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EVALUATING HABITAT USE BY NEKTON IN WIDGEON GRASS (RUPPIA MARITIMA), SHOAL GRASS (HALODULE WRIGHTII), AND UNVEGETATED BOTTOM HABITATS IN THE GRAND BAY NATIONAL ESTUARINE RESEARCH RESERVE

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EVALUATING HABITAT USE BY NEKTON IN WIDGEON GRASS (*RUPPIA
MARITIMA*), SHOAL GRASS (*HALODULE WRIGHTII*), AND UNVEGETATED
BOTTOM HABITATS IN THE GRAND BAY NATIONAL ESTUARINE RESEARCH
RESERVE

by

Jessica N. Woodall

A Thesis
Submitted to the Graduate School,
the College of Arts and Sciences
and the School of Ocean Science and Engineering
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Master of Science

Approved by:

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ABSTRACT

Seagrass beds support high biodiversity and animal abundance, serve as feeding grounds for a variety of animals, offer shelter from predation, and act as a nursery habitat for juveniles. The species composition of seagrass beds can impact their use as habitat by animals. Two common species of seagrass in the Gulf of Mexico are *Ruppia maritima* (widgeon grass) and *Halodule wrightii* (shoal grass). The shallow coastal waters of the Grand Bay National Estuarine Research Reserve (NERR) support both species, but the use of each seagrass as habitat by nekton is poorly understood, which can limit management decision-making. Nekton communities were sampled in May, July, September, and November 2022 in the Grand Bay NERR within *R. maritima* and *H. wrightii*-dominated seagrass beds and unvegetated habitat. All nekton were collected, identified to species, weighed, and measured to quantify density, species richness, and species diversity within each habitat. Seagrass cores were also collected to quantify aboveground biomass, root to shoot ratio, and epiphyte density. Juveniles of several commercially fished nekton species including blue crabs (*Callinectes sapidus*), white shrimp (*Penaeus setiferus*), and brown shrimp (*Penaeus aztecus*) were collected, with higher densities and greater species diversity in seagrass beds compared to unvegetated bottom. *R. maritima* and *H. wrightii* were marginally different from each other in terms of habitat use by nekton. Percent cover of both species was the most important habitat feature influencing use by nekton, with greater cover hosting more nekton. These results reinforce the importance of seagrass within the Grand Bay NERR as essential habitat and can be used to inform management and long-term planning.

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DEDICATION

For my Grandpa- my biggest supporter and number one fan. I know he would be proud of me for following my dreams and reaching my goals.

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LIST OF ABBREVIATIONS

<i>ANOVA</i>	Analysis of Variance
<i>BIOENV</i>	Biota and Environmental Matching Routine
<i>CW</i>	Carapace Width
<i>DO</i>	Dissolved Oxygen
<i>GCRL</i>	Gulf Coast Research Laboratory
<i>HW</i>	<i>Halodule wrightii</i>
<i>ISA</i>	Indicator Species Analysis
<i>NERR</i>	National Estuarine Research Reserve
<i>N Rigolets</i>	North Rigolets
<i>RM</i>	<i>Ruppia maritima</i>
<i>TL</i>	Total Length

CHAPTER I – DIFFERENCES IN NEKTON COMMUNITY COMPOSITION IN *R. MARITIMA*, *H. WRIGHTII*, AND UNVEGETATED BOTTOM IN THE GRAND BAY NATIONAL ESTUARINE RESEARCH RESERVE

1.1 Introduction

1.1.1 Seagrasses

Seagrasses are submerged flowering plants that grow in marine ecosystems. There are over 60 species that reside in shallow coastal waters around the world (Larkum et al., 2006). Seagrasses require sunlight, as photosynthesis is an important factor in their growth and survival, and this influences where they are able to colonize and grow (Larkum et al., 2006). Seagrasses are mainly found in temperate to tropical regions, and their depth range spans the mid-intertidal to 90 m deep (Duarte, 1991; Larkum et al., 2006).

Seagrass beds are important coastal habitats because of the many ecosystem functions and services they provide (Barbier, 2017; Heck et al., 2003). The belowground root and rhizome structure of seagrasses stabilizes the sediment and reduces resuspension of organic matter into the water column, which can lead to clearer waters (Barbier, 2017; Orth et al., 2006). Sediment stabilization is increasingly important in reducing coastal erosion that is being amplified by climate change (Duarte et al., 2013). Because of their ability to generate and store an influx of organic carbon, seagrasses also serve as important carbon sinks in marine ecosystems, which may help mitigate impacts of climate change (Duarte et al., 2013; Fourqurean et al., 2012; Orth et al., 2006). Seagrasses also help to protect coastlines from severe weather events, like hurricanes, by buffering wave action and preventing damage to coastal human developments (Duarte et al., 2013). As climate change continues to impact coastal regions, seagrass beds are essential to combating its

effects. In addition to these ecosystem functions and services, seagrasses also serve as essential habitat, food, and shelter for many animals.

1.1.2 Seagrasses as Habitat

It is well known that seagrass beds support high biodiversity and animal abundance, serve as feeding grounds for a variety of nearshore animals, offer shelter from predation, and act as a nursery habitat for juveniles (Barbier, 2017; Vaslet et al., 2012). According to Beck et al. (2001), for a habitat to be considered a nursery, it must contribute more individuals to the pool of adults than other locations that also host juveniles. There are many studies that support the role of seagrasses as nursery habitat, including a meta-analysis by McDevitt-Irwin et al. (2016) who reported that across all temperate and subtropical regions globally, seagrasses supported high juvenile densities and growth compared to other habitats, and that seagrasses were especially important for juvenile invertebrates. Additionally, connections between eelgrass (*Zostera marina*) and the Atlantic cod (*Gadus morhua*) were identified, noting that the finfish species received several benefits from residing in seagrass including increased foraging opportunities and enhanced growth and survival (Lilley & Unsworth, 2014). It is also important to note that seagrass beds are essential habitats for juveniles of many commercially fished species including blue crabs (*Callinectes sapidus*), white shrimp (*Penaeus setiferus*), and brown shrimp (*Penaeus aztecus*) (Fry, 1981; Rozas & Minello, 1998). Patterns of stable carbon isotopes in brown shrimp, a common penaeid shrimp in the Gulf of Mexico, have shown that seagrass beds were key in producing individuals that supported commercial fisheries (Fry, 1981).

1.1.3 Driving Factors of Habitat Use

Seagrasses typically create structure in otherwise unstructured habitats such as bare sediment (Heck et al., 2003; Rozas & Minello, 1998; Tokeshi & Arakaki, 2012). Different components of seagrass structure can influence animal abundance, including canopy height, leaf length, width, and number of leaves per shoot, which have all been reported as good gauges of finfish abundance within seagrass beds (Bell & Westoby, 1986; Gullström et al., 2008; Jones et al., 2021). Because of the increased abundance of food and other essential resources, juvenile finfish and shellfish inhabiting seagrass beds tend to have higher growth and survival rates compared to unstructured habitats, further highlighting the importance of seagrass to many organisms (Heck et al., 2003).

The species composition of seagrass beds can also impact their use as habitat by animals, as different species of seagrass have different physical characteristics. Seagrass features like biomass and epiphyte load can impact faunal community composition and abundance due to their influence on faunal behavioral preferences for habitat (Edgar, 1992; Hamilton et al., 2012; Hyndes et al., 2003). For example, Ray et al. (2014) reported significantly different communities of nekton when comparing *H. wrightii*, *Thalassia testudinum*, and *Syringodium filiforme* beds in Texas. Additionally, different species of seagrasses were host to different types and abundances of epiphytes, which are common food sources for small nekton, and may, in part, contribute to patterns of habitat use among seagrass species (Ray et al., 2014). Several studies have also concluded that changes in seagrass species dominance have ecosystem-wide effects, including changes in biodiversity (Hamilton et al., 2012; Micheli et al., 2008; Ray et al., 2014), trophic connections, and food webs (Lopez-Calderson et al. 2010).

1.1.4 Global Status of Seagrasses

Despite their importance, seagrass cover has been declining rapidly, with the rate of loss increasing in recent years (Waycott et al., 2009). A global assessment found that there has been ~30% reduction in total seagrass cover since the late 1800s, and that seagrass is being lost at a rate of about 7% per year globally (Waycott et al., 2009). One of the main causes for seagrass decline is a reduction in water quality due to anthropogenic impacts to coastal systems (Borum et al., 2004). Nutrient runoff from human activity (industrial, agricultural, and residential) frequently leads to high concentrations of nutrients in waterways, which can cause algal blooms that decrease water clarity and light availability to the benthos (Breininger et al., 2017; Grech et al., 2012). Seagrasses require light for photosynthesis and metabolism and reduced light can stress plants and results in die-offs (Borum et al., 2004). Other human impacts can lead to seagrass decline, such as direct physical damage and removal from dredging operations, fishing equipment, and boat propellers. Another overarching cause for decline that targets all ecosystems is climate change; warming waters and increased storm frequency increase the chance of seagrass die-offs and dislodgement via wave action (Borum et al., 2004).

1.1.5 Gulf of Mexico Seagrasses

Two common species of seagrass in the Gulf of Mexico coastal region are *Ruppia maritima* (widgeon grass) and *Halodule wrightii* (shoal grass) (Figure 1). *R. maritima* is a submerged aquatic plant that has a broad salinity tolerance and can grow in both freshwater and saltwater (Kantrud, 1991). This species tends to be found in salinities between 2 and 19, but *R. maritima* can grow in extremely high salinities up to 70-77 (Joanen & Glasgow, 1965; Kantrud, 1991; Murphy et al., 2003). It has a cosmopolitan distribution, growing in

many coastal bays and estuaries throughout North America (Kantrud, 1991). *R. maritima* has relatively short and shallow roots, extending only about 5-10 cm belowground, with narrow leaves (<1-1 mm wide) with serrated leaf tips that end in a single point (Kantrud, 1991). This species of seagrass is known as a pioneer species and frequently colonizes areas after a disturbance. When reproductive, its leaf structure changes to long, branching leaves that can extend up to 2.5m (Cho et al., 2009; Kantrud, 1991; Larkum et al., 2006).



Figure 1. Comparison of *H. wrightii* (A.) (texasseagrass.org) and *R. maritima* (B.) morphologies (Radloff et al., 2013)

H. wrightii is a sub-tropical and tropical seagrass species that can survive in salinities from 1 to 52 and is mainly found in coastal marine waters (Rivera-Guzmán et al., 2017a; Zieman & Zieman, 1989). It grows from North Carolina through South America, in northwestern Africa, the Indian Ocean, and along the west coast of Mexico (Eiseman, 1980). It has a shallow to deep root system, relatively high underground biomass, and narrow leaves with flat tips that end in a trident shape (Figure 1) (Eiseman, 1980; Pérez-Estrada et al., 2021). *H. wrightii* can be tolerant to stressful environmental conditions and is known as a pioneer species, similar to *R. maritima* (Biber & Cho, 2017).

1.1.6 Grand Bay National Estuarine Research Reserve (NERR)

Both *R. maritima* and *H. wrightii* grow within the waters of the Grand Bay National Estuarine Research Reserve (NERR). The Grand Bay NERR is one of 27 reserves around the country designated to protect, monitor, and restore coastal ecosystems, and to promote ecological stewardship. It is located on the Mississippi-Alabama border in southeast Jackson County, Mississippi, encompassing over 18,000 acres of protected land (Grand Bay NERR, 2022) (Figure 2). Grand Bay NERR managers have identified several topics as a high priority for management including Reserve-focused research that fills in data gaps concerning habitat protection and improvement, habitat nursery function, water quality, fisheries, and other aspects of the Reserve (Grand Bay NERR, 2022). There is currently a data gap in understanding the function of seagrasses within the NERR as habitat used by nekton, which has limited Reserve managers' ability to make management and/or restoration decisions within the Reserve boundaries.

Within the Grand Bay NERR, a handful of studies have focused on seagrass. These studies have investigated seasonal changes in seagrass percent cover (Cho & May, 2008), temporal variation and depth of seagrass (Cho & Nica, 2009), seed propagation of *R. maritima* (Cho et al., 2009), and seagrass seasonal and annual dynamics (Cho et al., 2017) (Table 1). These studies have reported that, within the NERR, *R. maritima* typically dominates cover during the summer and *H. wrightii* typically dominates during the fall (Cho & May, 2008; Cho & Nica, 2009); that there is distinct interannual variability in seagrass cover (Cho et al., 2017), and that both species are resilient to environmental stressors and disturbances like hurricanes (Cho et al., 2009, 2017).

