalassinus</i>	Green Goby	-	-	0.002	-	-	0.001	-	0.002
<i>Parablennius marmoreus</i>	Seaweed Blenny	-	-	-	-	-	0.001	-	0.001
<i>Cyprinodon variegatus</i>	Sheepshead Minnow	-	-	-	0.002	-	-	-	0.001

Appendix B. Scientific and common names of all species captured throughout the study and their CPUE in each bay system. G = Galveston Bay, M = Matagorda Bay, S = San Antonio Bay, A = Aransas Bay, C = Corpus Christi Bay, U = Upper Laguna Madre, L = Lower Laguna Madre.

Scientific Name	Common Name	G	M	S	A	C	U	L	Grand Total
<i>Eucinostomus spp.</i>	Eucinostomus	-	0.003	-	-	-	-	-	0.001
<i>Orthopristis chrysoptera</i>	Pigfish	-	-	-	-	0.002	-	-	0.001
<i>Lactophrys triqueter</i>	Smooth Trunkfish	-	-	-	-	-	-	0.001	0.001
<i>Porcellanidae spp</i>	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 C:

DWARF SEAHORSE CATCH, MEASUREMENTS, AND SEX

Appendix C. The catch location, height (mm), tail length (mm), snout length (mm), weight (mg), and sex of each Dwarf Seahorse.

Indiv ID	Site Number	Date	Gear	Rep	Height (mm)	Tail Length (mm)	Snout Length (mm)	Weight (mg)	Sex (M/F)
1	SABAY64	6/23/2020	PN	2	11.48	6.7	1.13	5.1	J
2	SABAY64	6/23/2020	PN	5	30.29	18.01	2.6	141.4	F
3	SABAY29	6/24/2020	PN	2	12.88	7.62	1.35	6.9	J
4	SABAY29	6/24/2020	PN	6	21.56	12.84	1.88	57	M
5	SABAY47	6/25/2020	PN	4	15.86	9.3	1.15	19.9	F
6	NERR28	6/30/2020	PN	2	17.83	11.25	1.4	28.2	M
7	NERR28	6/30/2020	PN	2	22.9	13.45	2.21	56.6	F
8	NERR28	6/30/2020	PN	3	10.84	6.61	0.85	4	J
9	NERR28	6/30/2020	PN	5	21.02	13.5	1.88	63.6	F
10	NERR28	6/30/2020	PN	5	7.98	5.16	0.57	2	J
11	NERR32	6/30/2020	PN	3	15.53	8.64	1.19	15.6	F
12	NERR32	6/30/2020	PN	4	14.15	8.73	1.38	14.3	J
13	NERR31	7/7/2020	PN	1	27.85	17.9	1.86	104.2	M
14	NERR31	7/7/2020	PN	1	25.05	16.07	2.18	101.2	M
15	NERR31	7/7/2020	PN	1	25.84	15.77	2.71	101.1	F
16	NERR31	7/7/2020	PN	3	21.17	12.77	1.85	43.4	M
17	NERR31	7/7/2020	PN	3	26.62	16.23	2.05	99.5	F
18	NERR31	7/7/2020	PN	3	25.07	14.1	1.93	89.1	F
19	NERR31	7/7/2020	PN	3	28.61	16.07	2.62	129.5	F
20	NERR5	7/8/2020	PN	3	29.47	17.59	2.26	143.9	F

Appendix C. The catch location, height (mm), tail length (mm), snout length (mm), weight (mg), and sex of each Dwarf Seahorse.

