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CHANGES IN THE MORPHOLOGY OF WIDGEON GRASS (RUPPIA MARITIMA) WITH THE ONSET OF REPRODUCTION AND IMPACTS ON FISH ASSEMBLAGES AT THE CHANDELEUR ISLANDS, LA

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CHANGES IN THE MORPHOLOGY OF WIDGEON GRASS (RUPPIA MARITIMA) WITH THE ONSET OF REPRODUCTION AND IMPACTS ON FISH ASSEMBLAGES AT THE CHANDELEUR ISLANDS, LA

by

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ABSTRACT

Seagrasses are important submerged coastal habitats that support nearshore communities. *Ruppia maritima* (widgeon grass) is a widespread species whose leaves branch extensively when reproductive. The goal of this study was to understand use of reproductive and non-reproductive *R. maritima* by fishes, recognizing the morphological change undergone by the plant when flowering. During the peak reproductive season in August and September 2021, *R. maritima* meadows at the Chandeleur Islands, LA were sampled to describe the spatial distribution and morphology of reproductive plants and investigate habitat use by fish assemblages. We assessed spatial trends in *R. maritima* presence and occurrence of reproduction and evaluated differences in shoot morphology using the metrics of longest leaf length, surface area, number of branches, and root to shoot ratios. We calculated density, Shannon diversity, and species richness to describe fish assemblages in reproductive and non-reproductive meadows. Additionally, general additive models were used to predict drivers of fish assemblage metrics. Results indicate that *R. maritima* was distributed along the entire length of North Chandeleur Island, but reproductive plants were located in the central, protected portion of the island. Reproductive plants were more morphologically complex than non-reproductive plants, but this did not drive fish assemblages. Rather, fish abundance was related to *R. maritima* biomass. This study provides information on patterns and drivers of habitat use by fish in *R. maritima*-dominated ecosystems that can be used to inform management and restoration.

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DEDICATION

To my family and friends who never leave my side through all the chaos of life. Also, I would be remiss if I failed to acknowledge my dog Cash who has been offering critical reviews as I write this document.

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1.1 Introduction

1.1.1 Seagrasses

Seagrasses are submerged angiosperms that inhabit marine environments, and include over 60 species across 13 genera (Larkum et al., 2006). The majority of seagrass genera occur at temperate and tropical latitudes in the northern and southern hemispheres (Larkum et al., 2006), and species' distributions are determined by taxon-specific light, nutrient, salinity, temperature, pH, and substrate requirements (Hemminga & Duarte, 2000; Larkum et al., 2006; McKenzie et al., 2016).

Seagrass expansion can occur through asexual or sexual processes. Clonal reproduction (asexual propagation) through fragmentation or rhizome extension is considered the primary mechanism for meadow formation and maintenance (Olesen et al., 2004); however, seagrasses also have the capacity for sexual reproduction, with species either being monoecious (i.e., both male and female organs on a single plant) or dioecious (i.e., separate male and female plants) (Larkum et al., 2006). A majority of seagrasses (42 species) produce dormant seeds that create seed reserves in the sediment near the parent plants that provide a buffer against disturbance (Darnell et al., 2021); in highly disturbed environments, dormant seeds are essential to the recolonization process (Larkum et al., 2006; Olesen et al., 2004).

1.1.2 Seagrass as Habitat

Seagrasses are essential submerged coastal habitats that support nearshore communities around the globe. These habitats often support faunal communities that are highly diverse and productive (Castillo-Rivera et al., 2002) and include valuable recreationally and commercially fished species (Bertelli & Unsworth, 2014; Nordlund et al., 2018). Seagrasses provide a variety of ecosystem functions specifically to fishes including shelter from predators and productive feeding grounds (Heck et al., 2003; Nordlund et al., 2018; Vaslet et al., 2012). Animals often rely on seagrass habitats for particular life stages, most importantly as nursery habitat for juveniles. McDevitt-Irwin et al. (2006) conducted a meta-analysis of 51 studies and found that seagrasses tended to support greater abundances of juveniles with higher survival rates compared to marsh, reef, mangrove, and other benthic habitats. Juveniles of many species utilize the abundant food and resources within the seagrasses to achieve faster growth rates prior to moving to more competitive adult habitats (McDevitt-Irwin et al., 2016).

1.1.3 Drivers of Fish Assemblage Composition

Drivers of site-specific fish assemblages in seagrass habitats are widespread and include local environmental conditions as well as the species and morphology of the seagrasses present, and at a broader scale, climate patterns. In a review of fish assemblages of northern Florida sub-tropical seagrasses, Fodrie et al. (2010) reported that more tropical fishes were present as sea surface temperatures increased, indicating “tropicalization”. Similar observations have been reported along the Texas coast, with increases in temperature and sea level corresponding to an increased presence of tropical species (Fujiwara et al., 2019). Hyndes et al. (2016) hypothesizes that these changes will initially cause an increase in biodiversity until a new biological equilibrium develops. Climatic changes can also affect abiotic conditions (e.g., dissolved oxygen) that may impact seagrass habitat suitability for certain species (Francis, 2013).

Distinct fish assemblages among seagrass species can be linked to differences in plant morphology (Nakaoka, 2005; Rotherham & West, 2002). Morphological characteristics (e.g., leaf number and length and shoot density) create microhabitats that may be favored by certain species because of the food, shelter, and/or other ecological benefits they provide. Hyndes et al. (1996), for example, reported that whiting species (Sillaginidae) in southwestern Australia showed preference for *Zostera* spp. because the less dense canopy allows for easier movement when compared to the other local seagrasses *Posidonia australis* and *P. sinuosa*.

1.1.4 Role of Seagrass Complexity

Plant complexity impacts the function of seagrasses as hunting grounds, shelter, and juvenile nursery habitat (Larkum et al., 2006). When discussing complexity, it is often categorized by spatial scales starting at the seascape level then moving to finer resolutions within patches and at the individual plant level (Horinouchi, 2007; Hyndes et al., 2003; Skilleter et al., 2017). Seascape complexity involves the arrangement of habitats within systems. This can include systems which are characterized by monotypic seagrass beds, mixed species beds, the spatial arrangement and/or sized of meadows, or a combination of nearshore habitats such as seagrass, coral reef, oyster reef, and mangroves (Nagelkerken et al., 2002; Olds et al., 2012; Staveley et al., 2017, 2020). Fish species have variable responses to increased complexity, depending on their on their size and life history. Cruising predators, for example, benefit from meadow fragmentation as more edge habitat is created, which increases their hunting efficiency (Mahoney et al., 2018). Despite this, less complex seagrass seascapes generally have more diverse fish assemblages (Staveley et al., 2020), with many species benefitting from the shelter and resources provided by large continuous seagrass meadows (Hovel et al., 2002; Williams et al., 2016). Individual seagrass patches can vary in density and substrate features (e.g., the presence of rocks or corals) (Horinouchi, 2007). Patches which are more dense may provide more niche spaces for predator avoidance and food when compared to less dense patches that are easier for predators to move through (Ambo-Rappe, 2016), and substrate features may increase fish diversity and abundance by creating unique microhabitats and increased shelter for smaller prey species (Cuadros et al., 2017). At the finest resolution, complexity is driven by individual plant morphology (e.g., leaf length, width, and number) (Larkum et al., 2006). Micheli et al. (2008) reported that beds of *Z. marina* with greater leaf surface area supported higher fish abundances when compared to shoal grass (*Halodule wrightii*) that has a lower leaf surface area. Different fish species also exhibit preferential distribution during certain life stages, with smaller individuals frequently associating more closely with morphologically complex seagrass species (Middleton et al., 1984).

1.1.5 Widgeon grass (*Ruppia maritima*)

Ruppia maritima is a widely distributed seagrass with a wide tolerance to environmental conditions that allows it to thrive in waters ranging from fresh to hypersaline (Hartog & Kuo, 2006) in both the northern and southern hemispheres from tropical to temperate latitudes (Orth & Moore, 1988; Reyes & Merino, 1991). *R. maritima* is an early successional species that is often the first species to colonize an area following a disturbance event (Cho et al., 2009). This is due to high rates of shoot turnover and the species’ reliance on sexual reproduction that creates a persistent seed bank buried in the sediment (Kilminster et al., 2015). In some areas, *R. maritima* is perennial, whereas in more ephemeral areas, plants are predominantly annual (Malea et al., 2004). *Ruppia maritima* is monecious, having both male and female reproductive structures on a single plant. When reproductive, the morphology of *R. maritima* shoots change dramatically; whereas non-reproductive *R. maritima* shoots have several narrow (1–2 mm) leaves ranging from 5–20 cm in length (Kantrud, 1997; Larkum et al., 2006), reproductive shoots branch extensively and can reach a length of 2.5 meters (Larkum et al., 2006).

Figure 1.1 *Comparison of non-reproductive and reproductive* R. maritima *shoots: (A) non-reproductive* R. maritima *(Radloff et al., 2013) ; (B) reproductive* R. maritima *(Kantrud, 1991)*

R. maritima plants at temperate latitudes undergo one annual reproductive cycle, typically in the summer months (Bigley & Harrison, 1986), while those found in more tropical regions usually complete two reproductive cycles (Orth & Moore, 1988; Pulich, 1985). Cho and Poirrier (2005) reported that *R. maritima* in Lake Pontchartrain, LA, flowers in Spring (March to May) and again in late Summer/early Fall (August to October).

The change in plant growth form with the onset of reproduction increases structural complexity and has the potential to impact habitat use by fish and invertebrates, as the reproductive shoots may indirectly provide increased opportunities for food and shelter through the creation of additional microhabitats. It is critical to understand patterns in the distribution of reproductive plants as a predictor of the habitat value of *R. maritima*, especially in areas experiencing changes in seagrass distribution and shifts in species composition.