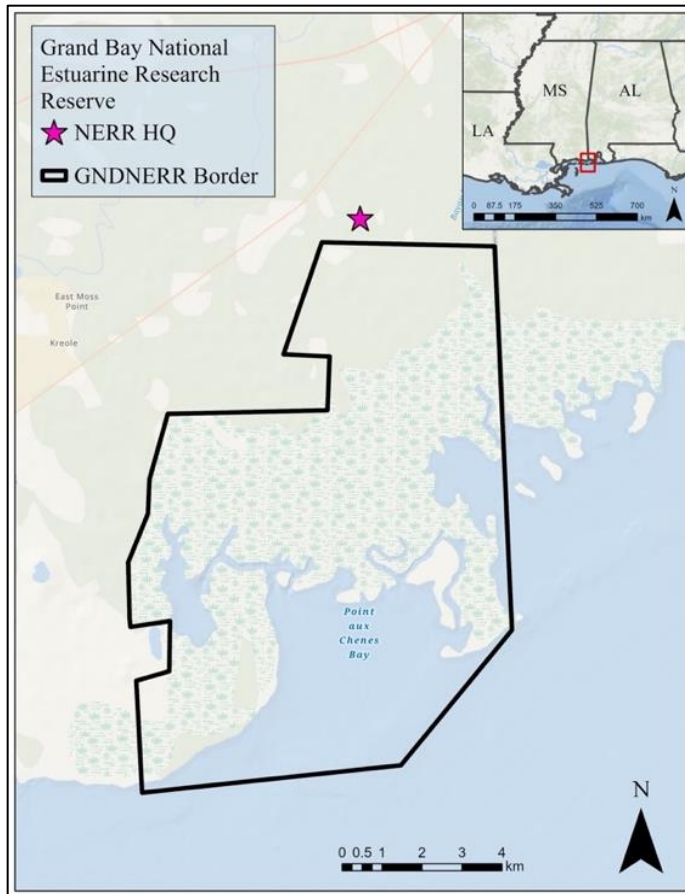


Figure 2. The borders of the Grand Bay National Estuarine Research Reserve on the border of Mississippi and Alabama (top), and the known seagrass distribution within the NERR boundaries (bottom, source: Grand Bay NERR). The star indicates the location of the reserve headquarters (top). Seagrass beds within the NERR are found in several different bays (bottom).



Although previous studies have investigated dynamics in seagrass distribution and cover, few studies have investigated habitat use within the NERR, and only two have focused on seagrasses, despite the central role of seagrass as habitat in coastal ecosystems (Barbier, 2017; Duarte et al., 2013; Heck et al., 2003; Orth et al., 2006). Previous studies have investigated plant-animal interactions, but did not look into habitat usage or community dynamics (Drury-McCall et al., 2009; Drury-McCall & Rakocinski, 2007). Research focused on ecosystem effects and animal sampling has taken place in other habitats within the NERR. For example, one study assessed differences in white shrimp (*Penaeus setiferous*) density, growth, and predation mortality across oyster reefs, vegetated marsh edge, and non-vegetated bottom habitats within the Grand Bay NERR, and reported more shrimp in structured habitat (oyster reef and vegetated marsh edge) compared to non-vegetated bottom (Shervette & Gelwick, 2008a). In addition to white shrimp, fish and invertebrate communities have been sampled in those same three habitats during spring, summer, and fall, with animal density and diversity highest in vegetated marsh edge and oyster reef habitats compared to unvegetated bottom (Shervette & Gelwick, 2008b). Furthering this research, a species-specific study of six common invertebrates (*Eurypanopeus depressus*, *Panopeus simpsoni*, *Palaemon pugio*, *Callinectes sapidus*, *Penaeus setiferus*, and *Penaeus aztecus*) reported that all species had greater abundances in vegetated and oyster reef habitats compared to unvegetated bottom (Shervette et al., 2011).

The few studies that have investigated habitat use by fish and invertebrates within the Grand Bay NERR have focused on intertidal salt marsh, oyster reefs, and vegetated marsh edge, but have ignored seagrasses. Seagrasses grow throughout the Grand Bay

NERR, and previous studies have reported changes in seagrass species distribution and cover over time (Cho et al., 2009, 2017; Cho & May, 2008; Cho & Nica, 2009). This, coupled with the lack of information on the role of seagrass for providing habitat within the Grand Bay NERR, presents a challenge for management and future planning for resources within reserve boundaries.

Table 1. Past seagrass and nekton studies conducted at Grand Bay NERR and surrounding areas. These studies focused on seagrass, finfish, and shellfish. Not much seagrass research was conducted from 2013- present.

Authors	Title	Location	Habitats	Species
Drury, D. M. (2004).	“Effects of grass shrimp (<i>Palaemonetes spp.</i>) density manipulations and nutrient enrichment on widgeongrass (<i>Ruppia maritima</i>) condition, epiphyte load, and epiphyte functional groups”	Grand Bay NERR	Seagrass beds	Grass shrimp (<i>Palaemonetes spp.</i>), <i>Ruppia maritima</i> , epiphytes
McCall, D. D., & Rakocinski, C. F. (2007).	“Grass Shrimp (<i>palaemonetes Spp.</i>) Play a Pivotal Trophic Role in Enhancing <i>Ruppia Maritima</i> .”	Grand Bay NERR	Seagrass beds	Grass shrimp (<i>Palaemonetes spp.</i>), <i>Ruppia maritima</i>
Cho, H. J., & May, C. (2008).	“Short-term Spatial Variations in the Beds of <i>Ruppia maritima</i> (<i>Ruppiaceae</i>) and <i>Halodule wrightii</i> (<i>Cymodoceaceae</i>) at Grand Bay National Estuarine Research Reserve, Mississippi, USA”	Grand Bay NERR	Seagrass beds	<i>Ruppia maritima</i> , <i>Halodule wrightii</i>
Shervette, V. R., & Gelwick, F. (2008a).	“Seasonal and Spatial Variations in Fish and Macroinvertebrate Communities of Oyster and Adjacent Habitats in a Mississippi Estuary.”	Grand Bay NERR	Oyster reef, vegetated marsh edge, unvegetated bottom	White shrimp (<i>Litopenaeus setiferus</i>), brown shrimp (<i>Farfantepenaeus aztecus</i>), blue crab (<i>Callinectes sapidus</i>), other nekton
Shervette, V. R., & Gelwick, F. (2008b).	“Relative nursery function of oyster, vegetated marsh edge, and nonvegetated bottom habitats for juvenile white shrimp <i>Litopenaeus setiferus</i> .”	Grand Bay NERR	Oyster reef, vegetated marsh edge, unvegetated bottom	White shrimp (<i>Litopenaeus setiferus</i>)
McCall, D. D., Rakocinski, C. F., & Pinckney, J. L. (2009).	“Effects of grass shrimp versus nutrient addition on epiphytic algae associated with the ephemeral widgeongrass <i>Ruppia maritima</i> .”	Grand Bay NERR	Seagrass beds	Grass shrimp (<i>Palaemonetes spp.</i>), <i>Ruppia maritima</i> , epiphytes
Cho, H. J., Biber, P., & Nica, C. (2009).	“The rise of <i>Ruppia</i> in seagrass beds: Changes in coastal environment and research needs.”	Grand Bay NERR	Seagrass beds	<i>Ruppia maritima</i>
Cho, H. J., & Biber, P. D. (2010).	“Seed Propagation Protocol for Widgeongrass (<i>Ruppia maritima</i>) (Mississippi)”	Grand Bay NERR	Seagrass beds	<i>Ruppia maritima</i>
Shervette, V. R., Gelwick, F., & Hadley, N. (2011).	“Decapod Utilization of Adjacent Oyster, Vegetated Marsh, and Non-Vegetated Bottom Habitats in a Gulf of Mexico Estuary.”	Grand Bay NERR	Oyster reef, vegetated marsh edge, unvegetated bottom	White shrimp (<i>Litopenaeus setiferus</i>), brown shrimp (<i>Farfantepenaeus aztecus</i>), blue crab (<i>Callinectes sapidus</i>), other nekton
Pham, L., Biber, P., & Carter, G. (2014).	“Seagrasses in the Mississippi and Chandeleur Sounds and Problems Associated with Decadal-Scale Change Detection.”	Grand Bay NERR, Chandeleur Sounds	Seagrass beds	<i>Ruppia maritima</i> , <i>Halodule wrightii</i> , <i>Thalassia testudinum</i>
Cho, H. J., & Biber, P. D. (2016).	“Habitat Characterization for Submerged and Floating-Leaved Aquatic Vegetation in Coastal River Deltas of Mississippi and Alabama”	Mobile Bay, Grand Bay NERR, and Pascagoula River	Seagrass beds	Various SAV/FAV, <i>Ruppia maritima</i> , <i>Halodule wrightii</i> , etc.
Cho, H. J., Biber, P. D., Darnell, K. M., & Dunton, K. H. (2017).	“Seasonal and Annual Dynamics in Seagrass Beds of the Grand Bay National Estuarine Research Reserve, Mississippi”	Grand Bay NERR	Seagrass beds	<i>Ruppia maritima</i> , <i>Halodule wrightii</i>

1.1.7 Objectives

1.1.7.1 Objective 1

The purpose of this study was to examine habitat use of *R. maritima*-dominated seagrass beds, *H. wrightii*-dominated beds, and unvegetated bottom by nekton (finfish and shellfish) in the Grand Bay NERR. The null hypothesis for this objective is outlined below:

H₀₁: There will be no difference in the nekton community composition at species level, total nekton density, species richness, and Shannon diversity between *R. maritima*-dominated beds, *H. wrightii*-dominated beds, and unvegetated bottom habitats.

1.1.7.2 Objective 2

This study also compared seagrass morphology metrics (aboveground biomass, root to shoot ratio, and epiphyte density) across months for *R. maritima* and *H. wrightii*. The null hypothesis for this objective are outlined below:

H₀₁: There will be no difference in the aboveground biomass, epiphyte density, and root to shoot ratio across months.

1.1.7.3 Objective 3

This study also aimed to compare growth of brown shrimp (*P. aztecus*) between *R. maritima*-dominated beds, *H. wrightii*-dominated beds, and unvegetated bottom in the Grand Bay NERR. The null hypothesis for objective is outlined below:

H₀₁: There will be no difference in growth of brown shrimp between *R. maritima*-dominated beds, *H. wrightii*-dominated beds, and unvegetated bottom habitats.

1.2 Methods

1.2.1 Station Selection

General locations for community sampling to quantify nekton species composition, total density, species richness, and Shannon diversity were identified in May 2022 when historic seagrass monitoring sites in the Grand Bay NERR were revisited and monitored for seagrass species distribution and cover (Wilson, 2023). At this time, the locations (coordinates) of *R. maritima*-dominated beds, *H. wrightii*-dominated beds, and unvegetated bottom were noted. These three habitats were found in Middle Bay and near North Rigolets Island. Each time the nekton communities were sampled, exact sampling locations within each of the target habitats were chosen haphazardly throughout Middle Bay and the North Rigolets Island. After community sampling, station coordinates were recorded to avoid resampling the exact location during the same survey period. Sampling stations in unvegetated habitat were occasionally located near *R. maritima*-dominated beds and/or *H. wrightii*-dominated beds but were sufficiently spaced (>2m from meadow edge) to prevent spillover effects (Jelbart et al., 2006).

1.2.2 Nekton Sample Collection

To assess habitat use by nekton in seagrass and unvegetated habitats in the Grand Bay NERR, nekton communities were sampled in May, July, September, and November 2022 (Figure 3). Sampling was conducted using a 1m² aluminum frame throw trap lined with 3.175-mm mesh and a 0.5m vertical extension. Five replicate throw trap samples were taken in each habitat type across the four sampling months, except for all sites in May (n = 4) and *H. wrightii* sites in November (n = 2).

Using information from the seagrass monitoring effort in May 2022 (described above), an area of a known habitat type was approached slowly with a boat, and once in the target habitat, the throw trap was deployed off the side of the boat and immediately pushed into the sediment to ensure full contact with the bottom and to prevent animals from escaping. After deployment of the throw trap, water temperature (C°), dissolved oxygen (DO; mg/L), and salinity were measured at the site using a YSI handheld multiparameter meter (YSI Pro 2030, YSI Inc., Yellow Springs, OH). Secchi depth (cm) was measured, and three water depth (cm) measurements were also collected inside the trap to calculate mean water depth. Light availability as photosynthetically active radiation ($\mu\text{mol m}^{-2} \text{s}^{-1}$) was also measured with spherical quantum sensors placed within the water column, and three replicate readings were taken, from which the light attenuation coefficient was calculated. The light meter included two sensors spaced 30 cm apart with one sensor located below the surface of the water and the other 30 cm below the water surface. The light attenuation coefficient was calculated using the Beer-Lambert equation:

$$k_d = -[\ln(I_z/I_0)]/z$$

Where k is the attenuation coefficient (m^{-1}) and I_z and I_0 are irradiance ($\mu\text{mol photons m}^{-2} \text{sec}^{-1}$) at a depth of z (meters) and at the surface.

To determine seagrass species composition of the target habitats, nine seagrass grabs were collected from inside the throw trap by hand. Two grabs were collected along each of the four sides of the trap, and one was collected from the center of the trap. Within each grab, the percent composition of each seagrass species was visually estimated, and this information was used to determine the dominant seagrass species at that site. Sites with greater than 50% *H. wrightii* were characterized as *H. wrightii*-dominated, and sites with

greater than 50% *R. maritima* were characterized as *R. maritima*-dominated, while unvegetated bottom was characterized by less than 10% vegetation cover. If both seagrass species were present in even amounts, a new site was selected. Total percent cover of seagrass (both species combined) within the throw trap was estimated by touch due to low water clarity, and the percent cover by species was scaled accordingly using the percent composition of the nine grass grabs.