Indiv ID	Site Number	Date	Gear	Rep	Height (mm)	Tail Length (mm)	Snout Length (mm)	Weight (mg)	Sex (M/F)
21	NERR18	7/8/2020	PN	1	22.14	12.99	1.67	58.2	F
22	NERR18	7/8/2020	PN	2	28.03	16.04	2.58	109.9	F
23	NERR18	7/8/2020	PN	2	28.6	17.03	2.16	108.8	F
24	NERR18	7/8/2020	PN	2	-	-	-	-	
25	NERR55	7/9/2020	PN	6	16.76	9.63	1.43	17.2	F
26	CCBAY34	7/14/2020	PN	6	25.38	15.05	2.17	65.3	F
27	CCBAY25	7/15/2020	PN	1	19.2	10.95	1.75	35.7	F
28	CCBAY2	7/15/2020	PN	3	15.88	9.3	1.84	15.9	J
29	CCBAY3	7/16/2020	PN	4	18.26	10.52	1.67	30.8	F
30	ULM6	7/21/2020	PN	2	26.12	15.99	2.34	85	M
31	ULM6	7/21/2020	PN	4	17.06	10.09	1.55	11.6	F
32	ULM6	7/21/2020	PN	4	21.83	13.52	2.21	55.4	M
33	ULM6	7/21/2020	PN	5	20.66	12.59	1.64	29.5	F
34	ULM16	7/21/2020	PN	5	21.52	13.06	2.12	65.1	F
35	ULM46	7/22/2020	PN	2	23.37	13.83	2.22	46.5	F
36	ULM130	7/28/2020	PN		18.03	10.93	1.9	20.5	F
37	ULM130	7/28/2020	PN		23.63	14.63	2.23	48.5	F
38	ULM133	7/29/2020	PN	2	8.45	4.94	0.999	0.5	J
39	ULM133	7/29/2020	PN	4	6.52	4.12	0.659	0.3	J
40	ULM91	8/4/2020	PN	1	25.59	14.74	2.62	79.1	F
41	ULM91	8/4/2020	PN	1	10.05	6.39	0.931	1.8	J
42	ULM91	8/4/2020	PN	1	21.67	13.44	1.82	32	M
43	ULM91	8/4/2020	PN	2	16.21	9.79	1.21	13.5	F
44	ULM61	8/5/2020	PN	1	10.11	6.04	0.878	1.9	J

Appendix C. The catch location, height (mm), tail length (mm), snout length (mm), weight (mg), and sex of each Dwarf Seahorse.

Indiv ID	Site Number	Date	Gear	Rep	Height (mm)	Tail Length (mm)	Snout Length (mm)	Weight (mg)	Sex (M/F)
45	ULM61	8/5/2020	PN	1	20.37	12.53	1.89	31.1	M
46	ULM61	8/5/2020	PN	4	18.23	10.98	1.66	22.3	M
47	ULM61	8/5/2020	PN	4	20.6	12.09	1.69	36.5	F
48	ULM61	8/5/2020	PN	5	19.5	11.44	1.7	25.2	F
49	LLM6	8/11/2020	PN	2	12.57	7.54	1.05	5.7	J
50	LLM6	8/11/2020	PN	3	12.4	7.11	1.01	6.5	J
51	LLM6	8/11/2020	PN	4	11.71	7.04	1.13	6.7	J
52	LLM6	8/11/2020	PN	4	22.19	12.95	2.13	55	F
53	LLM6	8/11/2020	PN	5	22.52	13.77	2.18	40.2	F
54	LLM6	8/11/2020	PN	6	7.42	4.95	0.834	0.9	J
55	LLM285	8/12/2020	PN	1	6.91	4.36	0.594	0.7	J
56	LLM285	8/12/2020	PN	2	8.14	4.91	0.799	0.8	J
57	LLM285	8/12/2020	PN	2	15.38	9	1.64	14.9	J
58	LLM285	8/12/2020	PN	3	6.56	4.6	0.885	1.4	J
59	LLM285	8/12/2020	PN	4	25.94	14.78	2.63	96.9	F
60	LLM285	8/12/2020	PN	4	17.08	10.37	1.47	19.8	M
61	LLM257	8/12/2020	PN	3	22.28	13.1	1.64	51.4	F
62	LLM257	8/12/2020	PN	3	9.35	5.72	0.903	3.9	J
63	LLM257	8/12/2020	PN	4	8.22	4.95	0.734	2.4	J
64	LLM257	8/12/2020	PN	6	18.68	10.71	1.65	26.2	F
65	LLM238	8/13/2020	PN	1	21.05	12.23	1.94	47.1	F
66	LLM118	8/18/2020	PN	5	28.4	18.83	2.54	90.1	M
67	LLM118	8/18/2020	PN	5	25.11	16.4	2.25	77.2	M
68	LLM109	8/18/2020	PN	5	12.49	8.21	1.3	5.6	J