1.1.6 Chandeleur Islands, LA

The Chandeleur Islands, LA, a chain of barrier islands in the northern Gulf of Mexico, represent an area of shifting seagrass species distribution and composition. The island chain stretches 72 km, with the northern end located 35 km south of Biloxi, MS and the southern end lying in an arch 25 km northeast of Venice, LA (Poirrier & Handley, 2007).

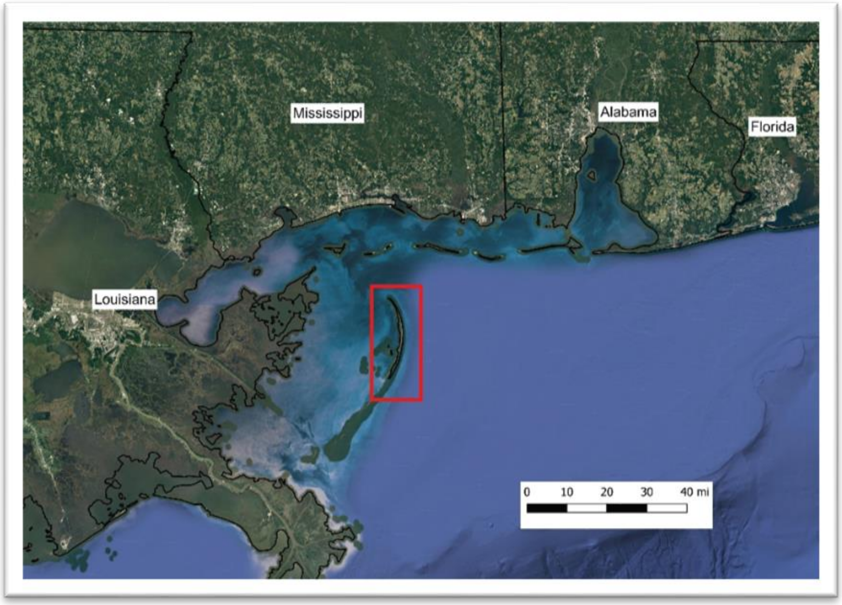


Figure 1.2 *Map of the northern Gulf of Mexico with the North Chandeleur Island, LA study site bounded by the red box*

Much of the land mass of the Chandeleur Islands is in the northern islands, with North Chandeleur Island being the northernmost and largest island. The islands are suffering chronic land loss due to a lack of sediment input, hurricane damage, and rising sea levels (Moore et al., 2014). Additionally, the *Deepwater Horizon* oil spill in 2010 impacted sediment and vegetation along the islands with both showing increased levels of total polycyclic aromatic hydrocarbons (Kenworthy et al., 2017).

The back barrier shelf to the west of the islands is protected from wave action which allows for the proliferation of extensive seagrass meadows (Ellinwood, 2008). There are five species of seagrass that grow along the leeward protected side of the islands, with the most common species being *R. maritima*, shoal grass (*Halodule wrightii*), and turtlegrass (*Thalassia testudinum*) (Kenworthy et al., 2017). Manatee grass (*Syringodium, filiforme*) and star grass (*Halophila engelmannii*) are also present, but are not as abundant (Kenworthy et al., 2017). Seagrass meadows at the Chandeleur Islands represent the only mixed meadows of these five species along 1,000 km of coastline from Perdido Key in Florida to the Texas Coastal Bend (Darnell et al., 2017). Seagrass cover has been decreasing along the island chain with 15,758 acres in 1969 to only 2,614 acres in 2011 (Handley & Lockwood, 2020; Pham et al., 2014). The observed decline is linked to a reduction of shallow protected areas through the combined processes of changes in island geomorphology and storm-related land loss (Darnell et al., 2017). For example, in the aftermath of Hurricane Katrina, the Chandeleur Islands lost approximately 70% of their land mass and 20% of the seagrass cover (Bethel & Martinez, 2008). In the wake of these disturbances, there has also been an observed shift in seagrass species composition, with increase in cover of *R. maritima* and decrease in cover of other species such as *T.*

testudinum (Kenworthy et al., 2017). Large seed banks and rapid growth are characteristics that allow *R. maritima* to thrive after large disturbances and out-compete other seagrass species (Cho et al., 2009; Poirrier & Handley, 2007). As disturbances increase in frequency, as projected with impacts of climate change (Collins et al., 2019), it is likely that *R. maritima* will continue to increase in cover at the Chandeleur Islands, with unknown impacts to the seagrass-associated communities.

1.1.7 Objective

The goal of this study was to understand the distribution of *R. maritima* and describe the morphology of reproductive plants at the Chandeleur Islands. Then, to quantify the impacts of reproductive and non-reproductive *R. maritima* on habitat association by fishes, testing the following null hypotheses:

H_{01}: Reproductive *R. maritima* plants will be homogenously distributed across the Chandeleur Islands

H_{02}: Fish assemblages will not differ between reproductive and non-reproductive *R. maritima.*

1.2 Methods

1.2.1 Vegetation Sample Collection

To describe the distribution and morphology of reproductive and non-reproductive *R. maritima* at the Chandeleur Islands, *R. maritima* was surveyed across its extent at the islands during late summer (August/September) 2021. Prior to sampling, sites with historic presence of reproductive and non-reproductive *R. maritima* were identified using seagrass monitoring data collected in previous years (K. Darnell, unpub. data). Those sites where *R. maritima* previously occurred (n = 30) were targeted for this study, while also ensuring sites were distributed across the entire area of seagrass occurrence at the Chandeleur Islands.

At each sampling site, a YSI handheld meter (Pro 2030, YSI Inc., Yellow Springs, OH) was used to measure salinity, dissolved oxygen (mg/L), and temperature (C°); water depth (cm) was measured using a pole marked in 5 cm increments; and light irradiance at the surface and at ~58–cm depth (μm photons m^{-2} s^{-1}) was measured using two, four pi (spherical quantum) sensors and a data logger (LI-1500, LI-Cor., Lincoln, NE). Light attenuation coefficients (k_d) were calculated as the inverse natural log of the deeper sensor value divided by the surface value, then divided by 0.58-m (linear distance between sensors). At each site, seagrass percent cover by species and bare sediment were quantified in three quadrats (50-cm × 50-cm), and the percent cover of reproductive and non-reproductive *R. maritima* was estimated. One seagrass core (9-cm diameter × 15-cm depth) was collected within *R. maritima* in each quadrat to quantify shoot density and biomass of reproductive and non-reproductive shoots. Quadrats were only collected from sites with seagrass present and cores were only collected from quadrats with seagrass cover. Cores were placed in plastic bags and transported to the Gulf Coast Research Lab (GCRL) where they were frozen until processing. Five reproductive and five non-reproductive plants were retained from the site for image analysis, with care taken to collect all aboveground (leaf and flower) and belowground (root and rhizome) tissue for each plant.

1.2.2 Fish Sample Collection

Fish were sampled at the Chandeleur Islands, LA, during a two-week period from September 6–11, 2021, at the height of the *R. maritima* reproductive season in this area.

This sampling time frame captures the greatest contrast in plant complexity between reproductive and non-reproductive plants. Fish were sampled using a throw trap at a subset of sites surveyed for differences in plant distribution and morphology. Throw trapping offers a targeted approach to sampling patchy habitat that is not possible with other gears such as benthic sled or trawl which cover larger areas (Camp et al., 2011; Jordan et al., 1997). The throw trap consisted of a 1-m × 1-m × 0.6-m aluminum frame. Nylon mesh (3.175 mm) was sewn onto the frame sides as well as extended above the frame and attached to floats to extend the trap height to 1.5 m. After trap deployment, all vegetation was removed and retained for processing. A bar seine was used to remove organisms from within the trap. The bar seine (90-cm × 50-cm) consisted of a PVC frame with handles and 3.175-mm mesh stretched between. The pattern for seining organisms was modeled from Shakeri et al. (2020) and involved three sweeps from each of the trap sides, with sweeps continuing until three consecutive sweeps yielded no additional fish. Collected fish were placed into plastic bags and stored on ice until returned to GCRL, where they were frozen until processing.

Sampling was conducted in *R. maritima* patches across a range of reproductive plant coverage. Patches with desired characteristics (e.g., target cover of reproductive and non-reproductive plants) were identified from those sampled in the survey for plant distribution and morphology and marked with PVC poles prior to sampling. Sampling depth was limited <1.5-m due to the height of the throw trap, and sites were separated by at least 15-m to minimize effects of disturbance. Thirty sites were selected to span the range of reproductive cover values.

1.2.3 Vegetation Sample Processing

Seagrass cores were rinsed over a 500-um sieve and plants were separated by species. The number of shoots of each species was counted, but only *R. maritima* plants were retained. The number of shoots, the reproductive status of each shoot, and the number of branching nodes per plant were recorded. Epiphytes were removed from leaves by gently scraping both sides of each leaf with a razor blade; epiphytes were then placed in a drying oven for 48 h at 60°C to obtain a dry weight to the nearest thousandth of a gram. Shoots were classified as reproductive or non-reproductive and separated. Reproductive shoots were defined as those with branching leaves and/or the presence of inflorescences. The aboveground and belowground tissues were then separated, and the aboveground and belowground tissues for each shoot type (reproductive or non-reproductive) were grouped and placed in a drying oven for 48 h at 60°C, after which they were weighed to the nearest thousandth of a gram to obtain a dry weight. Dried biomass was used to calculate a core-level root to shoot ratio (RSR), which is a useful proxy for plant condition and productivity (Hitchcock et al., 2017). Measurements from the 9-cm diameter core were then extrapolated to obtain bed characteristics per square meter.