A 1m-wide bar seine with 3.175-mm mesh was used to sweep the interior of the throw trap to remove all nekton, following the methods described in Shakeri et al (2020). The interior of the throw trap was swept from each side three times, and then once from each side until three consecutive sweeps yielded no nekton. The total sweep time did not exceed one hour at any site. All organisms were stored in labeled Ziploc bags on ice and returned to the Gulf Coast Research Laboratory (GCRL) in Ocean Springs, Mississippi where they were frozen until further processing.

1.2.3 Vegetation Sample Collection

Two seagrass cores (9.5cm diameter x 15cm deep) were collected at each site adjacent to the outside perimeter of the throw trap and rinsed in the field to remove sediment. The seagrass samples were stored on ice, then retained in the freezer until processing.

1.2.4 Nekton Sample Processing

All animals collected in the throw trap were retained in the freezer until processing. Nekton were identified to the lowest taxonomic level possible. The number of individuals of each species (or lowest taxonomic level) was counted, and total length (fish and shrimp, mm) or carapace width (crabs, mm) was measured. Total wet weight (g) for each species

in each sample was also measured. All non-nekton animals were identified to the lowest taxonomic level possible but were not included in subsequent analysis.

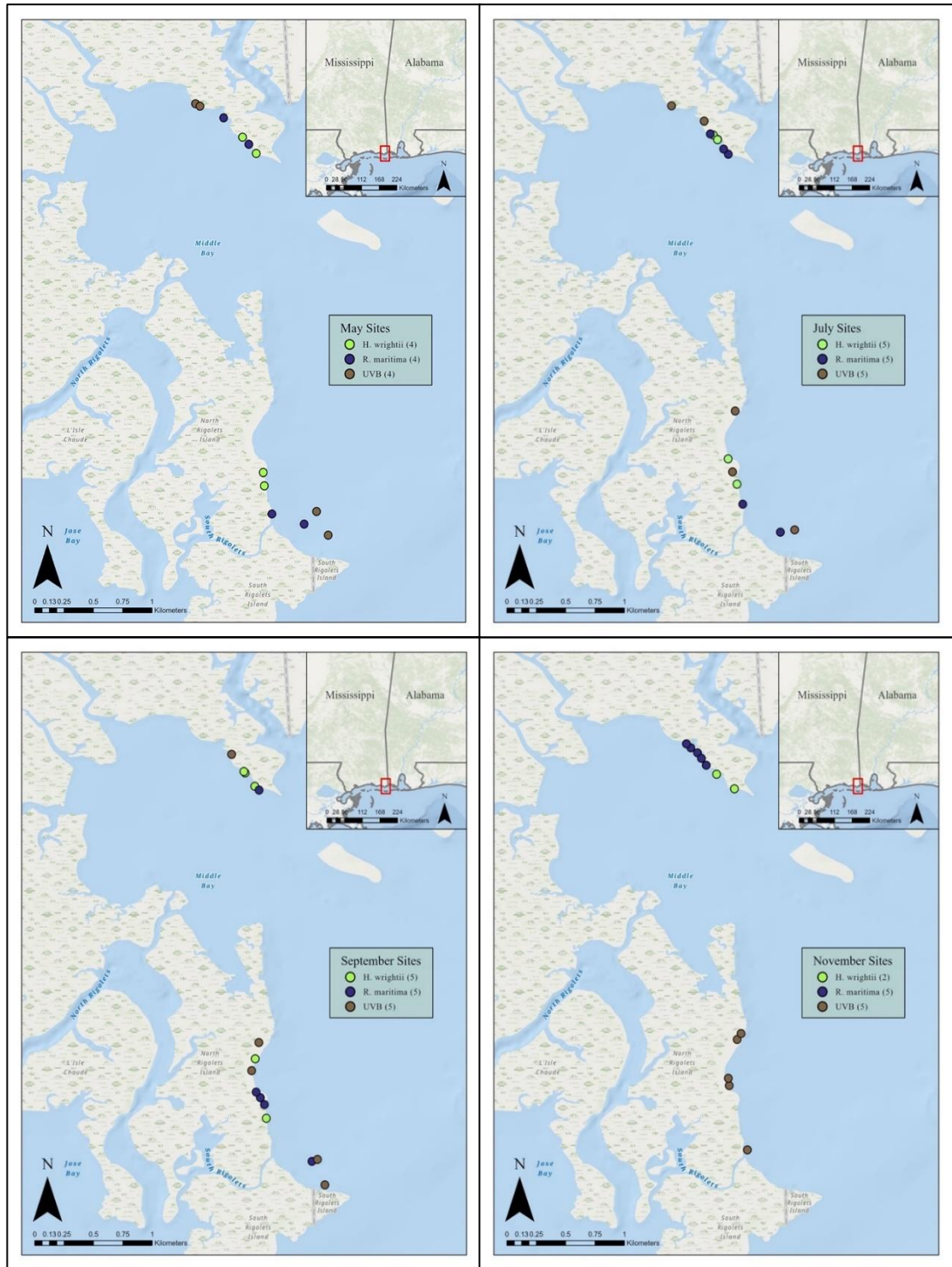


Figure 3. Nekton sample sites by habitat type (*R. maritima*, *H. wrightii*, or unvegetated bottom) and month (May, July, September, or November 2022) at the Grand Bay NERR. Green points are *H. wrightii* sites, blue points are *R. maritima*, and brown points are unvegetated bottom.

1.2.5 Vegetation Sample Processing

Once core samples were thawed, seagrass shoots were identified to species when possible. Plant above- and belowground biomass was separated, and epiphytes were gently scraped from the leaves using a razor blade. Seagrass aboveground biomass, belowground biomass, and epiphytes were dried in an oven 60 C° for >48 hours until a constant weight, then weighed to determine dry weight (g) (Frankovich & Fourqurean, 1997). The following seagrass metrics were calculated: epiphyte biomass (g dry weight epiphyte / g dry weight seagrass), aboveground biomass (g/m²), and root to shoot ratio.

1.2.6 Shrimp Growth

To quantify growth of brown shrimp in each habitat type, an *in situ* growth experiment was conducted during September 2022, when brown shrimp were most abundant (Farfante, 1969). The growth study was conducted for 14 days, which is sufficient time for differences in growth to be observed in this area (Shervette & Gelwick, 2008). Cages consisting of a bottomless cylinder measuring 1.07-m in diameter and 0.76-m tall with 3.2-mm mesh surrounding it (Figure 4) were anchored into the sediment using rebar in *H. wrightii*-dominated seagrass beds, *R. maritima*-dominated seagrass beds, and unvegetated sediment. A total of 12 cages were deployed, with five replicate cages in each habitat type. Each cage contained a small closeable opening in the top of the cage to allow access inside.

Brown shrimp were collected from the Grand Bay NERR 48 hours before the start of the growth experiment using a seine and were returned to the GCRL in aerated containers. Shrimp were stored overnight in large, aerated tubs prior to tagging. Shrimp were then implanted with unique Visible Implant Elastometer (VIE) tags (Northwest

Marine Technologies) for identification of individuals, then held for 12 hours in the lab after being implanted to identify any mortalities (Figure 5). All shrimp were measured to the nearest mm (total length) and were within the size range of 21-45mm, which falls within



Figure 4. Mesh cages used for the brown shrimp growth experiment in the Grand Bay NERR shown out of the water (left) and in the water with a marker pole (right). Cages were 1.07-m in diameter and 0.76-m tall with 3.2-mm mesh. 15 cages were deployed with 5 in each habitat type (*Ruppia maritima*, *Halodule wrightii*, and unvegetated bottom).

the juvenile size range (O’Connell et al., 2017). Other studies with shrimp and crabs have used between four and eight individuals in similar $\sim 1\text{m}^2$ sized enclosures, whereas some studies with larger cages have included 800 shrimp in a 4m^2 cage (Baker & Minello, 2010; Hayes et al., 2022; Rozas & Minello, 2011; Vaz et al., 2004). Before cage deployment, the site was swept with dip nets and a bar seine to remove large animals from the area, as well as any macroalgae to eliminate potential confounding variables.

The following abiotic parameters were measured at the start, middle, and end of the 14-day experiment within each cage: water temperature ($^{\circ}\text{C}$), DO (mg/L), and salinity

using a YSI (Pro 2030, YSI Inc., Yellow Springs, OH); Secchi depth (cm); and three water depth (cm) measurements were taken beside the cage to obtain average water depth. Light availability as photosynthetically active radiation ($\mu\text{mol m}^{-2} \text{s}^{-1}$) was also measured with spherical quantum sensors placed within the water column, and three replicate readings were taken, from which the light attenuation coefficient was calculated.



Figure 5. Inserting VIE tags into brown shrimp (left). Shrimp were tagged inside the last abdominal segment using a variety of colors (right). Shrimp were tagged using a variety of colors and color combinations to allow identification of individuals. Shrimp were held overnight to identify any mortalities from tagging.

Sites for cages were selected and cages were deployed and marked with identification poles on September 6th, 2022 (n=15). Brown shrimp were collected from the Grand Bay NERR following cage deployment on September 6th, 2022 and were transported to the GCRL in aerated containers, and stored in a large, aerated tank overnight. During the morning of September 7th, 187 shrimp were tagged with VIE tags in the colors: red, red-yellow, orange, orange-yellow, green, green-yellow, blue, blue-yellow, pink, and pink-yellow. More shrimp were tagged than needed due to the expectation that there would be some fatalities following tagging. Combinations of colors were used to allow for

individuals to be identified. Shrimp were held overnight and placed into cages starting at 8:00 am on September 8th, 2022. One shrimp per color combination was placed into small, aerated containers for transport, and then one container full of shrimp was deployed into each cage. Each cage contained 9-10 shrimp at the start of the experiment.

At the end of the experiment, a throw trap was placed around the cage and the cage was removed from the substrate. The interior of the throw trap was swept with dip nets and a bar seine to retrieve the shrimp, which were placed into Ziploc bags and stored on ice and returned to the GCRL. Finally, the percent cover of seagrass by species was quantified, canopy height of three plants per species were measured, and one seagrass core was collected.

1.3 Data Analysis

1.3.1 Environmental Data – Nekton Sampling

Before beginning analysis, sites were given location identifiers of either Middle Bay or North Rigolets. The abiotic data recorded during sampling was analyzed using Analysis of Variance (ANOVA) tests in R (v4.3.0; R Core Team, 2022). The packages “multcomp” (Hothorn et al., 2008) and “emmeans”(Lenth, 2023) were used to perform ANOVAs and post-hoc Tukey tests. Temperature was transformed with a natural log prior to analysis. A two-way ANOVA was performed to assess the effect of month and location on salinity, temperature, and DO. Levene’s and Shapiro-Wilks tests were performed to ensure each variable met the assumptions of homogeneity of variances and normality, respectively. Tukey post-hoc comparisons were performed on each variable to assess the differences among groups indicated as being significantly different through the two-way ANOVA.

1.3.2 Nekton Density, Diversity, and Richness

To identify if there were differences in the nekton assemblages (identified to species level) between the three habitat types (*R. maritima*, *H. wrightii*, and unvegetated bottom), a full-factorial distance-based permutational multivariate ANOVA (PERMANOVA, permutations = 9999) was conducted using PRIMER v7. Before beginning analysis, all non-nekton and any individuals that were too damaged to identify to species were removed from the dataset. The unknown individuals accounted for 6% of the data set, and a large majority were *Palaemon spp.* shrimp. Nekton data were log x+1 transformed to down-weight the influence of a few species with high densities, and a Bray-Curtis similarity resemblance matrix with a +1 dummy variable was calculated so sites containing no nekton could be included. Non-metric multi-dimensional scaling (nMDS) ordination was also performed to visualize the similarities and dissimilarities in nekton communities by month and habitat type.

Pairwise post-hoc comparisons were used to identify differences among habitat types within each month and differences among months within each habitat type. A permutational multivariate dispersion (PERMDISP) test was used to check for homogeneity of multivariate dispersion among months and habitat types. When both PERMANOVA and PERMDISP tests are significant for a given factor, this indicates differences in dispersion between group centroids but not necessarily differences in locations between group centroids (Anderson et al., 2008), thus significant results from the PERMANOVA should be cautiously interpreted.

In order to identify nekton habitat associations and seasonal abundance changes, we conducted an indicator species analysis (ISA) test using R (v4.3.0; R Core Team,

2022). The ISA generates indicator values (IV) for each species in each habitat or month and determines significance using the Monte Carlo randomization technique. The R packages “indicspecies” (De Cáceres & Legendre, 2009) and “dplyr” (Wickham et al., 2023) were used. Months were compared individually by using the “duleg” function in the “labdsv” package (Roberts, 2013), while habitats were compared together.