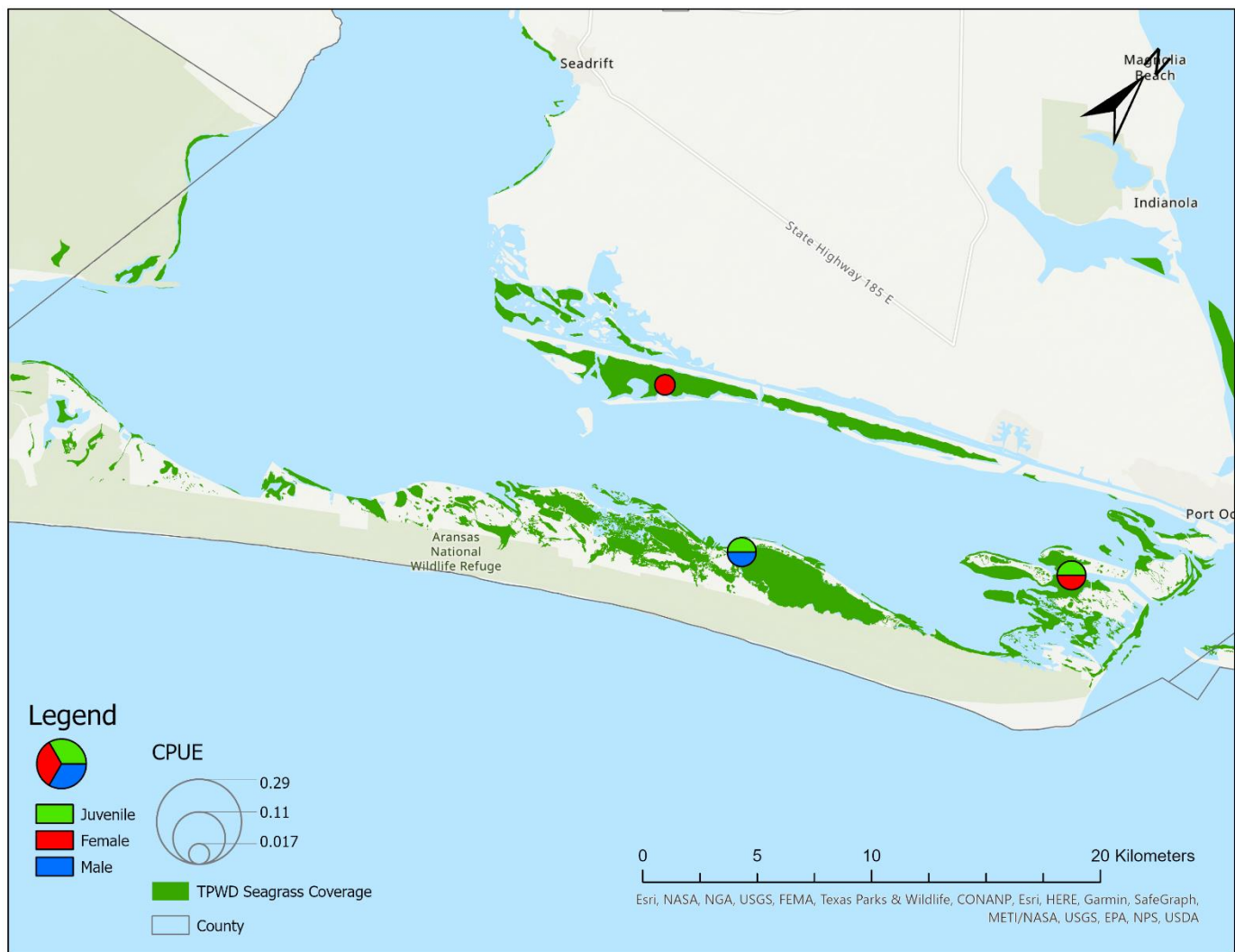
Appendix C. The catch location, height (mm), tail length (mm), snout length (mm), weight (mg), and sex of each Dwarf Seahorse.