Digital images (600 dpi) were taken of the handpick plants using a high-resolution flatbed scanner (Epson WF-3640). Prior to scanning, epiphytes were removed from each shoot and the aboveground and belowground tissues were separated. The aboveground biomass was scanned to produce a JPEG format image. The total leaf area (mm^2) was calculated using ImageJ (Version 1.53) with a threshold processing procedure (Easlon & Bloom, 2014). Additionally, the number of branching nodes and shoot length (mm) were recorded from the image. Total leaf surface area, number of branching nodes, and shoot length were used to quantify overall plant complexity. Each individual shoot's epiphyte, and aboveground and belowground biomass was dried separately in a drying oven for at least 48 h at 60 °C, after which they were weighed to the nearest thousandth of a gram to obtain dry weights (g). This information was used to calculate an individual plant RSR.

Seagrass collected from the throw trap was returned to GCRL and sorted for nekton, then the plant biomass was spun for 90 seconds using an industrial sized salad spinner to remove excess water and sorted and weighed by seagrass species to obtain a species-specific wet weight (g). Individual seagrass species wet weights were then combined to get a total wet weight. Finally, a random subset of reproductive and non-reproductive *R. maritima* plants (at least 3 reproductive and 3 non-reproductive plants per throw trap sample) were imaged and dried as described above to quantify total leaf area and biomass.

1.2.4 Fish Sample Processing

Fish were identified to species and the number of individuals of each species was counted. All individuals were measured for standard length (SL mm) (except for Dwarf Seahorses, which were only measured for total length), total length (TL mm), and weighed (g).

1.2.5 Vegetation Data Analysis

Sampling sites were mapped using QGIS (version 3.18.1) to display the spatial distribution of *R. maritima* along the Chandeleur Islands. Sites with reproductive *R. maritima* were also mapped to show the spatial distribution of reproductive plants. Each map was then evaluated for qualitative trends such as spatial clustering. The mean and standard error were calculated for abiotic parameters across all sites to characterize the distribution of site conditions across the study area.

Several metrics were selected to compare plant morphology between reproductive and non-reproductive shoots. These metrics included surface area, shoot length, leaf number, and RSR. Metrics were compared using unpaired two-sample t-tests (alpha = 0.05). Prior to testing each metric, the data were evaluated for violations of the parametric testing assumptions. If assumptions were violated the sample was bootstrapped with resampling and the test statistic calculated. This was repeated for 10,000 permutations and the reported test statistic was represented by the mean of bootstrapped test statistic distribution.

1.2.6 Fish Data Analysis

Fish assemblages were compared across sites using density, Shannon diversity, and species richness. Density was calculated as the number of individuals per square meter. Shannon diversity (H') was calculated using standard methods (Freeman et al., 1984). Species richness was calculated as the number of unique species.

General Additive Models (GAM) were formulated to evaluate drivers of fish assemblage metrics (Shannon diversity, species richness, and density) with three potential predictor variables: water depth (cm), biomass of vegetation recovered from throw trap (g), and the proportion of reproductive *R. maritima* in the throw trap. Prior to running the GAMs, the predictor variables were plotted against each other to identify any significant relationships. Biomass of vegetation recovered from throw trap and the proportion of reproductive *R. maritima* in the throw trap were found to co-vary; the final GAM formulations only included biomass of vegetation recovered from throw trap and depth as predictor variables. Poisson distributions were used to model species richness and density, while a gaussian distribution was used for modeling Shannon diversity.

1.3 Results

1.3.1 Reproductive Plant Spatial Distribution

Twenty-seven sites were sampled from August 5 to September 3, 2021 spanning the length of seagrass distribution along the Chandeleur Islands (N 30.0224°, W- 88.86616° to N 29.77237°, W -88.88668°)(Figure 1.1). Abiotic parameters were within the known ranges for seagrass meadows at the Chandeleur Islands (Darnell et al., 2017; Hayes, 2021) (Table 1.1).

Figure 1.3 *Sites sampled for* R. maritima *reproductive status during August and September 2021. Each point represents an individual sample site, sampled with three* $0.25m^2$ *quadrats.*

Abiotic Metric	Mean ± SE
Depth (cm)	97.2 ± 7.60
Temperature (°C)	30.8 ± 0.44
Dissolved Oxygen (mg L^{-1})	7.82 ± 0.52
Salinity (ppt)	18.97 ± 0.67
Light Attenuation Coefficient m^{-1}	1.08 ± 0.06

Table 1.1 *Abiotic measurements for 27 sites sampled for the presence and distribution of reproductive* R. maritima

Of the sites sampled, 24 (88.9%) had *R. maritima* present, and 17 of the sites (63.0%) had reproductive plants (Figure 1.2). Among the 72 quadrats sampled across the 27 sites, mean (± SE) percent cover of non-reproductive *R. maritima* was 44.5 ± 3.5, mean percent cover of reproductive *R. maritima* 21.5 ± 3.2, and mean canopy height was 216.8 ± 6.3 mm. Sites with reproductive plants showed a latitudinal central tendency along the islands (Figure 1.2).

A total of 65 cores were collected across the 24 sites with *R. maritima* present. The mean (± SE) overall shoot density was 3,866.71± 293.01 shoots per m^2, mean non-reproductive *R. maritima* shoot density was 3,843.13± 299.37 shoots per m^2, mean reproductive *R. maritima* shoot density was 895.94 ± 194.22 shoots per m^2, and mean core RSR was 1.097 ± 0.111. Within the cores, the density of reproductive shoots was significantly greater than the density of non-reproductive shoots (t = 8.2589, $P < 0.05$). Out of the 65 cores, only 19 cores had measurable epiphyte cover (0.068 ± 0.041 g for all cores combined). Mean epiphyte biomass for cores with only non-reproductive shoots (n=10) was 0.098 ± 0.075 g and epiphyte biomass for cores containing both non-reproductive and reproductive shoots (n = 9) was 0.035 ± 0.026 g.

Figure 1.4 *Presence of reproductive* R. maritima *at the Chandeleur Islands, LA during August and September 2021. White symbols indicate sampling stations with reproductive* R. maritima *present, while red symbols indicate stations where only non-reproductive* R. maritima *was present. Black symbols indicate stations where* R. maritima *was completely absent.*

1.3.2 Plant Morphology

A total of 74 reproductive and 112 non-reproductive plants were hand-collected to assess differences in plant morphology. Reproductive plants had significantly longer leaves (t = 7.054, $p<0.05$), greater total surface area (t = 11.509, $p<0.05$), and a greater number of leaves (t = 15.005, $p<0.05$), while non-reproductive plants had a higher RSR (t = 10.440, $p<0.05$), indicating a larger contribution of belowground biomass (Table 1.2).

Plant Metric	Reproductive Mean ± SE	Non-Reproductive Mean ± SE
Shoot Length (mm)	229.37 ± 8.83 *	150.72 ± 7.04
Total Surface Area (mm^2)	1,507.92 ± 126.06*	310.36 ± 21.65
Root-Shoot Ratio	0.192 ± 0.025	1.033 ± 0.078 *
Leaf Number	25.04 ± 1.84 *	3.125 ± 0.09

* Value significantly greater ($p<0.05$) than the compared value

Table 1.2 *Mean morphological metrics for individual reproductive and non-reproductive hand-collected* R. maritima *plants.*

1.3.3 Fish Assemblages

A total of 22 sites were sampled to investigate habitat use by fishes. Sites were predominant located the center of the island where the most reproductive *R. maritima* plants were observed. The mean (± SE) depth was 71.6 ± 2.8 cm, mean salinity was 19.46 ± 0.37 ppt, mean temperature was 28.81 ± 8.64 °C, and mean dissolved oxygen was 8.15 ± 0.02 mg/L. Mean (± SE) total percent cover seagrass was 69.1 ± 2.9 %, mean percent cover non-reproductive *R. maritima* was 38.9 ± 5.2 %, mean percent cover reproductive *R. maritima* was 30.2 ± 4.3 %, mean total *R. maritima* wet biomass was (371.58 ± 27.61 g).

Figure 1.5 *Sites sampled for fish assemblages within* R. maritima. *Each white circle represents a general sampling area, with the number inside the white circle indicating the number of samples taken in that area.*

Across all sites, a total of 224 individual fish were collected across 15 species. Mean (± SE) fish abundance was 10.2 ± 1.7 per m^2, mean species richness was 3.4 ± 0.2, and mean Shannon diversity was 0.97 ± 0.07 (Table 1.4). The five most abundant species accounted for 86.6% of all individuals collected and included Darter Goby (n=107), Blackcheek Tonguefish (n=33), Gulf Pipefish (n=23), Rough Silverside (n=20), and Code Goby (n=11). The mean weights, total lengths, and standard lengths for all species are listed in Table 1.3**.**

Species	Count	Mean Standard Length (mm) ± SE	Mean Total Length (mm) ± SE	Mean Individual Wet Weight (g) ± SE
Darter Goby	107	24.05 ± 0.66	30.81 ± 0.76	0.29 ± 0.02
Blackcheek Tonguefish	33	32.21 ± 1.5	34.03 ± 1	0.38 ± 0.03
Gulf Pipefish	23	62.3 ± 4.4	64.96 ± 4.59	0.25 ± 0.05
Rough Silverside	20	46.7 ± 0.93	57.5 ± 1.21	1.43 ± 0.1
Code Goby	11	23.45 ± 1.48	28.64 ± 1.81	0.33 ± 0.05
Pinfish	7	72.29 ± 8.59	90.71 ± 11.49	14.3 ± 3.35
Chain Pipefish	6	70 ± 7.45	73 ± 7.93	0.3 ± 0.11
Scaled Sardine	5	47.4 ± 1.17	59.6 ± 1.29	2.26 ± 0.19
Speckled Seatrout	4	38.75 ± 4.64	47.75 ± 6.02	1.14 ± 0.33
Atlantic Threadfin	2	46.5 ± 3.5	56 ± 4	1.79 ± 0.31
Dwarf Seahorse	2	NA ± NA	18.5 ± 0.5	0.03 ± 0.02
Bay Anchovy	1	30 ± NA	37 ± NA	0.27 ± NA
Freckled Blenny	1	25 ± NA	28 ± NA	0.12 ± NA
Gulf Killifish	1	19 ± NA	24 ± NA	0.14 ± NA
Silver Perch	1	70 ± NA	86 ± NA	7.29 ± NA

Table 1.3 *Count and morphological metrics for fish collected, NA values indicate data not collected or not applicable for metric.*

Fish Assemblage Metric	Mean ± SE
Total Number of Fish	10.2 ± 1.7
Species Richness	3.4 ± 0.2
Shannon Diversity	0.97 ± 0.07

Table 1.4 *Fish assemble metrics for 22 sites sampled*

Three GAMs were formulated to identify potential drivers of the fish assemblage metrics of density, species richness, and Shannon diversity. The first model with total number of fish as the response variable identified the wet biomass *R. maritima* of vegetation recovered from throw trap ($\chi 2 = 6.992$, $p<0.05$) as the only significant predictor variable (Table 1.5), and the second and third models identified no significant predictor variables for species richness and Shannon diversity (Tables 1.6 and 1.7).