To identify the seagrass and environmental parameters that were most correlated with nekton assemblages, a Biota and Environmental Matching Routine (BIOENV) was conducted using PRIMER v7 (Clarke & Ainsworth, 1993). The seagrass parameters that were included in this test were *R. maritima* canopy height (mm), *H. wrightii* canopy height (mm), *R. maritima* percent cover, and *H. wrightii* percent cover that were all measured in the field. The environmental parameters included were water temperature (C°), DO (mg/L), salinity, Secchi depth (cm), mean water depth (cm), and the light attenuation coefficient (m⁻¹). Prior to performing the analysis seagrass and environmental parameters were checked for collinearity. Seagrass and environmental parameters were “normalized” by subtracting the mean and dividing by the standard deviation for each variable across all months (May, July, September, November) and an environmental matrix was calculated between samples (each station per month) using Euclidean distance. The nekton assemblage similarity matrix followed the same procedure described above for the PERMANOVA. Explanatory variables from the top BIOENV models were selected as the best predictor variables for general additive mixed effects models (GAMMs).

General Additive Mixed Effects Models (GAMM) were used to identify patterns in nekton community metrics (total nekton density, species richness, Shannon diversity) with four predictor variables identified through BIOENV analysis: *R. maritima* percent cover,

H. wrightii percent cover, *R. maritima* canopy height, and *H. wrightii* canopy height. Two GAMMs were formulated for each nekton community metric: one with percent cover and another for canopy height. A negative binomial distribution was used to model total nekton density, Poisson distribution was used to model species richness, and a Gaussian distribution was used to model Shannon diversity. Both density and richness were converted to a 1m² scale, which resembled count data and was right skewed. Negative binomial and Poisson distributions were chosen because they had the best fit diagnostics. Shannon diversity was log+1 transformed before beginning GAMM analysis, and a Gaussian distribution was used because the data were normally distributed.

1.3.3 Seagrass Habitat Metrics

Several metrics were calculated from the collected seagrass cores. The root to shoot ratio was calculated by dividing the dried root biomass by the dried aboveground biomass. Epiphyte biomass (g dry weight epiphyte / g dry weight seagrass) was calculated by dividing the total dried epiphyte biomass by the dried aboveground biomass they came from, and aboveground biomass (g) was recorded as the total dry biomass of all aboveground leaves and shoots after removing epiphytes. Individual seagrass leaves which were in poor condition (detached from roots and broken leaf tips) and could not be reliably identified were labeled as unknown, and the average percentage of unknown aboveground biomass across samples was 22.265% ± 1.980. The data were analyzed together and not by species due to the high ratio of unidentifiable biomass.

Differences in seagrass metrics between months were compared using the Kruskal–Wallis one-way analysis of variance test using the "stats" package in R (v4.3.0; R Core Team, 2022). Nonparametric methods were used because the data violated the test

assumption of homogeneity of variance for parametric analyses. Additionally, outliers were present, and nonparametric tests are more robust in accommodating this. If the main effect was identified as significant, a Dunn's test of multiple comparisons using rank sums with a Holm adjustment was conducted to determine differences between groups using the “DescTools” package in R (Signorell, et mult. al., 2017). Figures were plotted using “ggplot2” and “patchwork” packages.

1.4 Results

1.4.1 Environmental Parameters

Environmental parameters varied across month and location (Middle Bay or North Rigolets) (Table 2). Across all months and locations, water temperature ranged from 18.7 to 37.2 °C, salinity from 10.8 to 26, and DO from 4.1 to 11.4 mg/L. Mean water depth ranged from 18 to 120cm, with Secchi depth ranging from 20 to 100cm, and the light attenuation coefficient ranging from 0.353 to 9.253m⁻¹. Mean salinity was highest in November for both locations, although mean salinity in July at the North Rigolets was also high (Table 2). Mean DO was only marginally higher in November for Middle Bay and in September for the North Rigolets compared to all other months, respectively (Table 2). Secchi depth was lowest on average in September (46.7 cm), while the highest Secchi depth on average was recorded in July (67.1 cm) (Table 2). Water clarity was also recorded as the lowest on average in September with a light attenuation coefficient of 4.2m⁻¹, and the highest water clarity on average was recorded in May with a coefficient of 1.6m⁻¹ (Table 2).

Comparison of environmental parameters across months and locations indicated that temperature differed between months ($p < 0.001$), locations ($p < 0.05$), and their

interaction ($p < 0.001$). Similarly, salinity was different across months ($p < 0.001$), locations ($p < 0.001$), and their interaction ($p < 0.001$). Meanwhile, DO was significantly different between months ($p = 0.05$) and the interaction between months and locations ($p < 0.05$) but not for the main effect of location ($p > 0.05$).

Tukey post-hoc comparisons were used to identify differences in temperature, salinity, and DO between months and locations. Water temperature was similar between both locations for all months ($p > 0.05$) except November ($p < 0.001$), where Middle Bay was slightly warmer compared to North Rigolets (Figure 6). Notably, both locations had the lowest average water temperature in November, while July and September had the highest (Figure 6). Additionally, Middle Bay and North Rigolets had similar water temperatures for July and September ($p > 0.05$), while May and November water temperatures differed from all other months for the two locations ($p \leq 0.001$).

Within Middle Bay, salinities were similar in May and July but differed across all other months ($p < 0.001$). By comparison, salinity within North Rigolets was different across all months ($p < 0.001$). Salinities for Middle Bay and North Rigolets were similar during May and November ($p > 0.05$) but differed during July and September ($p < 0.001$). During November, Middle Bay sites had significantly higher DO compared to July ($p < 0.05$) and September ($p < 0.05$). Additionally, DO at North Rigolets was significantly higher in September compared to DO in Middle Bay during July ($p < 0.05$) and September ($p = 0.05$) (Figure 6.)

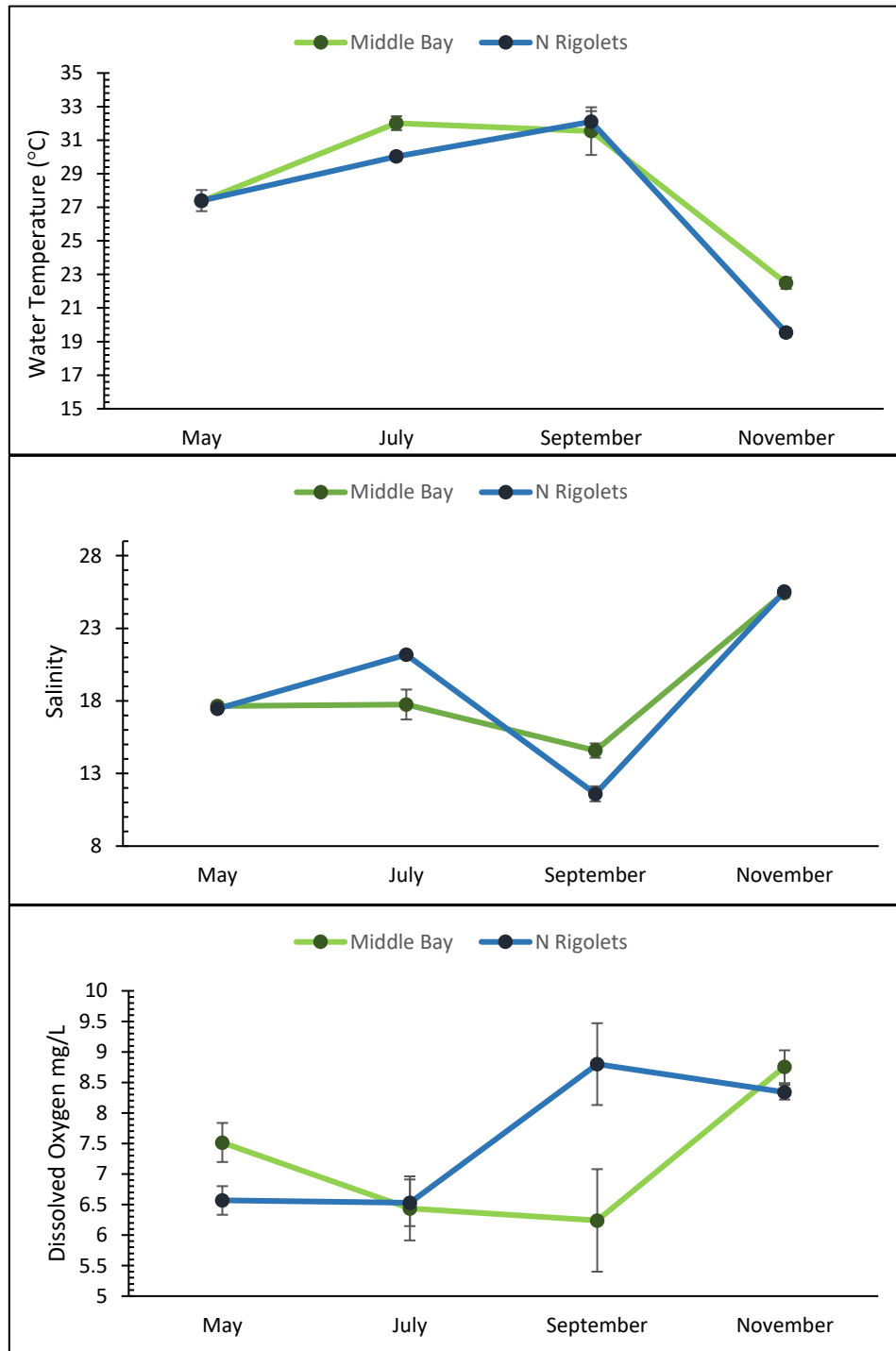


Figure 6. The average water temperature (°C) (top), salinity (middle), and dissolved oxygen (mg/L) (bottom) in Middle Bay and North Rigolets in the Grand Bay NERR \pm SE. Two values with the same letter indicate the values are not significantly different from each other. The temperature dataset was transformed with a natural log.

Table 2. Mean salinity, water temperature, dissolved oxygen, Secchi depth (cm), water depth (cm), and light attenuation coefficient (m^{-1}) \pm the standard error for Middle Bay and North Rigolets sites during all sampling periods. November had the highest salinity on average, while September had the lowest. Water temperature was warmest on average in September and coolest in November. DO averages ranged from 6.24 mg/L to 8.76. Secchi depth averages ranged from 41.167 cm to 74.167 cm. Lowest average water depth was in November and highest was in May. Water clarity was the highest on average in May (Middle Bay) and lowest in September (Middle Bay).

Month	Location	Mean Salinity \pm SE	Mean Temp ($^{\circ}C$) \pm SE	Mean DO (mg/L) \pm SE	Mean Secchi Depth (cm) \pm SE	Mean Water Depth (cm) \pm SE	Mean Light Attenuation Coef. (m^{-1}) \pm SE
May	Middle Bay	17.65 \pm 0.270	27.367 \pm 0.239	7.52 \pm 0.320	74.167 \pm 7.791	96.944 \pm 1.287	1.564 \pm 0.386
May	N. Rigolets	17.45 \pm 1.031	27.4 \pm 0.415	6.57 \pm 0.525	69.375 \pm 11.894	75.125 \pm 5.028	3.170 \pm 0.615
July	Middle Bay	17.75 \pm 0.493	32.01 \pm 1.420	6.44 \pm 0.839	48 \pm 5.831	72.533 \pm 7.337	2.944 \pm 0.986
July	N. Rigolets	21.17 \pm 0.296	30.03 \pm 0.341	6.53 \pm 0.269	65.429 \pm 4.017	67.857 \pm 2.526	1.825 \pm 0.329
Sept.	Middle Bay	14.58 \pm 0.220	31.54 \pm 0.630	6.24 \pm 0.235	41.167 \pm 1.302	91.833 \pm 4.408	4.155 \pm 1.737
Sept.	N. Rigolets	11.6 \pm 0.152	32.1 \pm 0.259	8.8 \pm 0.382	71 \pm 4.711	80.714 \pm 3.457	2.895 \pm 0.173
Nov.	Middle Bay	25.43 \pm 0.522	22.49 \pm 0.631	8.76 \pm 0.670	46 \pm 1.633	64.1 \pm 2.313	2.004 \pm 0.328
Nov.	N. Rigolets	25.52 \pm 0.037	19.54 \pm 0.258	8.34 \pm 0.121	52 \pm 2	57.667 \pm 3.201	1.847 \pm 0.145

1.4.2 Nekton Community Dynamics

A total of 2,568 individuals were collected over the four sampling months, with the dataset comprised of 82% crustaceans across 9 species and 18% fishes across 25 species (Table 4). 98% of all nekton were collected from vegetated habitats (both *R. maritima* and *H. wrightii* together). The four most abundant crustaceans were: *Palaemon mundusnovus* (brackish grass shrimp), *Palaemon pugio* (daggerblade grass shrimp), *Penaeus aztecus* (brown shrimp), and *Callinectes sapidus* (blue crab). The four most abundant fish species were: *Anchoa mitchelli* (bay anchovy), *Ctenogobius boleosoma* (darter goby), *Lagodon rhomboides* (pinfish), and *Symphurus plagiusa* (black cheek tonguefish). Species richness ranged from 0 to 12, with the highest average species richness occurring in July in *R. maritima*, and lowest in May in unvegetated bottom. Nekton total densities ranged from 0 to 284 individuals/m². Highest average total nekton densities were recorded in July for both *H. wrightii* and *R. maritima*, while lowest densities were consistently recorded in unvegetated bottom. Shannon diversity ranged from 0 to 1.970, with the highest overall average Shannon diversity recorded in November in *R. maritima*, while *H. wrightii* and unvegetated bottom had the highest average Shannon diversity in September (Table 3).