Indiv ID	Site Number	Date	Gear	Rep	Height (mm)	Tail Length (mm)	Snout Length (mm)	Weight (mg)	Sex (M/F)
69	LLM109	8/18/2020	PN	5	14.34	8.54	1.26	11.9	J
70	LLM170	8/19/2020	PN	1	25.61	15.58	2.42	86.4	F
71	LLM151	8/19/2020	PN	6	23.1	13.39	2.13	84.3	F
72	LLM3	8/20/2020	PN	3	26.66	15.71	2.28	95.8	F
73	LLM3	8/20/2020	PN	5	12.8	7.4	1.17	7.7	J
74	CCBAY34	7/14/2020	PN	6	12.1	7.61	1.35	7.1	J
75	CCBAY2	7/15/2020	PN	6	14.07	8.65	1.44	8	J
76	LLM285	8/12/2020	PN	2	7.08	4.38	0.861	0.7	J
77	LLM285	8/12/2020	PN	6	6.62	4.07	0.711	1.2	J
78	LLM3	8/20/2020	PN	3	6.77	4.17	0.748	1.5	J
79	MAT7	6/17/2020	PN	6	20.63	12.65	1.78	19.8	M

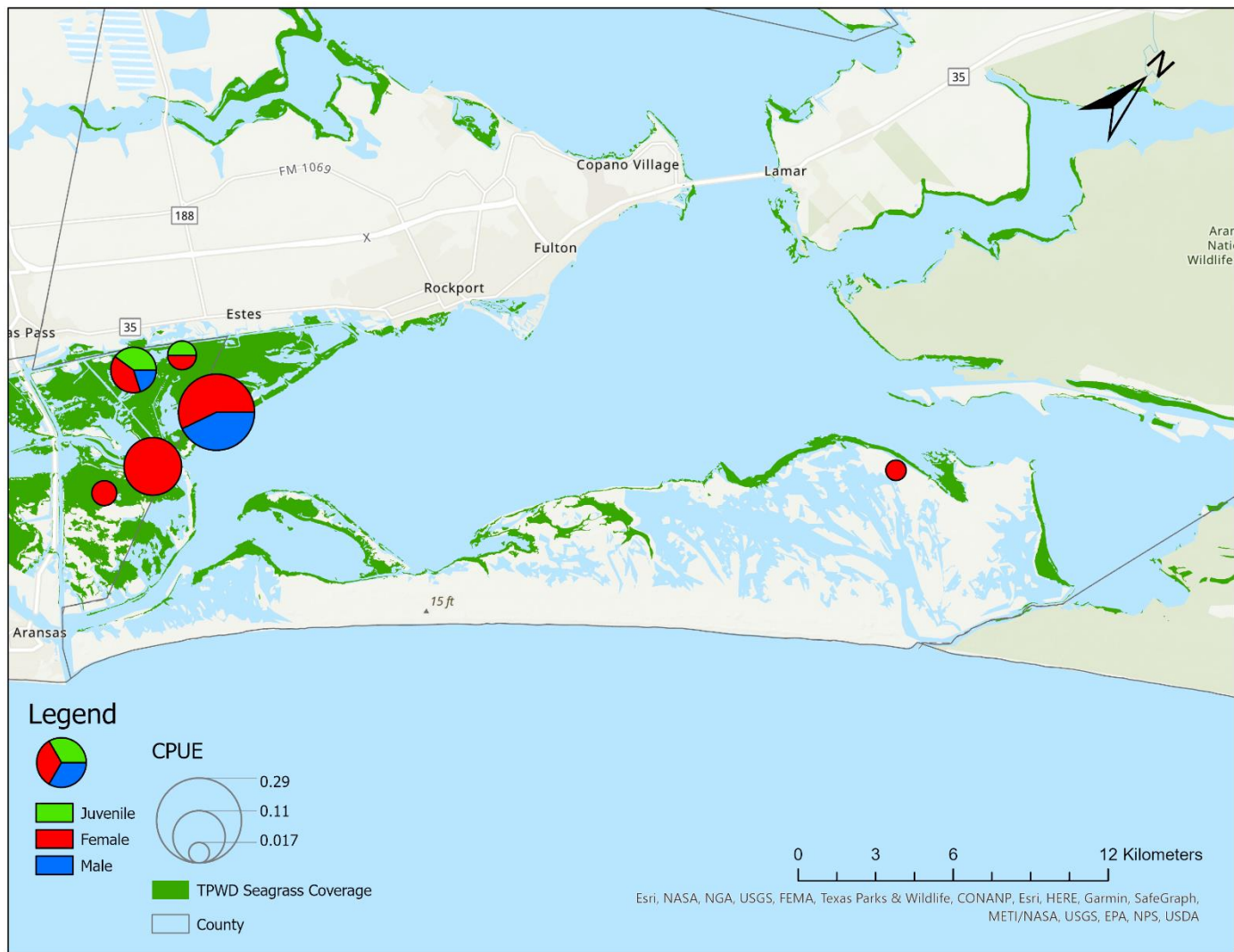
APPENDIX D:
DWARF SEAHORSE SEX DISTRIBUTION IN EACH BAY



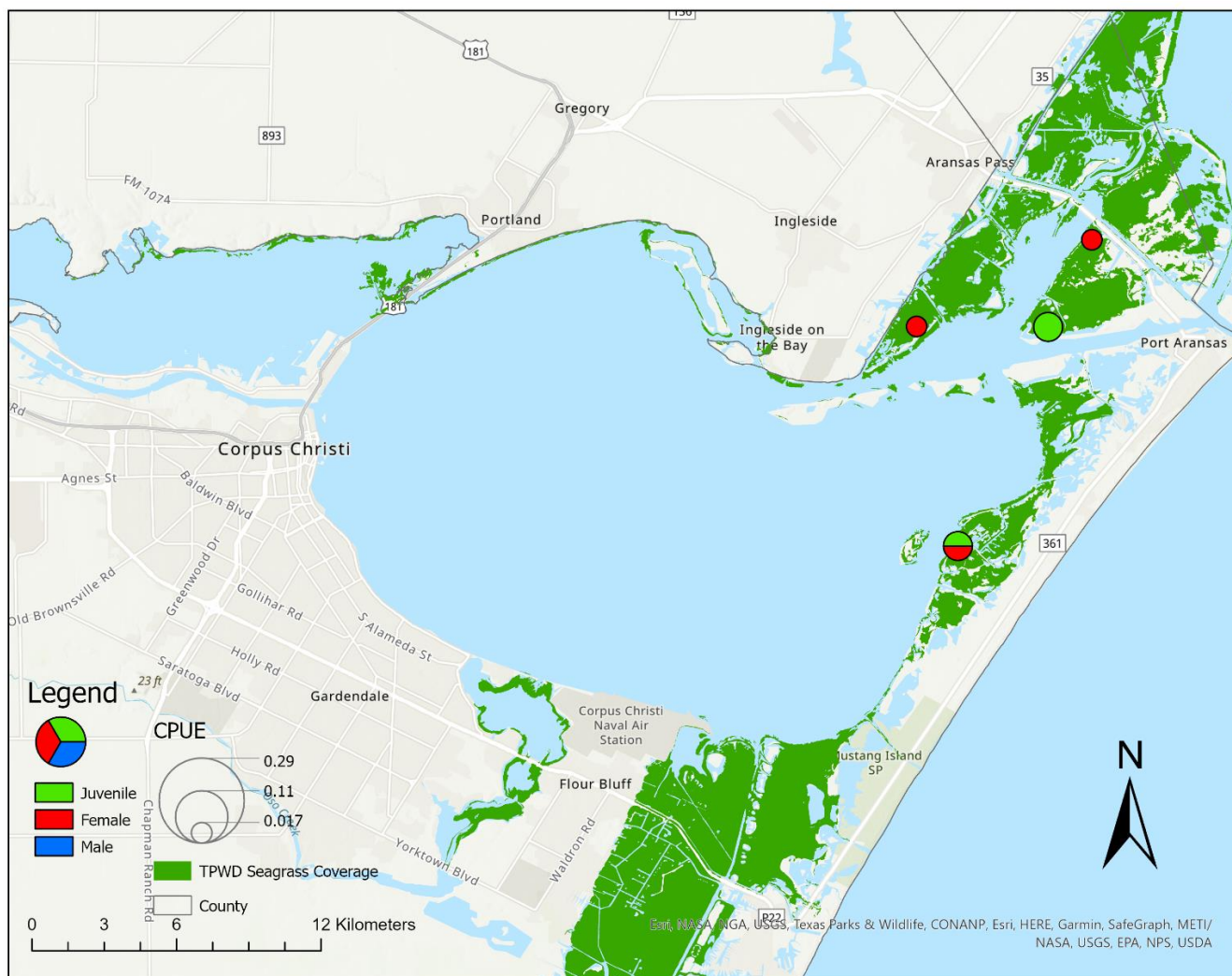
Appendix D-1. Sex distribution of captured Dwarf Seahorses at each site in Matagorda Bay. Size of the circle indicates CPUE of Dwarf Seahorse at each site.