Variable	$\chi 2$	*P*
Depth (cm)	1.88	0.161
Wet biomass (g) of *R. maritima* recovered from throw trap	6.992	0.008*

* Value significant ($p<0.05$)

Table 1.5 *GAM output for potential drivers of site density*

Variable	χ2	*P*
Depth (cm)	0.021	0.885
Wet biomass (g) of *R. maritima* recovered from throw trap	0.287	0.592

* Value significant (p<0.05)

Table 1.6 *GAM output for potential drivers of site species richness*

Variable	χ2	*P*
Depth (cm)	0.008	0.931
Wet biomass (g) of *R. maritima* recovered from throw trap	0.117	0.759

* Value significant (p<0.05)

Table 1.7 *GAM output for potential drivers of site Shannon diversity*

1.4 Discussion

The goal of this project was to describe the spatial distribution of reproductive *R. maritima* across the Chandeleur Islands and investigate differences in seagrass-associated fishes between reproductive and non-reproductive plants. To accomplish these goals, we conducted extensive seagrass surveys along the length of the Chandeleur Islands and used throw trapping to describe fish assemblages.

Ruppia maritma was found along the entire distribution of seagrass at North Chandeleur Island. Given the robustness of *R. maritima* to environmental stress, the absence of the species at only three of the sampled sites is likely linked to localized physical disturbance. The only other species present at the stations sampled was turtlegrass (*Thalassia testudinum*). Turtlegrass is a climax species that requires relatively stable environmental conditions and low levels of physical disturbance (Larkum et al., 2006). Turtlegrass occurred in centralized portions of the islands that likely represent areas with relatively more stable environmental and physical conditions.

Similar to turtlegrass, reproductive *R. maritima* plants were primarily restricted to the center of the island's back shelf. The complex reproductive shoots have reduced structural tissue and rely on support from the surrounding water (Kantrud, 1997), making them susceptible to physical disturbances. The central portion of the island represents an area with increased sheltering from wind driven wave action that dominates the system. The most northern and southern portions of the islands are characterized by reduced island relief and more exposed shallow waters (Kahn, 1986; Miselis & Plant, 2021). It is possible that *R. maritima* is reproductive at the northern and southern tips of North Chandeleur island, but that shoots cannot sustain the physical disturbance and are dislodged, suggesting that the environmental requirements for sexually reproductive *R. maritima* are stricter than those of non-reproductive plants.

Core samples showed distinct differences between reproductive and non-reproductive shoot densities. Shifts in shoot density have been described for *R. maritima* during the growth phase leading up to reproduction, where resources may be selectively partitioned to the reproductive shoots to promote vegetative growth, resulting in lowered survivorship of the non-reproductive shoots (Bigley & Harrison, 1986). Among the cores collected, less than one third (29.2%) showed measurable levels of epiphyte cover. The lack of epiphytes may be due to changes in bed characteristics as described above, where older non-reproductive shoot density is reduced when beds rapidly produce fresh reproductive vegetation, as well as due to the rapid growth of reproductive plants. The rapid growth may not provide sufficient time for epiphytes to colonize a measurable amount.

R. maritima exhibits morphological plasticity across its range in distribution (Cho et al., 2009; Ito et al., 2013; Lopez-Calderon et al., 2010; Martínez-Garrido et al., 2017), and the current study represents the first known description of *R. maritima* reproductive shoot morphology at the Chandeleur Islands. Results from this study of increased surface area and leaf number for reproductive shoots relative to non-reproductive shoots are consistent with majority of descriptions for the species (Bigley & Harrison, 1986; Kantrud, 1991), but plasticity in reproductive plant morphology has been observed among habitat types (Richardson, 1983). In this study, the mean shoot length for reproductive shoots was 229.37 mm, which is much shorter than the maximum described shoot length of 2.5 m (Larkum et al., 2006). The observed difference may be linked to the presence of large physical disturbances that limit the growth of the fragile reproductive stems, differences in population structure, and/or may be due to a variety of other environmental drivers (Richardson, 1983).

This study describes patterns and potential drivers of habitat use by fish in reproductive and non-reproductive *R. maritima* and suggests that total plant biomass most influences density in *R. maritima* meadows. Similar relationships have been described for nekton communities of *R. maritima* in brackish ponds in Louisiana (Kanouse et al., 2006). The significant relationship between total plant biomass and proportion of *R. maritima* present implies that biomass can be a proxy for overall complexity. GAMs for species richness and Shannon diversity showed no predictor variable relationships were significant, further suggesting that the impacts of biomass and complexity are marginal when compared to the overall influence of seagrass presence for shaping the fish community. This conclusion aligns with previous studies investigating relationships between seagrass presence and biomass and animal abundance, diversity, and richness (Heck et al., 2003; Strayer et al., 2003; Wyda et al., 2002). These results suggest that future studies to investigate the role of *R. maritima* in structuring fish communities in similar study systems may be better served to focus effort on robust measures of seagrass complexity (e.g. biomass) rather than more laborious measures of plant complexity such as cores and image analyses.

The lack of significant predictors among GAMs for species richness and Shannon diversity implies that other predictors are driving the relationships. One possible driver may be prey availability, which is linked to the function of seagrass as foraging grounds. Invertebrates were collected during the present study but were found to be outside the size range of those consumed by the fishes collected. Future studies designed to collect infauna and smaller invertebrates could help elucidate the relationship between prey availability and habitat use. Additionally, other environmental drivers such as salinity (Matheson et al., 1999), turbidity (Blaber & Blaber, 1980), and the availability of dissolved nutrients (Deegan et al., 2002) may impact the distribution of fishes within seagrass habitats. Although salinity was measured in the present study, there was little variation in the measured values which limited power in any subsequent analysis.

The fish collected within *R. maritima* meadows in this study were predominately small benthic species with individuals under 100-mm total length. The majority of individuals were adults of their respective species, with notable exceptions being spotted seatrout (*Cynoscion nebulosus*) and pinfish (*Lagodon rhomboides*) which were composed of young-of-year individuals (*FishBase*, 2022). The family Gobiidae made up the majority (52.7%) of all species collected. Gobiidae are benthic-associated fish and are known to live within seagrass meadows during all life stages where they feed on meiofaunal prey and use the structure provided by seagrasses as shelter from predators (Ara et al., 2010; Carle & Hastings, 1982). Although adult pinfish are known to be abundant in seagrass beds at the Chandeleur Islands (e.g., Hayes 2021), they were collected in low numbers during this study. This may be due to the life history characteristics of the species, where adults leave seagrass beds between May and October to spawn (Faletti et al., 2019), which could have led to a decrease in abundance within seagrass beds at the time of this study (September).

Interestingly, the fish collected in this study included no tropical species. Recent studies have reported the occurrence of tropical fishes in Chandeleur Islands seagrass meadows, indicating a northward movement of these species and tropicalization (Fodrie et al., 2010; Hayes, 2021). However, the previous studies collected relatively low abundances of tropical species (e.g. Lane Snapper (*Lutjanus synagris*) 0.056 per m^2 and Gag Grouper (*Mycteroperca microlepis*) 0.002 per m^2; (Hayes, 2021)), sampled using trawls and benthic sleds which covered a greater area than the throw trap, and targeted different seagrass species (e.g. *Thalassia testudinum*, Hayes 2021). The absence of tropical species in our small-scale (1 m^2) samples indicates that, although they may be present in seagrass meadows at the Chandeleur Islands, tropical species are not likely abundant in *R. maritima* meadows.

One potential limitation of the current study is the throw trap sampling method which may underrepresent the abundance of highly mobile species (Freeman et al., 1984;

Kushlan, 1981) such as members of family Mugilidae. Despite this limitation, throw traps have been shown to have high rates of accuracy when describing fish assemblages (Jordan et al., 1997). Additionally, the removal of all vegetation within the throw trap contributed to clearing efficiency, as animals were recovered from the collected plant material during processing at GCRL.

1.5 Conclusion

R. maritima occurred along the entire latitudinal range of seagrass distribution at North Chandeleur Island. Despite the widespread distribution of non-reproductive plants, there was a central spatial trend of reproductive activity. Where reproductive plants occurred, their density was lower than that of non-reproductive plants, and reproductive shoots had significantly greater shoot lengths, number of leaves, and surface area when compared to non-reproductive plants. Non-reproductive plants showed significantly greater RSR, which indicates resource allocation to belowground biomass rather than aboveground biomass. These results of distinct spatial and morphological characteristics for reproductive *R. maritima* plants suggest that the ecosystem functions of *R. maritima* may change with the onset of reproduction.