Nekton assemblages at the species level differed significantly by month (pseudo-F = 4.146, $p < 0.001$), habitat type (pseudo-F = 33, $p < 0.001$), and their interaction (pseudo-F = 2.0478, $p < 0.001$) based on the PERMANOVA analysis. Habitat type showed the strongest separation in nekton assemblage structure as indicated through a larger pseudo-F value compared to month and the interaction between month and habitat type. These results were further supported through the nMDS plots which showed a clear separation between

vegetated (*R. maritima* and *H. wrightii* together) and unvegetated sites, whereas month-to-month separations were less prominent (Figure 7).

Pairwise post-hoc comparisons were used to further investigate differences in nekton community species composition for the main effects of month and habitat type as well as their interaction with comparisons being made across months within each habitat type, and for habitat types within each month. Comparisons across months suggested that nekton community species composition was significantly different between months ($p \leq 0.042$). Additionally, comparisons across habitat types identified that *R. maritima* and *H. wrightii* nekton species composition were both significantly different from unvegetated bottom ($p < 0.001$), and also different from each other ($p = 0.014$). Assessment of the interaction between month and habitat type showed that within *R. maritima* habitat, nekton community compositions across all months were significantly different from each other ($p \leq 0.043$) besides May and July ($p = 0.0687$). Within *H. wrightii* habitat, nekton community compositions were only different between May and September ($p = 0.015$) and July and September ($p = 0.009$). Nekton communities for unvegetated sites were not significantly different between any paired months ($p > 0.05$). Meanwhile, pairwise tests indicated that nekton community species compositions were different between vegetated and unvegetated habitats within each month, with marginally significant differences between *R. maritima* and *H. wrightii* only occurring during November ($p = 0.0463$). The PERMDISP test for homogeneity of multivariate dispersion was non-significant for the main effect of month ($F = 0.285$, $p = 0.8837$). However, the PERMDISP test was significant for habitat types ($F = 8.599$, $p = 0.002$), indicating heterogeneity of multivariate

dispersions and therefore respective significant PERMANOVA results should be cautiously interpreted (Anderson et al., 2008; Rakocinski et al., 2023).

Table 3. The mean total nekton density, species richness, and Shannon diversity per m² ± SE for each habitat type during each sampling month. Highest mean species richness was recorded in R. maritima (RM) in July, lowest was recorded in unvegetated bottom (UVB) in May. Highest mean total nekton densities were recorded in July for both H. wrightii (HW) and R. maritima. Lowest densities were consistently recorded in UVB. Overall highest mean Shannon diversity was recorded in November in RM, while HW and UVB had the highest Shannon diversity in September.

Habitat	Month	Mean Total Nekton Density	Mean Richness	Mean Shannon Diversity
HW	May	27.75 ± 4.644	5.75 ± 0.75	1.163 ± 0.092
RM	May	81 ± 27.316	8 ± 0.707	1.458 ± 0.102
UVB	May	11.75 ± 11.75	0.25 ± 0.25	0 ± 0
HW	July	125.4 ± 47.962	7.4 ± 0.872	1.127 ± 0.123
RM	July	129 ± 19.837	8.2 ± 1.281	1.257 ± 0.087
UVB	July	0.8 ± 0.374	0.8 ± 0.374	0.139 ± 0.139
HW	Sept.	38.2 ± 23.451	6.6 ± 1.435	1.398 ± 0.110
RM	Sept.	46 ± 20.729	7 ± 1.549	1.365 ± 0.234
UVB	Sept.	1.4 ± 0.678	1.4 ± 0.678	0.439 ± 0.269
HW	Nov.	38.5 ± 2.5	5.5 ± 0.5	1.132 ± 0.251
RM	Nov.	60.6 ± 10.966	7.6 ± 0.812	1.570 ± 0.066
UVB	Nov.	0.4 ± 0.245	0.4 ± 0.235	0 ± 0

Table 4. Fish and crustaceans collected in each habitat type (*Ruppia maritima*, *Halodule wrightii*, unvegetated bottom) and month (May, July, September, November). Fish and crustaceans are ordered from most abundant to least abundant. The most abundant fish species was *Anchoa mitchelli* (bay anchovy) and the most abundant crustacean was *Palaemon mundusnovus* (brackish grass shrimp).

Species	Total	Habitat			Month			
		<i>R. maritima</i>	<i>H. wrightii</i>	UVB	May	July	Sept.	Nov.
Fish								
<i>Anchoa mitchelli</i>	199	12	139	48	54	0	117	28
<i>Ctenogobius boleosoma</i>	84	70	14	0	2	8	19	55
<i>Lagodon rhomboides</i>	41	32	9	0	27	10	4	0
<i>Symphurus plagiusa</i>	38	28	10	0	2	4	13	19
<i>Bairdiella chrysoura</i>	26	19	7	0	10	7	8	1
<i>Syngnathus scovelli</i>	15	10	3	2	2	6	7	0
<i>Lucania parva</i>	11	8	3	0	0	11	0	0
<i>Gobiosoma bosc</i>	10	9	1	0	0	7	0	3
<i>Membras martinica</i>	8	8	0	0	8	0	0	0
<i>Latreutes parvulus</i>	4	4	0	0	4	0	0	0
<i>Cynoscion nebulosus</i>	3	2	1	0	0	1	2	0
<i>Menidia beryllina</i>	3	1	2	0	1	2	0	0
<i>Myrophis punctatus</i>	3	0	3	0	0	0	1	2
<i>Anchoa lyolepis</i>	2	1	1	0	0	0	2	0
<i>Citharichthys spilopterus</i>	2	0	2	0	2	0	0	0
<i>Erotelis smaragdus</i>	2	2	0	0	0	0	0	2
<i>Gobiosoma robustum</i>	2	2	0	0	1	0	0	1
<i>Syngnathus louisianae</i>	2	0	2	0	0	0	2	0
<i>Eucinostomus argenteus</i>	1	0	0	1	0	0	1	0
<i>Hippocampus zosterae</i>	1	0	0	1	0	0	0	1
<i>Lutjanus griseus</i>	1	0	1	0	0	1	0	0
<i>Menidia peninsulae</i>	1	0	0	1	0	0	1	0
<i>Microgobius guiosus</i>	1	1	0	0	0	0	0	1
<i>Ophidiidae sp.</i>	1	1	0	0	0	0	0	1
<i>Opsanus beta</i>	1	1	0	0	0	1	0	0
Total Fishes	462	211	198	53	113	58	177	114
Crustaceans								
<i>Palaemon mundusnovus</i>	730	441	289	0	130	402	116	82
<i>Palaemon pugio</i>	535	335	199	1	53	473	9	0
<i>Penaeus aztecus</i>	416	208	205	3	146	175	61	34
<i>Callinectes sapidus</i>	267	191	75	1	35	77	31	124
<i>Penaeus setiferus</i>	87	81	5	1	1	80	4	2
<i>Palaemon vulgaris</i>	68	34	33	1	4	11	28	25
<i>Acetes americanus carolinae</i>	1	1	0	0	0	0	1	0
<i>Hippolytidae spp.</i>	1	0	1	0	0	0	1	0
<i>Rimapenaeus constrictus</i>	1	0	1	0	0	0	0	1
Total Crustaceans	2106	1291	808	7	369	1218	251	268
Grand Total	2568	1502	1006	60	482	1276	428	382

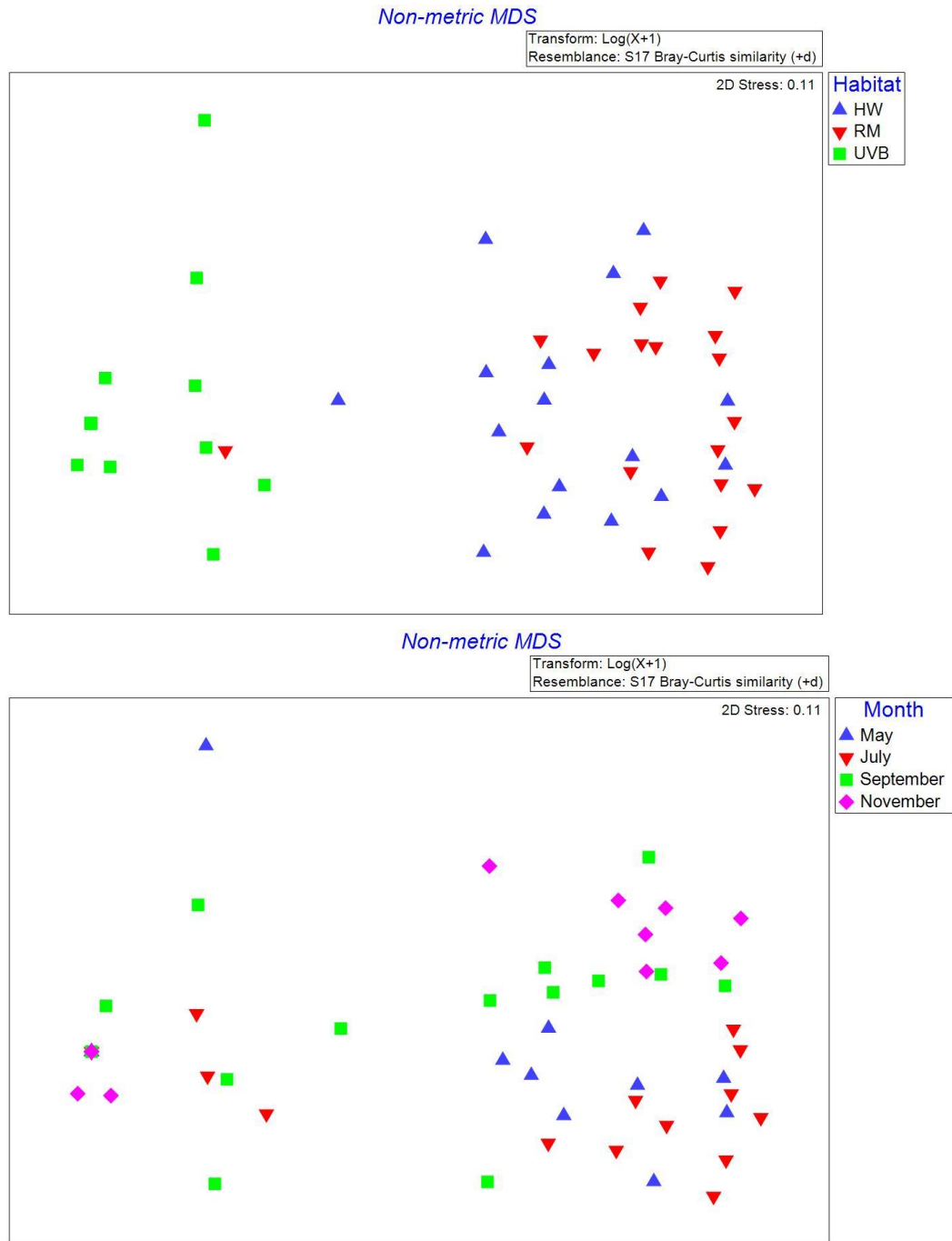


Figure 7. NMDS plots showing group similarities between habitat types (top) and months (bottom). The habitats are: *Ruppia maritima* (RM, red downward triangle), *Halodule wrightii* (HW, blue upright triangle), and unvegetated bottom (UVB, green square). The months are May (blue upright triangle), July (red downward triangle), September (green square), and November (pink diamond). Data was transformed with a log x+1 transform. Points that are close together indicate that they are similar in terms of nekton abundance and composition.

The ISA indicated there were species that characterized specific months, as well as habitats. May was characterized by *Lagodon rhomboides* only. July was characterized by *Palaemon pugio*, *Lucania parva*, and *Penaeus setiferous*. November was characterized by *Ctenogobius boleosoma* only, while September did not have any characterizing species. The ISA for habitat types indicated that *R. maritima* alone had indicator species of *Penaeus setiferus* and *Gobiosoma bosc*, while *H. wrightii* and unvegetated bottom respectively did not have any indicator species. Both *R. maritima* and *H. wrightii* habitats together had several characterizing species including *Penaeus aztecus*, *Callinectes sapidus*, *Ctenogobius boleosoma*, *Palaemon pugio*, *Palaemon mundusnovus*, *Symphurus plagiusa*, *Palaemon vulgaris*, *Bairdiella chrysoura*, and *Lagodon rhomboides*.

1.4.3 Influential Environmental Parameters

The BIOENV identified that out of the 10 environmental variables tested, the top four correlated variables were *R. maritima* canopy height, *H. wrightii* canopy height, percent cover *R. maritima*, and percent cover *H. wrightii* (Table 5). An additional BIOENV was also performed without seagrass variables to compare correlation values, and the best model for abiotic variables only had a very poor correlation (mean water temperature correlation = 0.115).

Table 5. The best BIOENV results from PRIMER showing which environmental variables (up to 4 variables) best explained the patterns observed in the nekton communities observed in the Grand Bay NERR. The best variables included: *Ruppia maritima* (RM) canopy height and percent cover, and *Halodule wrightii* (HW) canopy height and percent cover.