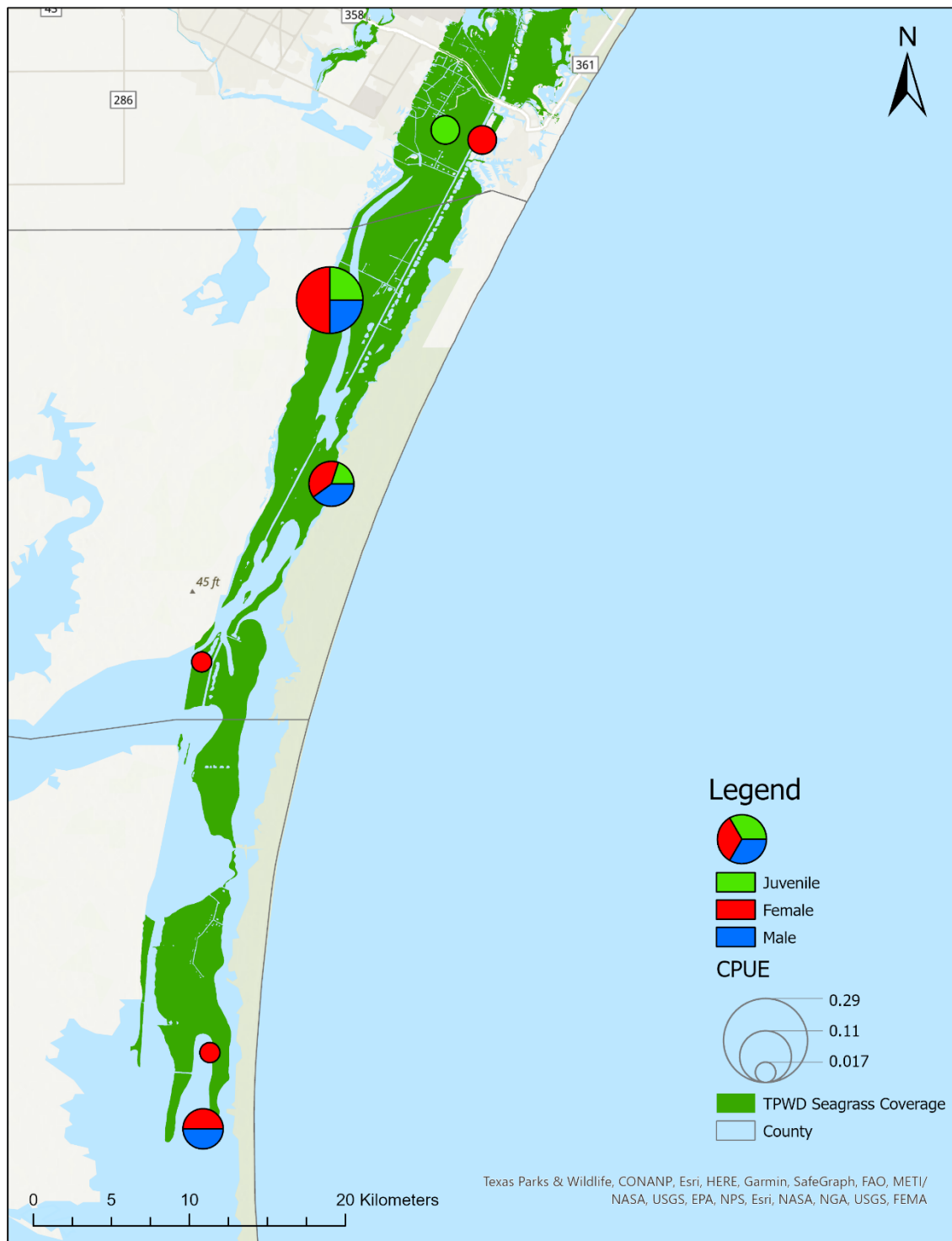
Appendix D-2. Sex distribution of captured Dwarf Seahorses at each site in San Antonio Bay. Size of the circle indicates CPUE of Dwarf Seahorse at each site.