Fish assemblages within *R. maritima* beds at the Chandeleur Islands are represented by an abundant group of small benthic species. The presence of morphologically complex reproductive *R. maritima* plants had little impact on the fish assemblages; rather, total biomass of seagrass was the primary drivers of habitat use. *R. maritima* has increased in cover by colonizing areas of disturbance along the islands and displacing climax species such as *T. testudinum*. Future studies should compare *R.*

maritima to other co-occurring seagrass species to better understand possible functional differences for associated fishes.

1.6 Project Significance

The Chandeleur Islands represent a highly dynamic system that supports the most diverse seagrass community in the region. The rise in occurrence of *R. maritima* within the system has been linked to large-scale disturbances that are increasing in frequency along the islands. This study described the spatial distribution and reproductive ecology of *R. maritima* at the Chandeleur Islands and is the first known description of reproductive morphology of this species for the area. *R. maritima* was present across most sites surveyed, often in monospecific meadows, suggesting that it has the ability to thrive in areas where other seagrass species may not. The occurrence of reproductive plants in high densities at the Chandeleur Islands also reinforces the predominance of this life history strategy for the species, and future research should investigate the role of sexual reproduction in the spread of *R. maritima* across the Chandeleur Islands. Results of this study also indicate that, despite the distinct morphologies between reproductive and non-reproductive plants, there were no discernible impacts on habitat use by fishes. Rather, the biggest driver was the more robust metric of total seagrass biomass.

Regardless of its reproductive status, *R. maritima* supported diverse and productive fish communities at the Chandeleur Islands. The recent decline in seagrass cover along the Chandeleur Islands has elicited attention by natural resources managers and in response to land and seagrass loss, a large-scale restoration project is in the early planning phase with a goal of preventing further decline. Managers seeking to restore seagrass along the Chandeleur Islands should view *R. maritima* as a species that hosts diverse and abundant fish communities and should consider this species in restoration efforts to provide long-term resilience and stability of seagrass ecosystem function within the system.

APPENDIX A–Sites Sampled for Reproductive *R. maritima* Presence and Distribution

Station	**Latitude**	**Longitude**
1897	29.85078	-88.8422
C101	29.90364	-88.8343
C113	29.92694	-88.8336
C115	29.93186	-88.831
C119	29.94002	-88.836
C125	29.95087	-88.8416
C127	29.95115	-88.8357
C129	29.95787	-88.8419
C134	29.96797	-88.8444
C138	29.97975	-88.8433
C146	29.99955	-88.8529
C153	30.0097	-88.8497
C155	30.01164	-88.8546
C156	30.01431	-88.8608
C160	30.0224	-88.8662
C24	29.80817	-88.869
C52	29.85123	-88.8419
C6	29.77237	-88.8867
C60	29.8569	-88.842
C68	29.86134	-88.84
C76	29.87076	-88.8368
C81	29.87721	-88.8349
C89	29.88226	-88.8363
C99	29.90052	-88.8363
M7	29.95135	-88.8346
M8	29.93984	-88.8411
M9	29.93599	-88.8378

APPENDIX B–Individual *R. maritima* Shoot Surface Area, Biomass, Root to Shoot Ratio, and Reproductive Status

Station	**Area (mm^2)**	**Number of Leaves**	**Aboveground Dried Biomass (g)**	**Belowground Dried Biomass (g)**	**Root to Shoot Ratio**	**Reprod-uctive**
1897	294.973	4	0.009	0.005	0.584	N
1897	585.090	3	0.017	0.014	0.811	N
1897	227.490	3	0.005	0.013	2.694	N
1897	366.621	3	0.010	0.008	0.857	N
1897	489.886	2	0.009	0.004	0.438	N
1897	4893.703	70	0.188	0.014	0.072	Y
1897	1930.005	25	0.075	0.009	0.126	Y
1897	1266.102	21	0.043	0.011	0.256	Y
1897	1442.344	19	0.060	0.004	0.067	Y
1897	1101.431	19	0.040	0.010	0.236	Y
C101	200.076	3	0.007	NA	NA	N
C101	268.251	3	0.009	NA	NA	N
C101	352.434	3	0.008	NA	NA	N
C101	308.179	3	0.008	NA	NA	N
C101	167.985	2	0.004	NA	NA	N
C101	1610.557	22	0.104	NA	NA	Y
C113	1898.590	5	0.067	NA	NA	N
C113	819.093	4	0.028	NA	NA	N
C113	989.616	4	0.036	NA	NA	N
C113	959.831	4	0.042	NA	NA	N
C113	533.139	3	0.023	NA	NA	N
C113	1949.759	48	0.102	NA	NA	Y
C113	1400.908	28	0.079	NA	NA	Y
C113	970.098	23	0.061	NA	NA	Y
C113	886.280	20	0.055	NA	NA	Y
C113	759.661	20	0.044	NA	NA	Y
C115	271.917	5	0.007	NA	NA	N
C115	258.675	4	0.012	NA	NA	N
C115	120.273	3	0.004	NA	NA	N
C115	76.385	2	0.003	NA	NA	N
C115	102.404	2	0.004	NA	NA	N
C115	2568.365	50	0.130	NA	NA	Y
C115	1786.163	42	0.107	NA	NA	Y

C115	1560.691	23	0.077	NA	NA	Y
C115	925.277	22	0.047	NA	NA	Y
C115	664.989	17	0.039	NA	NA	Y
C119	379.243	3	0.007	0.014	1.932	N
C119	251.272	3	0.011	0.012	1.018	N
C119	272.201	2	0.010	0.010	0.971	N
C119	253.638	3	0.008	0.012	1.457	N
C119	244.629	2	0.007	0.018	2.392	N
C119	1925.930	43	0.125	0.004	0.034	Y
C119	1362.068	30	0.080	0.019	0.240	Y
C119	862.648	28	0.058	0.011	0.180	Y
C119	800.784	17	0.050	0.019	0.386	Y
C119	732.941	11	0.041	0.017	0.410	Y
C127	296.825	3	0.007	NA	NA	N
C127	346.743	3	0.012	NA	NA	N
C127	536.353	3	0.017	NA	NA	N
C127	277.005	2	0.007	NA	NA	N
C127	155.233	1	0.002	NA	NA	N
C127	2060.169	33	0.162	NA	NA	Y
C127	1647.764	23	0.109	NA	NA	Y
C127	808.715	15	0.068	NA	NA	Y
C127	735.914	14	0.032	NA	NA	Y
C127	522.121	12	0.045	NA	NA	Y
C129	370.035	21	0.048	NA	NA	Y
C138	343.266	5	0.013	NA	NA	N
C138	164.456	4	0.006	NA	NA	N
C138	675.964	3	0.018	NA	NA	N
C138	381.201	3	0.014	NA	NA	N
C138	367.409	3	0.011	NA	NA	N
C146	311.399	3	0.010	NA	NA	N
C146	507.062	3	0.017	NA	NA	N
C146	250.033	2	0.008	NA	NA	N
C146	143.704	2	0.005	NA	NA	N
C146	360.287	2	0.019	NA	NA	N
C153	178.686	5	0.005	0.010	2.021	N
C153	90.734	2	0.002	0.003	1.684	N
C155	267.751	4	0.010	0.004	0.439	N
C155	168.806	4	0.005	0.006	1.118	N
C155	501.413	3	0.013	0.014	1.069	N
C155	560.407	3	0.013	0.012	0.899	N

C155	250.950	2	0.007	0.007	1.015	N
C155	3753.501	43	0.183	0.009	0.047	Y
C155	4307.327	43	0.194	0.014	0.073	Y
C155	3468.211	35	0.140	0.005	0.034	Y
C155	2641.617	31	0.125	0.014	0.109	Y
C155	1834.478	19	0.075	0.003	0.037	Y
C156	135.757	3	0.007	NA	NA	N
C156	152.393	3	0.005	NA	NA	N
C156	155.633	3	0.003	NA	NA	N
C156	162.740	3	0.007	NA	NA	N
C156	216.080	3	0.015	NA	NA	N
C156	2237.484	29	0.134	NA	NA	Y
C156	1566.612	23	0.100	NA	NA	Y
C156	1367.629	20	0.070	NA	NA	Y
C156	740.580	12	0.031	NA	NA	Y
C160	165.914	5	0.007	NA	NA	N
C160	542.922	3	0.020	NA	NA	N
C160	97.015	3	0.052	NA	NA	N
C160	215.642	2	0.008	NA	NA	N
C160	455.848	2	0.015	NA	NA	N
C52	174.729	5	0.011	0.004	0.406	N
C52	241.441	4	0.010	0.002	0.240	N
C52	264.236	3	0.007	0.015	2.083	N
C52	527.882	3	0.014	0.005	0.379	N
C52	438.006	3	0.015	0.007	0.462	N
C52	1852.897	30	0.080	0.007	0.085	Y
C52	590.878	14	0.028	0.012	0.418	Y
C52	695.288	14	0.028	0.011	0.380	Y
C52	589.024	11	0.025	0.010	0.423	Y
C52	410.608	9	0.021	0.014	0.668	Y
C60	277.888	5	0.012	0.006	0.504	N
C60	600.317	4	0.015	0.007	0.507	N
C60	167.225	3	0.009	0.014	1.621	N
C60	299.295	3	0.010	0.003	0.272	N
C60	441.967	3	0.012	0.008	0.677	N
C60	2745.981	53	0.144	0.007	0.049	Y
C60	2900.023	52	0.156	0.004	0.027	Y
C60	2375.668	39	0.138	0.006	0.043	Y
C60	705.981	10	0.031	0.013	0.406	Y
C60	731.624	9	0.036	0.009	0.245	Y