No. of Variables	Correlation	Variable Combinations
1	0.467	RM Canopy Height
2	0.514	HW Canopy Height, % RM
3	0.52	RM Canopy Height, HW Canopy Height, % RM
4	0.51	RM Canopy Height, HW Canopy Height, % RM, % HW

1.4.4 Patterns in Nekton and Seagrass Metrics

The generalized additive mixed model (GAMM) investigating percent cover influence on nekton density showed that both *R. maritima* and *H. wrightii* percent cover were significant predictor variables ($\chi^2 = 78.613$, $\chi^2 = 49.167$, $p < 0.05$) (Table 6). By contrast, the model investigating canopy height influence on nekton density identified that neither seagrass species' canopy height was a significant predictor ($\chi^2 = 3.591$, $\chi^2 = 1.970$, $p > 0.05$).

The GAMM model exploring percent cover influence on nekton species richness identified that both *R. maritima* and *H. wrightii* percent cover were significant predictor variables ($\chi^2 = 89.63$, $\chi^2 = 66.78$, $p < 0.05$) (Table 7). Conversely, the model focused on canopy height identified that neither seagrass species' canopy height significantly predicted nekton species richness ($\chi^2 = 2.286$, $\chi^2 = 0.240$, $p > 0.05$).

The GAMM model examining the influence of percent cover on Shannon diversity identified that both *R. maritima* and *H. wrightii* percent cover were significant predictor variables ($F = 113.196$, $F = 64.684$, $p < 0.05$) (Table 8). The model investigating the

influence of canopy height on Shannon Diversity found that canopy height of *H. wrightii* was a marginally significant predictor ($F = 4.603$, $p = 0.045$) while *R. maritima* canopy height was not significant ($F = 0.919$, $p > 0.05$).

Table 6. GAMM output for potential drivers of total nekton density. Only % cover of *R. maritima* and % cover of *H. wrightii* were significant. *Value significant (p<0.05)

Variable	χ^2	p
% cover <i>R. maritima</i>	78.613	< 0.001*
% cover <i>H. wrightii</i>	49.167	< 0.001*
Canopy height <i>R. maritima</i>	3.591	0.215
Canopy height <i>H. wrightii</i>	1.970	0.161

Table 7. GAMM output for potential drivers of nekton species richness. Only % cover of *R. maritima* and % cover of *H. wrightii* were significant. *Value significant (p<0.05)

Variable	χ^2	p
% cover <i>R. maritima</i>	89.63	<0.001*
% cover <i>H. wrightii</i>	66.78	<0.001*
Canopy height <i>R. maritima</i>	2.286	0.458
Canopy height <i>H. wrightii</i>	0.240	0.624

Table 8. GAMM output for potential drivers of Shannon diversity. % cover of *R. maritima*, % cover of *H. wrightii*, and canopy height of *H. wrightii* were significant. *Value significant (p<0.05)

Variable	F	p
% cover <i>R. maritima</i>	113.196	<0.001*
% cover <i>H. wrightii</i>	64.684	<0.001*
Canopy height <i>R. maritima</i>	4.603	0.3786
Canopy height <i>H. wrightii</i>	0.919	0.0451*

1.4.5 Patterns in Seagrass Vegetation

The range of the root to shoot ratio varied by month (Table 9). In May it ranged from 0–5.306, in July from 0–6.946, in September from 0–11.2, and in November from 0–9.929 (Figure 8). Epiphyte biomass ranged from 0–1.7 g dry weight epiphyte / g dry weight seagrass in May, 0–0.753 g dry weight epiphyte / g dry weight seagrass in July, 0–10 g dry weight epiphyte / g dry weight seagrass in September, and 0–0.570 g dry weight epiphyte / g dry weight seagrass in November (Figure 9). Lastly, aboveground biomass ranged from 0–145.312 g/m² in May, 0–120.623 g/m² in July, 0.014–297.987 g/m² in September, and 0.339–30.826 in November (Figure 10).

The root to shoot ratio differed significantly between months ($p < 0.001$; chi-square = 19.458 with 3 degrees of freedom). The post-hoc Dunn's test indicated that root to shoot ratio in November was significantly different from May, July, and September (Holm-adjusted $p < 0.01$). Epiphyte biomass differed significantly between months ($p < 0.001$; chi-square = 16.66 with 3 degrees of freedom). Pairwise post-hoc comparisons indicated that epiphyte biomass in May was significantly different from September and November (Holm-adjusted $p < 0.05$). Aboveground biomass differed significantly between months ($p < 0.01$; chi-square = 13.74 with 3 degrees of freedom). Post-hoc comparisons identified that aboveground biomass in July was significantly different from May and November (Holm-adjusted $p < 0.05$).

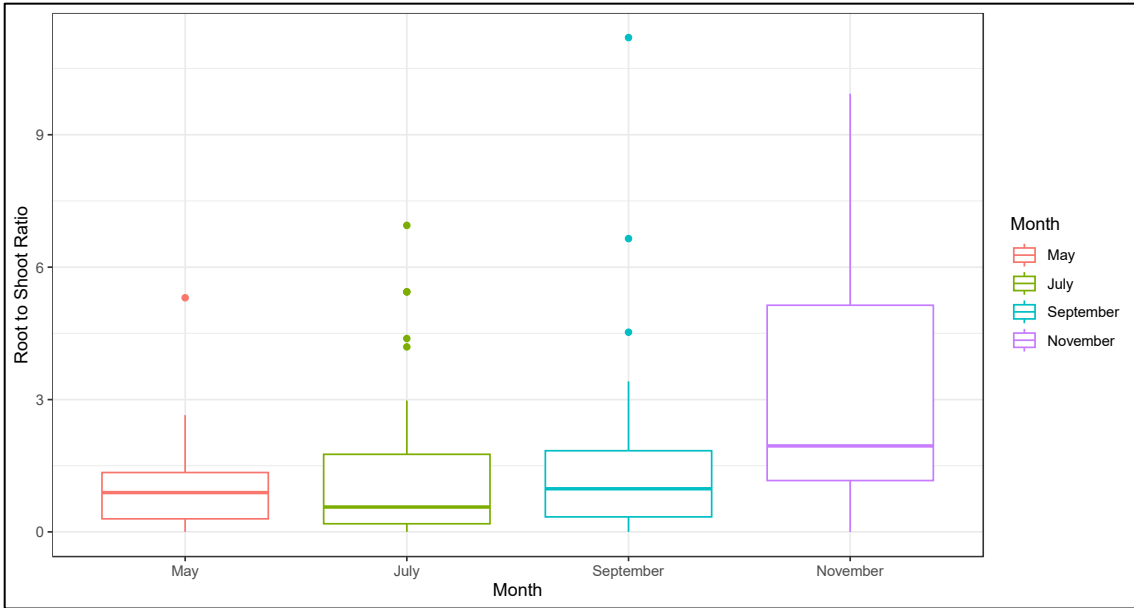


Figure 8. The root to shoot ratios for all seagrasses combined, by month. Points on the plots indicate the values for individual cores. Higher values indicate that more biomass was found in the roots compared to the shoots. November had the highest median root to shoot ratio while July had the lowest.

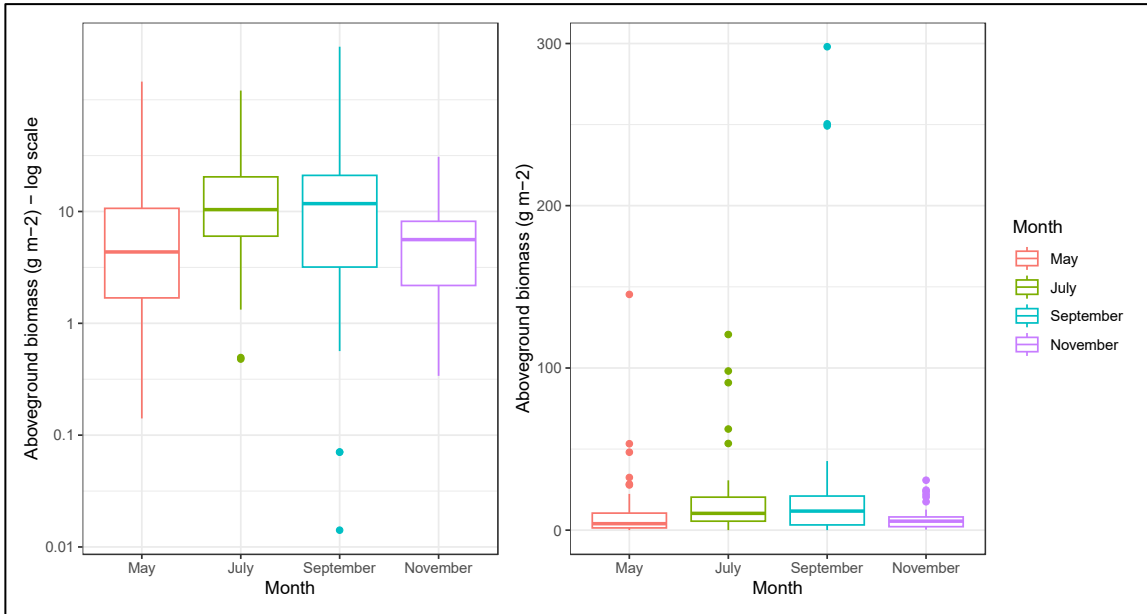


Figure 9. The epiphyte biomass for all seagrasses combined, by month. Points on the plots indicate the values for individual cores. The right plot shows epiphyte biomass (g dry weight epiphyte/ g dry weight seagrass) raw values, and the left plot also shows epiphyte biomass, but note the log y axis. Both are provided for understanding the magnitude of outliers (right) and displaying the variation without losing the outliers (left). May had the highest median epiphyte biomass, while November had the lowest.

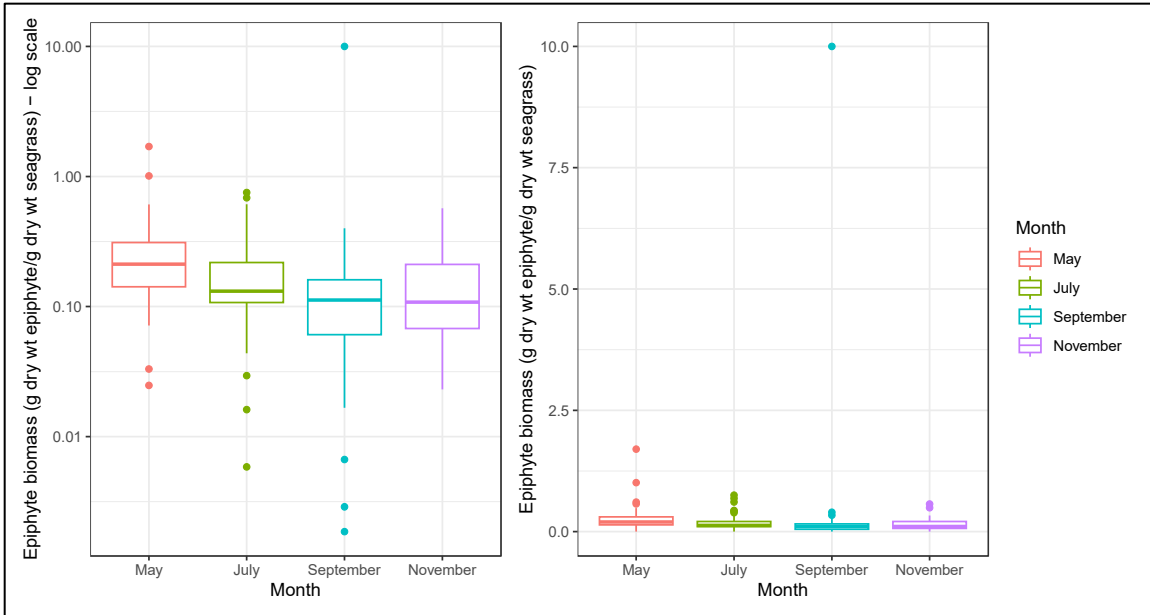


Figure 10. The aboveground biomass for all seagrasses combined, by month. Points on the plots indicate the values for individual cores. The right plot shows aboveground biomass (g m⁻²) raw values, and the left plot also shows aboveground biomass, but note the log y axis. Both are provided for understanding the magnitude of outliers (right) and displaying the variation in the data without losing the outliers (left). September had the highest median aboveground biomass, while May had the lowest.

Table 9. Mean seagrass core metrics by month including the root to shoot ratio, epiphyte biomass, and aboveground biomass. These are the metrics for all species present in the core. The data show the average value \pm the standard error.

Metric	May	July	Sept.	Nov.
Root to Shoot Ratio \pm SE	0.994 \pm 0.153	1.277 \pm 0.213	1.452 \pm 0.262	2.91 \pm 0.412
Epiphyte Density \pm SE	0.271 \pm 0.046	0.181 \pm 0.022	0.308 \pm 1.94	0.154 \pm 0.022
Aboveground Biomass (g m ⁻²) \pm SE	15.771 \pm 5.036	18.551 \pm 3.262	27.294 \pm 8.611	7.703 \pm 1.312

1.4.6 Shrimp Growth Experiment Summary

At the conclusion of the 14-day shrimp growth experiment, 13 out of 15 cages remained; however, of the 13 remaining cages, shrimp were only recovered from six cages. A total of 31 shrimp out of an initial 129 tagged shrimp were recovered. The low number of occupied cages (n=6) remaining at the conclusion of the experiment and the low number of shrimp (n=31) recovered did not allow for analysis of the data, as it is standard across growth studies to analyze the mean growth of animals per cage (Hayes et al., 2022; Rozas & Minello, 2011). Four of the six remaining cages were at *H. wrightii* sites, with just one replicate each for *R. maritima* and unvegetated bottom, thus further analyses beyond mean growth per cage could not be computed. Growth of individual shrimp over the two-week period ranged from 5 to 14 mm (Table 11).