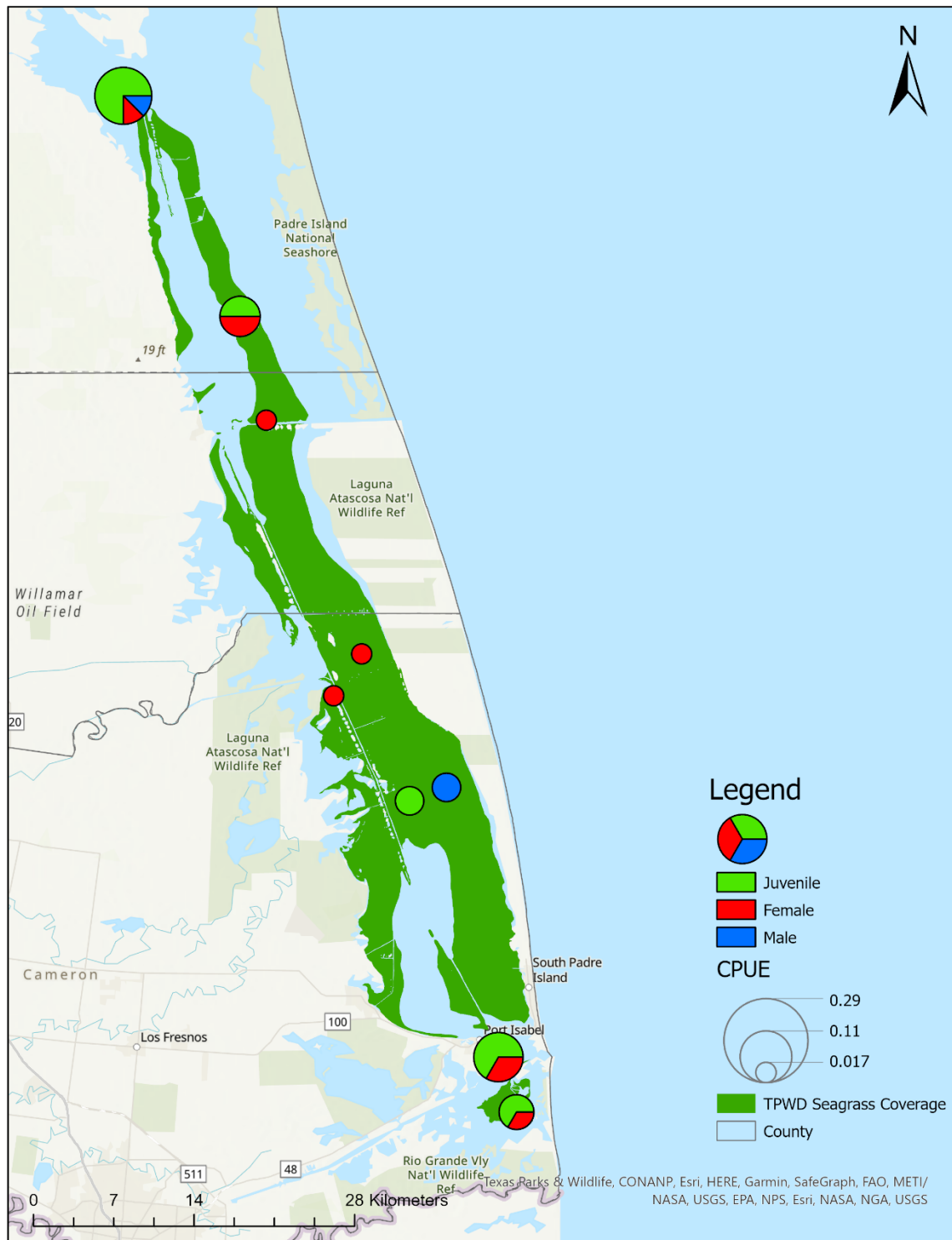
Appendix D-3. Sex distribution of captured Dwarf Seahorses at each site in Aransas Bay. Size of the circle indicates CPUE of Dwarf Seahorse at each site.



Appendix D-4. Sex distribution of captured Dwarf Seahorses at each site in Corpus Christi Bay. Size of the circle indicates CPUE of Dwarf Seahorse at each site.



Appendix D-5. Sex distribution of captured Dwarf Seahorses at each site in Upper Laguna Madre. Size of the circle indicates CPUE of Dwarf Seahorse at each site.



Appendix D-6. Sex distribution of captured Dwarf Seahorses at each site in Lower Laguna Madre. Size of the circle indicates CPUE of Dwarf Seahorse at each site.