C68	716.999	4	0.019	0.017	0.873	N
C68	310.763	3	0.011	0.007	0.657	N
C68	368.121	3	0.010	0.007	0.650	N
C68	324.791	3	0.007	0.005	0.703	N
C68	165.235	2	0.005	0.007	1.447	N
C68	6067.380	99	0.361	0.018	0.050	Y
C68	4064.073	65	0.224	0.006	0.028	Y
C68	1413.061	23	0.051	0.008	0.159	Y
C68	1706.138	19	0.068	0.009	0.133	Y
C68	830.316	16	0.038	0.009	0.237	Y
C76	216.287	5	0.009	0.006	0.604	N
C76	204.927	5	0.006	0.006	1.000	N
C76	169.751	5	0.006	0.006	0.906	N
C76	184.694	4	0.006	0.005	0.814	N
C76	113.705	3	0.002	0.004	1.714	N
C76	459.023	3	0.016	0.009	0.543	N
C76	272.311	2	0.008	0.004	0.525	N
C76	133.848	2	0.005	0.004	0.800	N
C76	93.771	2	0.004	0.004	1.054	N
C76	206.042	2	0.007	0.007	1.076	N
C76	1575.108	32	0.079	0.006	0.073	Y
C76	1330.467	23	0.070	0.016	0.223	Y
C76	1485.543	21	0.067	0.007	0.106	Y
C76	657.110	19	0.031	0.004	0.133	Y
C76	779.222	18	0.034	0.007	0.196	Y
C76	927.745	15	0.045	0.010	0.223	Y
C76	470.346	10	0.020	0.008	0.394	Y
C76	491.767	9	0.020	0.006	0.294	Y
C81	300.387	3	0.008	0.006	0.803	N
C81	298.685	3	0.008	0.010	1.190	N
C81	411.272	3	0.009	0.016	1.918	N
C81	135.680	2	0.004	0.003	0.744	N
C81	263.002	2	0.007	0.007	0.986	N
C89	400.755	4	0.011	0.010	0.891	N
C89	153.440	4	0.003	0.006	1.931	N
C89	168.030	3	0.005	0.005	0.923	N
C89	185.391	3	0.004	0.005	1.308	N
C89	203.521	3	0.005	0.005	1.109	N
C99	281.291	3	0.009	NA	NA	N
C99	550.450	3	0.019	NA	NA	N

C99	357.121	3	0.009	NA	NA	N
C99	340.737	3	0.012	NA	NA	N
C99	294.894	3	0.008	NA	NA	N
C99	1559.042	22	0.077	NA	NA	Y
C99	1662.499	20	0.097	NA	NA	Y
C99	1183.698	19	0.071	NA	NA	Y
C99	1069.597	18	0.060	NA	NA	Y
C99	1095.890	16	0.055	NA	NA	Y
M7	230.484	6	0.012	NA	NA	N
M7	265.726	4	0.010	NA	NA	N
M7	159.360	4	0.006	NA	NA	N
M7	190.420	3	0.009	NA	NA	N
M7	199.831	3	0.008	NA	NA	N
M7	2204.808	44	0.183	NA	NA	Y
M7	1151.471	19	0.069	NA	NA	Y
M7	967.029	18	0.072	NA	NA	Y
M7	925.762	15	0.070	NA	NA	Y
M7	808.715	13	0.056	NA	NA	Y
M8	289.312	3	0.014	NA	NA	N
M8	251.373	3	0.011	NA	NA	N
M8	255.190	3	0.015	NA	NA	N
M8	129.473	2	0.007	NA	NA	N
M8	158.345	2	0.011	NA	NA	N
M9	100.817	3	0.009	NA	NA	N
M9	195.801	3	0.012	NA	NA	N
M9	165.836	3	0.011	NA	NA	N
M9	165.680	3	0.006	NA	NA	N
M9	157.163	3	0.008	NA	NA	N
M9	1293.049	21	0.057	NA	NA	Y
M9	729.407	14	0.040	NA	NA	Y
M9	808.970	13	0.044	NA	NA	Y
M9	715.049	11	0.045	NA	NA	Y
M9	551.710	9	0.031	NA	NA	Y

APPENDIX C– Individual *R. maritima* Shoot Length and Reproductive Status

Station	Length (mm)	Reproductive
1897	279.8370	N
1897	115.7230	N
1897	135.8420	N
1897	213.0410	N
1897	168.7910	N
1897	306.7790	Y
1897	283.9330	Y
1897	137.5620	Y
1897	167.9770	Y
1897	256.3030	Y
C101	86.3360	N
C101	102.2200	N
C101	126.4970	N
C101	167.2720	N
C101	135.7330	N
C101	201.0470	Y
C113	327.0770	N
C113	282.7190	N
C113	325.3710	N
C113	401.5610	N
C113	369.9670	N
C113	291.8980	Y
C113	226.5350	Y
C113	425.3860	Y
C113	275.1820	Y
C113	383.3840	Y
C115	102.0620	N
C115	58.4650	N
C115	54.5310	N
C115	56.1590	N
C115	94.9900	N
C115	279.7120	Y
C115	193.3360	Y
C115	182.3730	Y
C115	263.5920	Y
C115	237.2680	Y
C119	185.7480	N
C52	99.3560	N
C52	210.4970	N
C52	197.9930	N
C52	100.9500	N
C52	101.9920	N
C52	144.3140	Y
C52	137.1010	Y
C52	172.5170	Y
C52	139.6640	Y
C52	128.2180	Y
C60	95.9500	N
C60	97.9680	N
C60	175.8340	N
C60	199.6340	N
C60	203.8590	N
C60	208.7940	Y
C60	306.0150	Y
C60	270.2420	Y
C60	165.9510	Y
C60	192.9630	Y
C68	220.2860	N
C68	224.0080	N
C68	108.5630	N
C68	179.1310	N
C68	277.0920	N
C68	286.5600	Y
C68	233.5820	Y
C68	385.8050	Y
C68	336.2330	Y
C68	186.6800	Y
C76	82.4710	N
C76	100.4860	N
C76	75.9030	N
C76	83.6530	N
C76	88.4150	N
C76	159.6720	Y
C76	130.1560	Y

C119	232.3700	N
C119	225.6440	N
C119	265.5400	N
C119	186.9290	N
C119	430.3870	Y
C119	260.4470	Y
C119	184.8930	Y
C119	284.2890	Y
C119	327.5670	Y
C127	152.2290	N
C127	204.9400	N
C127	224.8810	N
C127	151.8570	N
C127	244.3760	N
C127	242.9600	Y
C127	162.2050	Y
C127	106.0750	Y
C127	245.0900	Y
C127	236.1570	Y
C129	87.9840	Y
C138	76.0180	N
C138	102.9570	N
C138	259.0360	N
C138	248.4650	N
C138	214.0170	N
C146	164.5540	N
C146	148.5780	N
C146	248.1980	N
C146	127.7710	N
C146	247.7040	N
C153	66.1650	N
C153	62.6880	N
C155	71.5360	N
C155	251.2530	N
C155	152.4210	N
C155	80.9980	N
C155	261.8530	N
C155	352.5760	Y
C155	379.1520	Y
C155	336.8560	Y

C76	80.2330	Y
C76	199.9960	N
C76	241.5730	N
C76	107.8200	N
C76	71.9120	N
C76	156.0080	N
C76	148.5020	Y
C76	154.7260	Y
C76	219.5150	Y
C76	219.3010	Y
C76	203.5840	Y
C81	173.9250	N
C81	140.7610	N
C81	172.6920	N
C81	91.2370	N
C81	169.2730	N
C89	138.9170	N
C89	90.3030	N
C89	100.1640	N
C89	98.4300	N
C89	68.8350	N
C99	110.4260	N
C99	142.4010	N
C99	178.8480	N
C99	169.2750	N
C99	129.7030	N
C99	243.1980	Y
C99	261.3080	Y
C99	256.8720	Y
C99	229.7410	Y
C99	228.8080	Y
M7	105.3480	N
M7	128.1970	N
M7	95.2840	N
M7	80.8710	N
M7	64.3640	N
M7	180.5600	Y
M7	219.9830	Y
M7	171.9770	Y
M7	203.6210	Y

C155	255.6210	Y
C155	239.9350	Y
C156	87.7710	N
C156	82.0420	N
C156	95.6770	N
C156	79.8800	N
C156	97.0430	N
C156	298.5530	Y
C156	245.9590	Y
C156	116.8770	Y
C156	271.4050	Y
C160	267.6450	N
C160	109.5440	N
C160	61.5840	N
C160	228.6930	N
C160	56.5760	N

M7	211.8950	Y
M8	135.4590	N
M8	119.2840	N
M8	158.3570	N
M8	129.9320	N
M8	140.7090	N
M9	192.1940	Y
M9	202.9640	Y
M9	166.7800	Y
M9	216.7790	Y
M9	199.3440	Y
M9	87.0980	N
M9	88.9210	N
M9	72.1960	N
M9	80.6040	N
M9	88.0610	N

APPENDIX D–Sites Sampled for *R. maritima* Associated Fish Assemblages

Station	**Latitude**	**Longitude**
C60_1	29.8556	-88.8428
C60_3	29.85637	-88.8431
C60_2	29.85588	-88.8431
C60_4	29.85638	-88.8427
M9_3	29.93977	-88.8358
M9_1	29.93926	-88.8361
M9_2	29.9393	-88.8357
M7_3	29.95149	-88.8344
M7_2	29.95126	-88.8343
M7_1	29.95129	-88.8345
C68_1	29.86217	-88.8411
C76_9	29.87101	-88.8373
C76_7	29.87026	-88.8376
C76_4	29.87158	-88.8329
C60_5	29.85437	-88.8447
C76_3	29.87116	-88.8377
C76_2	29.87068	-88.8371
C76_1	29.87022	-88.8376
C76_8	29.87258	-88.8374
C76_6	29.87206	-88.8378
C76_5	29.87185	-88.8381
C68_2	29.86179	-88.8406