On September 16th, 2023, we returned to the cages for the mid-point check. Each cage was identified and assessed. Several cages needed to be reattached to rebar and several had collapsed and needed to be set back upright. Additionally, erosion occurred underneath many of the cages, creating openings between the bottom fiberglass rings and the sediment. These holes were filled-in to the best of our ability and all minimally collapsed cages were set back up. At this time, one unvegetated cage was unable to be located (UVB2) and one cage had significantly collapsed and had to be fully removed (UVB3).

On September 22nd, 2023, at the conclusion of the experiment, cages were retrieved, and remaining shrimp were removed. At this time, we were still unable to locate the cage from UVB3. All cages were treated as though shrimp were present, although several had significantly collapsed again or had large, eroded holes between the bottom fiberglass ring and sediment. Although the number of shrimp and cages retrieved was insufficient for

statistical analysis, all except two recovered shrimp had grown during the two-week period (Table 10).

The cages placed in Middle Bay were much more successful than those placed further south along the North Rigolets Island. It was observed that the southernmost cages received stronger wave action, and the sediment was much more prone to erosion underneath the cages, potentially leading to shrimp escape and predator entry. Middle Bay sediment was observed to be muddier, which made it easier to bury the bottom of cages and lessen the chance of erosion.

If this project were to be repeated, a pilot study should be first conducted to ensure that the equipment used can withstand the conditions in this area at the Grand Bay NERR. The outcome of this experiment suggests that stronger cages and anchoring mechanisms are needed. Although there is seagrass present south along the North Rigolets Island, the area is very exposed, and it is unlikely that a cage of the design used here would be able to withstand the wave action. Middle Bay is likely a better location, should another experiment such as this be attempted, although a different cage design would likely still be needed given that some areas with seagrass in Middle Bay are also exposed to wave action. Overall, there was much to be learned from the failure of this study which can provide insight for future endeavors in the Grand Bay NERR.

Table 10. Recovered brown shrimp from six sites including four *Halodule wrightii* (HW), one *Ruppia maritima* (RM), and one unvegetated bottom (UVB). Colors are denoted as: blue (B), blue-yellow (BY), red (R), red-yellow (RY), orange (O), orange-yellow (OY), green (G), green-yellow (GY), pink (P), and pink-yellow (PY). The measurements include their starting and ending total length (TL) and growth/14 days.

Site ID	Habitat	Color	Start TL (mm)	End TL (mm)	Growth (mm)
S1	HW	BY	32	46	14
S3	HW	R	21	28	7
S3	HW	RY	35	44	9
S3	HW	O	28	36	8
S3	HW	OY	21	30	9
S3	HW	G	22	28	6
S3	HW	B	40	45	5
S3	HW	BY	36	42	6
S4	HW	R	28	35	7
S4	HW	RY	28	33	5
S4	HW	O	31	36	5
S4	HW	OY	33	40	7
S4	HW	G	26	33	7
S4	HW	GY	28	39	11
S4	HW	BY	35	40	5
S5	HW	R	28	28	0
S5	HW	RY	24	30	6
S5	HW	O	32	40	8
S5	HW	G	25	30	5
S5	HW	B	22	30	8
S5	HW	BY	31	42	11
S5	HW	PY	36	43	7
S5	HW	PY	35	41	6
UVB5	UVB	R	26	26	0
UVB5	UVB	RY	29	38	9
UVB5	UVB	O	22	29	7
UVB5	UVB	G	35	42	7
UVB5	UVB	B	36	43	7
UVB5	UVB	BY	27	35	8
W5	RM	BY	31	31	0
W5	RM	P	24	34	10

Table 11. Mean growth of brown shrimp from six sites including four *Halodule wrightii* (HW), one *Ruppia maritima* (RM), and one unvegetated bottom (UVB). The mean growth includes the difference in their starting and ending total length (TL) per 14 days \pm standard error.

Habitat	Site #	# Individuals	Average Growth (mm)
HW	S1	1	14 \pm 0
HW	S3	7	7.143 \pm 0.225
HW	S4	7	6.714 \pm 0.808
HW	S5	8	6.375 \pm 1.117
UVB	UVB5	6	6.33 \pm 1.308
RM	W5	2	5 \pm 5

1.5 Discussion

The goal of this study was to assess the use of *R. maritima*, *H. wrightii*, and unvegetated bottom as habitat for nekton in the Grand Bay NERR. We also sought to identify potential drivers of habitat use and investigate monthly differences. Habitat type was the strongest factor influencing nekton habitat use. Pairwise comparisons indicated that nekton usage differed significantly between vegetated and unvegetated habitats. These results are similar to those from previous studies in the Gulf of Mexico and other areas that have investigated habitat use in vegetated and unvegetated areas (Heck et al., 2003; Kanouse et al., 2006; Orth & van Montfrans, 1990; Scott-Denton, 1998). A previous study conducted in the Grand Bay NERR reported that other types of vegetation, such as marsh edge, are also more valuable habitats for nekton than unvegetated bottom, as vegetated marsh edge had greater animal densities than unvegetated bottom (Shervette & Gelwick, 2008b).

Although nekton species composition was significantly different between *H. wrightii* and *R. maritima*, this was driven by a significant difference in November only. It

is important to note that in November, *H. wrightii* and *R. maritima* had begun a seasonal die-back, and we were only able to locate two *H. wrightii* sites, compared to five sites each for *R. maritima* and unvegetated bottom. We conclude that the marginal difference between *R. maritima* and *H. wrightii* during November is likely driven by the unbalanced sample design rather than true differences, as other months were not significantly different from each other in terms of nekton usage. These results are similar to those of Scott-Denton (1998) who conducted a similar study in Galveston Bay, Texas comparing faunal densities between *R. maritima* and *H. wrightii* and reported that these two species were not different in terms of overall faunal density, and that vegetated sites supported more animals than unvegetated. They did, however, find that *H. wrightii* supported more fish species than *R. maritima*. In contrast, we found that *H. wrightii* and *R. maritima* supported a similar number of fish species and total fish abundances.

Differences in the structural complexity of seagrass can impact their use as habitat (Bell & Westoby, 1986; Gullström et al., 2008; Jones et al., 2021). Characteristics like canopy height, epiphyte load, and percent cover all influence the structural complexity of a seagrass bed, and higher structural complexity has been linked to higher animal abundance, species richness, and diversity (Bell & Westoby, 1986; Gartner et al., 2013; Gullström et al., 2008; Heck et al., 2003; Hori et al., 2009). *R. maritima* and *H. wrightii* commonly grow together in the Gulf of Mexico (Cho et al., 2017; Pulich, 1985; Rozas & Minello, 1998), and their morphologies are relatively similar, with the exception of *R. maritima*'s reproductive shoot structure. The seagrass beds at the Grand Bay NERR are relatively well mixed with both species of seagrass, and their morphologies are similar,

making them appear as a cohesive environment for nekton. Therefore, it is likely that their habitat usage, even in single species-dominant patches, would be similar.

The nekton communities observed in the seagrasses varied between months. For *R. maritima*, May and July assemblages were similar, but both differed from September and November assemblages. For *H. wrightii*, May and July assemblages differed from September. This pattern in the nekton communities supports the concept that different species of nekton have peaks in abundance at different times of the year due to their lifecycle and reproduction. For example, blue crabs typically mate in the fall, and other studies have observed peaks in zoea and juveniles in late summer and fall (Milliken & Williams, 1984; Perry, 1975; Thomas et al., 1990). Similarly, in this study blue crabs were most abundant in November.

The indicator species analysis identified nekton species that were more abundant in certain months, and those that had significant habitat associations. *L. rhomboides* was most abundant in May, and this species is known to have a fall-winter-spring reproductive season in the Gulf of Mexico, possibly leading to many juveniles around May (Darcy, 1985). *P. setiferous* was most abundant in July, which aligns with what previous literature identified as their peak season in the Gulf of Mexico, but we did not witness a common second peak in September, as September did not have any indicator species (Farfante, 1969). *P. pugio*, another species that peaked in July, has a typical spawning season of February to October, but can spawn year-round in some areas of the Gulf (Anderson, 1985). There is some variability in when their densities are highest in the Gulf of Mexico (Cházaro-Olvera, 2009), which could be due to this long spawning phase, but previous studies at the Grand Bay NERR indicated that *P. pugio* peaked in the summer (Shervette

et al., 2011; Shervette & Gelwick, 2008b). *L. parva* was the final characterizing species for July. This species is present all year in the Gulf of Mexico, but there can be peaks in their abundance. In Texas, their spawning period is from May to June with the highest species densities in July, but in general *L. parva* has been known to mate in spring and summer (Crawford & Balon, 1994; Gunter, 1950; Talbot & Able, 1984) and other local studies have noted their high abundance in the summer (Duffy & Baltz, 1998; Kanouse et al., 2006). Our data indicate a similar July peak for *L. parva* in the Grand Bay NERR system.

In contrast to May and July, November only had one characterizing species, which was *C. boleosoma*. A previous study at Grand Bay NERR reported that *C. boleosoma* was most common in early October compared to late May and late July (Shervette & Gelwick, 2008b). Similarly, our data suggest that there was a peak in their abundance in November. *C. boleosoma* is one of the most common species of goby in the Mississippi Sound and neighboring estuaries (Dawson, 1969). This goby species spawns during the summer and fall (Wyanski & Targett, 2000), so this peak in their abundance observed in November could be an increase in reproductive individuals and developed juveniles.

Several species were good indicators of vegetated sites, as nekton were found most commonly in these areas compared to unvegetated sites, which did not have any indicator species. Among those with significant habitat associations with seagrass were commercially valuable species like *C. sapidus* (blue crabs) and *P. aztecus* (brown shrimp). Seagrass beds are important nursery habitats for juveniles of *C. sapidus*, and *P. aztecus* is known reside in seagrass beds (Heck et al., 1997; Heck & Orth, 1980; Orth & van Montfrans, 1990; Weinstein & Brooks, 1983).

Among the other indicator species for vegetated areas were three *Palaemon* species, which are known to feed in seagrasses; the presence of these shrimp can also increase the biomass and shoot density of the seagrasses they inhabit (Drury-McCall & Rakocinski, 2007; Rozas & Minello, 1998). *L. rhomboides*, *B. chrysourea*, *C. boleosoma*, and *S. plagiusa* are all common fishes found in seagrasses (Belgrad et al., 2021; Rozas & Minello, 1998), and unsurprisingly these were indicator species for vegetated sites. Additionally, the latter two species were among the most common fish captured in *R. maritima* beds at the Chandeleur Islands, Louisiana (Byrnes et al., 2022), highlighting how important these habitats are locally to these fish. Seagrass beds are also critical habitats for juveniles of *L. rhomboides* and *B. chrysourea* as they migrate into estuaries and submerged vegetation after birth (Grammer et al., 2009; Holt et al., 1985; Muncy, 1984).

There were two indicator species for *R. maritima* beds, *P. setiferus* (white shrimp) and *G. bosc* (naked goby), which are both commonly found in submerged vegetation. *P. setiferus* is known to utilize *R. maritima* beds (Howe & Wallace, 1999; Kanouse et al., 2006) and our data support that they frequently inhabit these beds at the Grand Bay NERR. Other studies have similarly found that *G. bosc* is more present in seagrass beds than unvegetated bottom but they can inhabit a variety of submerged vegetation species and are not exclusive to *R. maritima* (Duffy & Baltz, 1998; Rozas & Minello, 1998). There were no characterizing species found for *H. wrightii* or unvegetated bottom, which identifies that there were no nekton species that stood out as being different from the other habitats.

We investigated patterns between nekton community characteristics and seagrass metrics to evaluate potential drivers of habitat use in *H. wrightii* beds and *R. maritima* beds. We found that seagrass percent cover positively influenced total nekton density, species

richness, and Shannon diversity. Studies in Texas and Louisiana have similarly reported that increased coverage of vegetation can have positive effects on animal abundances (Belgrad et al., 2021; Hitch et al., 2011; Scott-Denton, 1998; Sheridan & Minello, 2003). Scott-Denton (1998) conducted similar analyses to identify drivers of faunal densities in *R. maritima* and *H. wrightii* and also reported that SAV coverage was the most important variable explaining animal densities. Additionally, seagrass biomass is known to influence nekton communities (Kanouse et al., 2006; Wyda et al., 2002), and percent cover captures a similar characteristic. Seagrass percent cover is an easily assessed metric that captures the living biomass present, and our results indicated that it had a significant positive influence on nekton communities in the Grand Bay NERR. In contrast, some studies have identified that percent cover was not influential in shaping nekton communities, and rather it is overall vegetation presence that drives nekton communities, with percent cover being less influential (Alsaffar et al., 2020; Heck et al., 2003; McDonald et al., 2016; Wyda et al., 2002). Additionally, the importance of seagrass percent cover can vary by species (Hitch et al., 2011; Rozas & Odum, 1987; Sheridan & Minello, 2003).