APPENDIX E–Table of Fish Species, Standard Length, Fork Length, Total Length, and Individual Wet Weight

Station	**Species**	**Standard Length (mm)**	**Fork Length (mm)**	**Total Length (mm)**	**Wet Weight (g)**
M9_2	Darter Goby	19	NA	25	0.402
M9_2	Darter Goby	26	NA	33	0.3027
M9_2	Blackcheek Tonguefish	31	NA	34	0.3434
M9_2	Gulf Pipefish	57	NA	60	0.1387
M9_2	Freckled Blenny	25	NA	28	0.1203
C60_1	Pinfish	80	90	99	13.9806
C60_2	Blackcheek Tonguefish	35	NA	37	0.3712
C60_2	Dwarf Seahorse	NA	NA	18	0.0133
C60_2	Bay Anchovy	30	33	37	0.2688
C60_3	Blackcheek Tonguefish	69	NA	40	0.5762
C60_3	Darter Goby	35	NA	47	0.6495
C60_3	Darter Goby	25	NA	31	0.2374
C60_3	Gulf Pipefish	71	NA	75	0.3038
C60_3	Gulf Pipefish	55	NA	58	0.1581
C60_3	Gulf Pipefish	48	NA	50	0.125
C60_4	Blackcheek Tonguefish	24	NA	26	0.1699
C60_4	Blackcheek Tonguefish	25	NA	28	0.1812
C60_4	Blackcheek Tonguefish	34	NA	36	0.4895
C60_4	Darter Goby	18	NA	22	0.0886
C60_4	Darter Goby	24	NA	28	0.1844
C60_4	Darter Goby	25	NA	32	0.2088
C60_4	Darter Goby	22	NA	28	0.1494
C60_4	Darter Goby	21	NA	25	0.1659
C60_4	Dwarf Seahorse	NA	NA	19	0.0478
C60_5	Blackcheek Tonguefish	26	NA	29	0.2156
C60_5	Scaled Sardine	47	52	60	2.235
C60_5	Scaled Sardine	46	51	59	2.171
C60_5	Scaled Sardine	50	54	62	2.7794
C60_5	Scaled Sardine	50	54	62	2.4616

C60_5	Scaled Sardine	44	48	55	1.65
C60_5	Atlantic Threadfin	50	54	60	2.0943
C60_5	Atlantic Threadfin	43	46	52	1.4817
C68_1	Blackcheek Tonguefish	33	NA	36	0.4233
C68_1	Blackcheek Tonguefish	31	NA	34	0.3257
C68_1	Blackcheek Tonguefish	27	NA	29	0.2225
C68_1	Darter Goby	22	NA	27	0.1884
C68_1	Darter Goby	22	NA	27	0.1878
C68_1	Darter Goby	24	NA	30	0.2292
C68_1	Darter Goby	22	NA	28	0.1948
C68_1	Darter Goby	19	NA	23	0.1058
C68_1	Darter Goby	24	NA	31	0.2582
C68_1	Darter Goby	23	NA	27	0.2201
C68_1	Darter Goby	20	NA	25	0.1154
C68_1	Darter Goby	22	NA	27	0.168
C68_1	Darter Goby	20	NA	25	0.124
C68_1	Gulf Pipefish	64	NA	67	0.1823
C68_1	Pinfish	24	25	26	0.1069
C68_2	Pinfish	73	85	93	11.9176
C68_2	Pinfish	83	95	106	18.8405
C68_2	Blackcheek Tonguefish	38	NA	41	0.6116
C68_2	Blackcheek Tonguefish	37	NA	40	0.5788
C68_2	Darter Goby	18	NA	24	0.131
C68_2	Darter Goby	23	NA	28	0.1904
C68_2	Darter Goby	19	NA	25	0.1395
C68_2	Darter Goby	20	NA	26	0.1427
C68_2	Speckled Seatrout	42	NA	51	1.4186
C68_2	Speckled Seatrout	39	NA	47	1.0039
C76_1	Speckled Seatrout	48	NA	61	1.8317
C76_1	Darter Goby	23	NA	28	0.1985
C76_1	Darter Goby	22	NA	28	0.268
C76_1	Darter Goby	19	NA	22	0.0992
C76_1	Chain Pipefish	53	NA	55	0.1035
C76_1	Chain Pipefish	59	NA	62	0.1375
C76_1	Chain Pipefish	99	NA	104	0.7617
C76_1	Chain Pipefish	86	NA	90	0.4788
C76_1	Gulf Pipefish	93	NA	97	0.6527
C76_1	Gulf Killifish	19	NA	24	0.1408

C76_2	Gulf Pipefish	75	NA	77	0.3426
C76_2	Darter Goby	11	NA	14	0.0231
C76_2	Darter Goby	28	NA	33	0.3411
C76_2	Darter Goby	20	NA	24	0.1218
C76_3	Rough Silverside	47	54	59	1.6153
C76_3	Rough Silverside	44	49	53	1.1368
C76_3	Rough Silverside	42	47	52	1.075
C76_3	Rough Silverside	47	53	59	1.4065
C76_3	Rough Silverside	42	47	52	1.1344
C76_3	Rough Silverside	42	47	52	0.9967
C76_3	Rough Silverside	49	55	60	1.5637
C76_3	Rough Silverside	44	49	53	1.0817
C76_3	Rough Silverside	47	52	56	1.3623
C76_3	Rough Silverside	48	52	57	1.4587
C76_3	Rough Silverside	43	48	52	1.0555
C76_3	Rough Silverside	42	47	52	1.0293
C76_3	Darter Goby	23	NA	29	0.2261
C76_3	Darter Goby	22	NA	28	0.187
C76_3	Darter Goby	18	NA	23	0.1078
C76_3	Gulf Pipefish	76	NA	79	0.3693
C76_4	Blackcheek Tonguefish	37	NA	40	0.5573
C76_4	Blackcheek Tonguefish	33	NA	35	0.399
C76_4	Darter Goby	26	NA	31	0.2815
C76_4	Darter Goby	25	NA	31	0.2411
C76_4	Darter Goby	18	NA	22	0.1385
C76_4	Darter Goby	32	NA	38	0.1758
C76_4	Darter Goby	17	NA	22	0.086
C76_4	Pinfish	72	88	90	10.0964
C76_4	Pinfish	96	111	122	29.2523
C76_4	Gulf Pipefish	65	NA	68	0.1906
C76_4	Gulf Pipefish	103	NA	106	0.7786
C76_5	Rough Silverside	50	56	60	1.5354
C76_5	Rough Silverside	48	54	59	1.6777
C76_5	Rough Silverside	58	66	73	2.7408
C76_5	Rough Silverside	54	61	66	2.2479
C76_5	Rough Silverside	48	56	61	1.6471
C76_5	Rough Silverside	48	56	61	1.5472
C76_5	Darter Goby	18	NA	23	0.1106
C76_5	Silver Perch	70	NA	86	7.2898

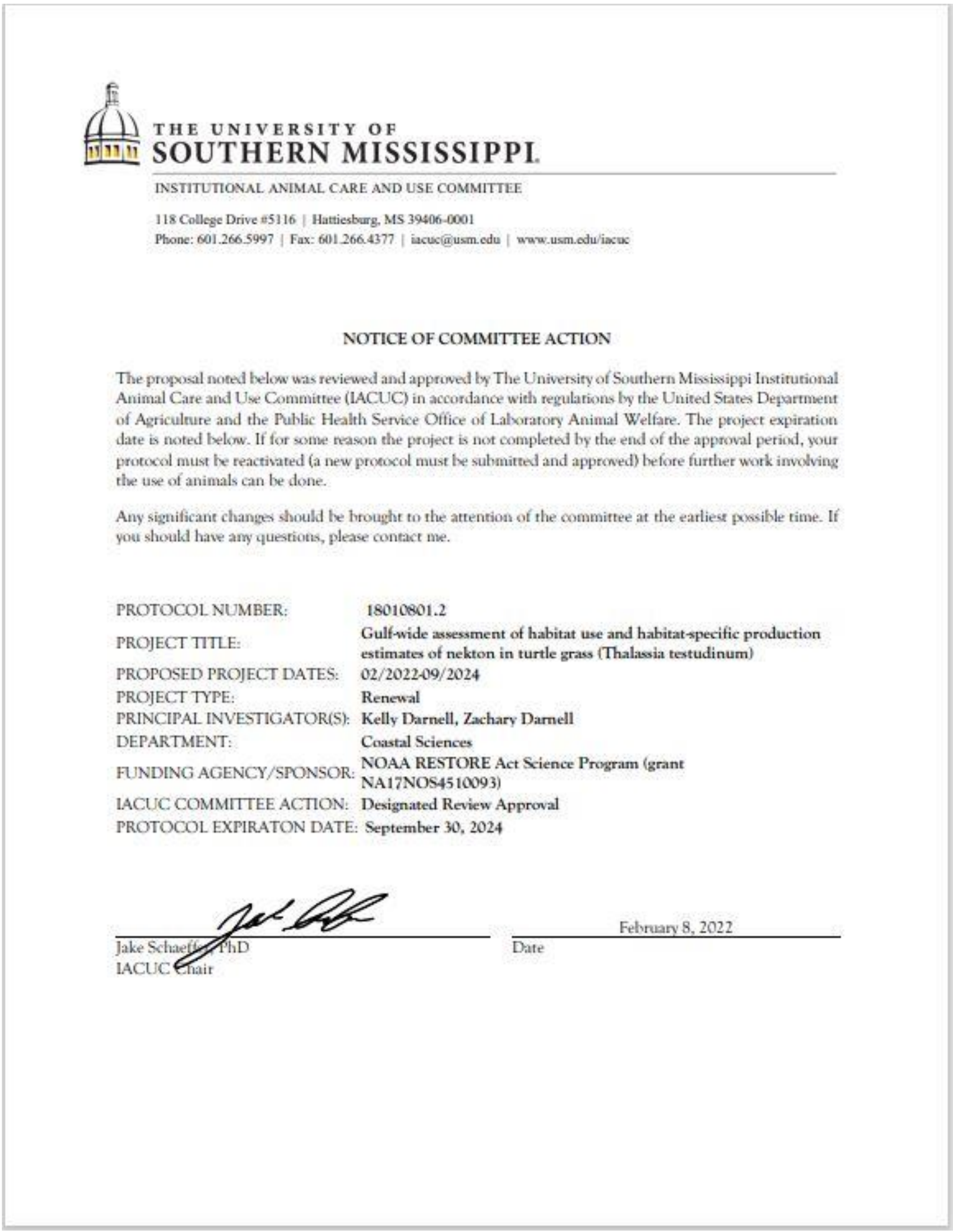
C76_6	Blackcheek Tonguefish	38	NA	41	0.6474
C76_6	Darter Goby	18	NA	23	0.0839
C76_7	Blackcheek Tonguefish	31	NA	34	0.3695
C76_7	Blackcheek Tonguefish	26	NA	28	0.2052
C76_7	Gulf Pipefish	59	NA	62	0.1483
C76_7	Rough Silverside	47	53	58	1.2872
C76_7	Rough Silverside	44	51	55	1.0421
C76_8	Blackcheek Tonguefish	30	NA	33	0.3806
C76_8	Blackcheek Tonguefish	21	NA	24	0.1191
C76_8	Darter Goby	24	NA	27	0.2001
C76_8	Darter Goby	19	NA	24	0.1336
C76_8	Darter Goby	18	NA	23	0.1125
C76_8	Darter Goby	24	NA	28	0.2263
C76_8	Darter Goby	19	NA	24	0.1145
C76_8	Gulf Pipefish	25	NA	26	0.0107
C76_9	Pinfish	78	89	99	15.8768
C76_9	Blackcheek Tonguefish	33	NA	36	0.393
C76_9	Blackcheek Tonguefish	33	NA	36	0.3955
C76_9	Blackcheek Tonguefish	19	NA	21	0.0846
C76_9	Darter Goby	25	NA	34	0.2976
C76_9	Darter Goby	21	NA	26	0.153
C76_9	Darter Goby	19	NA	24	0.1253
C76_9	Darter Goby	21	NA	27	0.1651
C76_9	Darter Goby	21	NA	27	0.1838
C76_9	Darter Goby	21	NA	27	0.1756
C76_9	Darter Goby	17	NA	22	0.096
C76_9	Darter Goby	22	NA	28	0.162
C76_9	Gulf Pipefish	95	NA	100	0.7628
C76_9	Gulf Pipefish	67	NA	70	0.1815
C76_9	Gulf Pipefish	62	NA	65	0.2121
M7_1	Blackcheek Tonguefish	38	NA	42	0.6629
M7_1	Blackcheek Tonguefish	28	NA	31	0.2577

M7_1	Blackcheek Tonguefish	30	NA	33	0.2999
M7_1	Gulf Pipefish	95	NA	99	0.5811
M7_1	Gulf Pipefish	38	NA	39	0.0327
M7_1	Gulf Pipefish	49	NA	52	0.0727
M7_1	Code Goby	28	NA	35	0.4825
M7_1	Darter Goby	24	NA	30	0.2383
M7_1	Darter Goby	35	NA	44	0.7633
M7_1	Darter Goby	39	NA	46	0.8609
M7_1	Darter Goby	32	NA	41	0.5338
M7_1	Darter Goby	31	NA	36	0.5038
M7_1	Darter Goby	39	NA	52	1.016
M7_1	Darter Goby	29	NA	38	0.4755
M7_1	Darter Goby	29	NA	37	0.4477
M7_1	Darter Goby	32	NA	41	0.5362
M7_1	Darter Goby	29	NA	37	0.4042
M7_1	Darter Goby	28	NA	35	0.3434
M7_1	Darter Goby	24	NA	30	0.1891
M7_1	Darter Goby	19	NA	25	0.1271
M7_1	Darter Goby	19	NA	25	0.1277
M7_1	Darter Goby	19	NA	25	0.1358
M7_1	Darter Goby	2	NA	25	0.1191
M7_1	Darter Goby	17	NA	23	0.081
M7_1	Darter Goby	17	NA	21	0.0706
M7_1	Darter Goby	34	NA	41	0.5487
M7_2	Blackcheek Tonguefish	25	NA	28	0.2165
M7_2	Darter Goby	28	NA	36	0.4494
M7_2	Code Goby	25	NA	31	0.3782
M7_2	Code Goby	15	NA	20	0.1289
M7_3	Blackcheek Tonguefish	37	NA	41	0.5199
M7_3	Blackcheek Tonguefish	22	NA	25	0.1401
M7_3	Blackcheek Tonguefish	36	NA	39	0.4411
M7_3	Blackcheek Tonguefish	35	NA	38	0.4481
M7_3	Gulf Pipefish	44	NA	45	0.0577
M7_3	Gulf Pipefish	41	NA	42	0.0406
M7_3	Gulf Pipefish	26	NA	27	0.0131
M7_3	Chain Pipefish	64	NA	66	0.1881
M7_3	Code Goby	30	NA	36	0.5611

M7_3	Code Goby	28	NA	33	0.4231
M7_3	Code Goby	20	NA	24	0.193
M7_3	Code Goby	19	NA	22	0.1606
M7_3	Code Goby	18	NA	21	0.119
M7_3	Darter Goby	3	NA	38	0.4346
M7_3	Darter Goby	31	NA	38	0.4452
M7_3	Darter Goby	24	NA	29	0.1806
M7_3	Darter Goby	32	NA	40	0.5033
M7_3	Darter Goby	31	NA	39	0.539
M7_3	Darter Goby	35	NA	46	0.7059
M7_3	Darter Goby	35	NA	46	0.8455
M7_3	Darter Goby	28	NA	35	0.352
M7_3	Darter Goby	39	NA	52	0.9185
M7_3	Darter Goby	31	NA	39	0.4926
M7_3	Darter Goby	30	NA	38	0.5338
M7_3	Darter Goby	19	NA	24	0.1204
M7_3	Darter Goby	21	NA	28	0.1777
M7_3	Darter Goby	21	NA	25	0.098
M7_3	Darter Goby	21	NA	26	0.1465
M7_3	Darter Goby	19	NA	24	0.1213
M7_3	Darter Goby	33	NA	41	0.4941
M7_3	Darter Goby	22	NA	28	0.1673
M7_3	Darter Goby	19	NA	24	0.1384
M7_3	Darter Goby	19	NA	24	0.1126
M7_3	Darter Goby	24	NA	30	0.259
M9_1	Gulf Pipefish	67	NA	70	0.2077
M9_1	Gulf Pipefish	58	NA	60	0.1133
M9_1	Darter Goby	18	NA	24	0.1019
M9_1	Code Goby	28	NA	35	0.4957
M9_3	Darter Goby	29	NA	38	0.4288
M9_3	Darter Goby	35	NA	41	0.6362
M9_3	Darter Goby	30	NA	39	0.5579
M9_3	Darter Goby	31	NA	40	0.5701
M9_3	Darter Goby	33	NA	41	0.6331
M9_3	Darter Goby	26	NA	34	0.2941
M9_3	Darter Goby	22	NA	28	0.1894
M9_3	Darter Goby	29	NA	36	0.4036
M9_3	Darter Goby	21	NA	28	0.1742
M9_3	Darter Goby	22	NA	28	0.1823
M9_3	Darter Goby	23	NA	29	0.2062
M9_3	Darter Goby	21	NA	27	0.1435
M9_3	Darter Goby	46	NA	58	1.2416

M9_3	Code Goby	24	NA	30	0.4327
M9_3	Code Goby	23	NA	28	0.2978
M9_3	Chain Pipefish	59	NA	61	0.1436
M9_3	Blackcheek Tonguefish	27	NA	30	0.2817
M9_3	Blackcheek Tonguefish	40	NA	42	0.7804
M9_3	Blackcheek Tonguefish	34	NA	36	0.4272
M9_3	Speckled Seatrout	26	NA	32	0.2921

APPENDIX F– IACUC Approval Letter



THE UNIVERSITY OF
SOUTHERN MISSISSIPPI.
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
118 College Drive #5116 | Hattiesburg, MS 39406-0001
Phone: 601.266.5997 | Fax: 601.266.4377 | iacuc@usm.edu | www.usm.edu/iacuc

NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER:	**18010801.2**
PROJECT TITLE:	**Gulf-wide assessment of habitat use and habitat-specific production estimates of nekton in turtle grass (Thalassia testudinum)**
PROPOSED PROJECT DATES:	**02/2022-09/2024**
PROJECT TYPE:	**Renewal**
PRINCIPAL INVESTIGATOR(S):	**Kelly Darnell, Zachary Darnell**
DEPARTMENT:	**Coastal Sciences**
FUNDING AGENCY/SPONSOR:	**NOAA RESTORE Act Science Program (grant NA17NOS4510093)**
IACUC COMMITTEE ACTION:	**Designated Review Approval**
PROTOCOL EXPIRATON DATE:	**September 30, 2024**

Jake Schaeffer, PhD
IACUC Chair

February 8, 2022
Date

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