Canopy height of both *H. wrightii* and *R. maritima* did not influence nekton community characteristics, with the exception that *H. wrightii* canopy height was marginally influential for Shannon diversity. This is similar to other previous studies that did not find a significant positive link between seagrass canopy height and animal density, diversity, or community structure (Alsaffar et al., 2020; Gross et al., 2019). In other studies, however, canopy height has influenced nekton community characteristics (Belgrad et al., 2021; Hori et al., 2009; Ruesink et al., 2019), but it is possible that in this system the abundance of seagrass is more important than the height. It is also possible that the canopy

height of both seagrass species was similar enough to not cause nekton to develop a major preference for canopy height (Gross et al., 2019). These results indicate that other studies seeking to investigate differences between the habitat use by nekton in the Grand Bay NERR or other similar systems should focus on more robust seagrass metrics such as percent cover or biomass, rather than canopy height.

Environmental parameters collected in the field indicated that there were seasonal fluctuations in abiotic parameters, which is expected for this region (Cho et al., 2017). We saw seasonal changes in nekton communities, with some species characterizing different months. Many of the species we collected have seasonal reproduction patterns, which were likely influenced by the changing environmental conditions. For example, fluctuations in water temperature can be a cue for many fish species to spawn (Bolland & Boeticher, 2005; Montie et al., 2015; Pankhurst & Munday, 2011; Zucchetta et al., 2012). A local study in Mobile Bay reported a correlation between reproduction in *S. scovelli* and water temperatures, with warmer waters increasing species abundance and breeding (Bolland & Boeticher, 2005). Comparably, we found *S. scovelli* to be most abundant in July and September, which had the warmest water temperatures on average across our sampling months. Invertebrates can also follow this temperature pattern; *C. sapidus*, for example, spawns from May to October in Chesapeake Bay, but ovigerous females have been found as early as March in the Gulf of Mexico (Graham et al., 2012; Hill et al., 1989). Their mating season is related to seasonal patterns, with water temperature being an important factor to trigger this (Graham et al., 2012; Hill et al., 1989).

Another environmental parameter to note is the changes in salinity. The Grand Bay NERR system is brackish, but salinity did fluctuate between seasons as well as between

the locations we sampled. In July, we collected the highest number of *L. parva*, which is known to have a wide salinity range, however it is found more commonly in brackish waters (Fuller, 2008; Fuller et al., 2007; Hardy Jr, 1978; Ross et al., 2001). *L. parva* can survive in salinities between 0 and 48 and southernmost individuals are more likely to venture into saltier waters than their northern relatives (Akin et al., 2003; Hardy Jr, 1978). July had an average salinity of 17.75 and 21.7 depending on the location. This is in the middle of the salinity range for *L. parva*, and this species spawns in the spring and summer, making it logical to have a July peak given the conditions (Crawford & Balon, 1994; Gunter, 1950; Talbot & Able, 1984).

It is also important to note the environmental tolerances and optima of *R. maritima* and *H. wrightii*. *R. maritima* has a wide salinity tolerance from 0 to 70. (Kantrud, 1991; Murphy et al., 2003). *H. wrightii* has a salinity tolerance of 1 to 52 and is primarily found in estuarine and marine waters (Rivera-Guzmán et al., 2017b). Given that we saw a maximum salinity of 25, this falls well within the tolerance range of both species. *H. wrightii* is a tropical and sub-tropical species, and has been recorded in temperatures between 12 and 36 °C (Rivera-Guzmán et al., 2017b). *R. maritima* has an optimum water temperature between 18 and 30 °C, outside of which growth can be hindered (Joanen & Glasgow, 1965; Setchell, 1924). The hottest months we observed were July and September, with average temperatures between 30–32°C and 31–32°C, respectively. We saw both species thrive in the summer and fall, when sites had the highest average percent cover for *H. wrightii* and second highest for *R. maritima*, and July specifically had the highest abundance of nekton in seagrass. Previous research has similarly noted that *R. maritima* and *H. wrightii* have peaks in growth in the summer at the Grand Bay NERR (Cho et al.,

2017). The coolest month we sampled was November, with an average water temperature between 19–22°C. While this falls within both species' temperature tolerances, we did see significant die-back during November. All our sites were limited to Middle Bay in November because there was very little seagrass along North Rigolets Island. These seagrasses are known to slow their growth and die off in winter due to colder water temperatures and less light availability (Cho et al., 2017; Dunton, 1990). Additionally, it has been noted that wintertime low tides are much lower than in the summer (Cho et al., 2017), which limited our ability to sample in seagrass beds near the shore where many *H. wrightii*-dominated beds were located. We likely observed both the die off and tidal limitations during our November sampling, which made it difficult to find *H. wrightii*-dominated beds.

The seagrass cores we collected support the concept that seagrass was beginning to senesce in November. The root to shoot ratio was significantly higher in November compared to all other months, indicating there was more biomass underground than above. Cho et al. (2017) similarly identified that in winter at the Grand Bay NERR (November-February) *R. maritima* had the lowest total biomass and shoot biomass, indicating senescence.

In this study, aboveground biomass for seagrass peaked in September. Cho et al. (2017) reported that *R. maritima* aboveground biomass peaked in August in 2011 and June in 2013, and *H. wrightii* peaked in August 2011 and June 2013 in the Grand Bay NERR. While our peak in biomass was later than what Cho et al. (2017) reported, it can be inferred that these seagrasses have a late summer to fall peak in aboveground biomass, as they do begin to die off in the late fall (Cho et al., 2017; Dunton, 1990). Additionally, we found

that seagrass aboveground biomass was lowest on average in November, which follows a similar pattern witnessed by Cho et al. (2017) in the Grand Bay NERR where November, December, and January had the lowest shoot biomass.

We found the highest epiphyte biomass in September, which is different from what was found in *R. maritima* beds at the Chandeleur Islands, Louisiana in August-September 2022. Byrnes (2022) found that only 29% of cores had measurable epiphytes on the *R. maritima* blades during late summer/early fall. Additionally, another study from the Gulf of Mexico found that June was a peak for epiphyte biomass on *H. wrightii*, while July had about 30% less epiphytes (Morgan & Kitting, 1984). While our results are different from other local studies, we can conclude that seagrass epiphyte biomass likely peaks in the summer to early fall in the Grand Bay NERR.

The root to shoot ratio can be used to identify how a plant is allocating its resources. Cho et al. (2017) found that the combined root to shoot ratio was highest in August-September and January-February in the Grand Bay NERR. They also identified that both *R. maritima* and *H. wrightii* had very little root biomass in the winter (November-February). In contrast, we found that there was a significantly higher root to shoot ratio in November compared to all other months, indicating that the seagrasses had more belowground material than above.

1.6 Conclusion

This study is the first known comparison of habitat use between *R. maritima* and *H. wrightii* by nekton in the Grand Bay NERR. Our results indicate that *R. maritima* and *H. wrightii* serve similar roles as habitat in this system, with vegetated areas supporting more nekton than unvegetated areas. Vegetated habitats also had characterizing nekton

species, whereas unvegetated sites did not. Commercially important nekton occupied vegetated habitats in greater densities than unvegetated sites, and seagrass beds are known to be important juvenile and nursery habitats for many of them. There were some seasonal influences on the nekton in vegetated areas, indicating that a variety of animals occupy these beds temporally, highlighting their long-term importance. Nekton density, species richness, and Shannon diversity were all highly positively correlated with percent cover of both *R. maritima* and *H. wrightii*. In comparison, canopy height of the seagrasses seems to be less important to animals compared to the density of the seagrass bed.

R. maritima and *H. wrightii* have similar morphologies and in this study supported similar densities of nekton. Overall, the function of these seagrasses as habitat for nekton appear to be similar in the Grand Bay NERR system, suggesting that future restoration and management activities could focus on either species or both together to support the abundance and diversity of the local fauna.

1.7 Project Significance

Resource managers at the Grand Bay NERR have expressed a need for information concerning ecosystem functions and services provided by habitats within the Reserve, and seagrass beds are a large part of their system. There has been a knowledge gap with respect to current information on the function of seagrass as habitat in the Grand Bay NERR, which has impeded management decisions. Understanding habitat use by commercially fished nekton is important to support these fisheries and their sustainability. Commercially important species like blue crabs (*Callinectes sapidus*), white shrimp (*Penaeus setiferus*), and brown shrimp (*Penaeus aztecus*) were found in significantly higher numbers within seagrass beds than in unvegetated areas, which highlights the importance of protecting,

restoring, and monitoring these areas within the Grand Bay NERR. The results of this study indicate that beds of both *R. maritima* and *H. wrightii* are important habitats for a variety of crustaceans and fish, which should help to inform decision-making regarding future management and restoration efforts within the Grand Bay NERR.

APPENDIX A – Average TL or CW for All Nekton Caught at All Sites

Species	Total	Mean TL/CW (mm) ± SE	Mean Total Wet Weight (g) ± SE
Fishes			
<i>Anchoa lyolepis</i>	2	31.5 ± 0.50	0.16 ± 0
<i>Anchoa mitchelli</i>	199	34.54 ± 0.81	4.11 ± 2.12
<i>Bairdiella chrysoura</i>	26	47.69 ± 5.52	6.10 ± 2.60
<i>Citharichthys spilopterus</i>	2	31 ± 5.00	0.30 ± 0.13
<i>Ctenogobius boleosoma</i>	84	27.66 ± 0.69	0.72 ± 0.14
<i>Cynoscion nebulosus</i>	3	99.33 ± 39.75	17 ± 15.08
<i>Erotelis smaragdus</i>	2	41 ± 5.00	0.88 ± 0
<i>Eucinostomus argenteus</i>	1	40 ± 0	0.83 ± 0
<i>Gobiosoma bosc</i>	10	27.5 ± 2.00	0.48 ± 0.24
<i>Gobiosoma robustum</i>	2	17.5 ± 0.5	0.06 ± 0.01
<i>Hippocampus zosterae</i>	1	18 ± 0	0.05 ± 0
<i>Lagodon rhomboids</i>	41	50.22 ± 3.81	14.48 ± 7.92
<i>Lucania parva</i>	11	22.36 ± 1.20	0.24 ± 0.08
<i>Lutjanus griseus</i>	1	29 ± 0	0.41 ± 0
<i>Membras martinica</i>	8	37.25 ± 1.54	2.85 ± 0
<i>Menidia beryllina</i>	3	71 ± 7.55	3.55 ± 1.69
<i>Menidia peninsulae</i>	1	44 ± 0	0.56 ± 0
<i>Microgobius guiosus</i>	1	50 ± 0	0.83 ± 0
<i>Myrophis punctatus</i>	3	113.33 ± 36.34	2.28 ± 2.08
<i>Ophidiidae sp.</i>	1	27 ± 0	0.12 ± 0
<i>Opsanus beta</i>	1	17 ± 0	0.1 ± 0
<i>Symphurus plagiusa</i>	38	26.97 ± 1.10	0.42 ± 0.07
<i>Syngnathus louisianae</i>	2	110 ± 37	0.58 ± 0.48
<i>Syngnathus scovelli</i>	15	69.47 ± 6.83	0.43 ± 0.14
Crustaceans			
<i>Acetes americanus carolinae</i>	1	17 ± 0	0.02 ± 0
<i>Callinectes sapidus</i>	267	8.55 ± 0.47	4.84 ± 2.36
<i>Hippolytidae sp.</i>	1	10 ± 0	0.01 ± 0
<i>Latreutes parvulus</i>	4	19.25 ± 0.63	0.72 ± 0
<i>Palaemon mundusnovus</i>	730	14.12 ± 0.12	2.25 ± 0.83
<i>Palaemon pugio</i>	535	18.47 ± 0.20	3.77 ± 1.25
<i>Palaemon vulgaris</i>	68	15.15 ± 0.37	0.34 ± 0.07
<i>Penaeus aztecus</i>	416	22.72 ± 0.49	2.42 ± 0.38
<i>Penaeus setiferus</i>	87	25.07 ± 1.11	2.05 ± 0.77
<i>Rimapenaeus constrictus</i>	1	21 ± 0	0.1 ± 0

APPENDIX B – 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 EXPIRATION DATE: September 30, 2024



Jake Schaeffer, PhD
IACUC Chair

February 8, 2022

Date

APPENDIX C – Percent Cover and Sites with Reproductive *R. maritima*

Metric	Spp.	May (n=8)	July (n=10)	Sept. (n=10)	Nov. (n=7)
Range of Percent Cover	HW	35.78-60.00%	55.00-66.2%	49.94-85%	45.50-47.67%
	RM	47.22-90.00%	53.67-77.9%	50.00-90.00%	50.00-76.89%
Mean Percent Cover	HW	45.7% ± 6.64	60.1% ± 7.00	67.99% ± 8.50	46.58% ± 5.12
	RM	76.52% ± 10.63	65.91% ± 7.75	69.65% ± 8.90	61.39% ± 8.92
% of Species Dominated Sites with Reproductive <i>R. maritima</i> present	HW	50%	80%	20%	0%
	RM	75%	100%	80%	0%

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