Biology Honors Thesis

Seasonal species assemblages in seagrass habitats of northeast Pamlico Sound

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I hereby declare I am the sole author of this thesis. It is the result of my own work and is not the outcome of work done in collaboration, nor has it been submitted elsewhere as coursework for this or another degree.

Signed:________________________________ Date:_ 12/14/22

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ABSTRACT – Large estuarine habitats support a wide variety different species that use these areas for habitat. However, species assemblages are still poorly described over large areas of estuarine habitat. The underrepresentation of species assemblages in estuarine habitats in the literature leaves room for research inquiring about how and why these assemblages are occurring. The objective of this study was to assess how estuarine habitat type and location, as well as season, influences nekton and invertebrate community diversity and composition, within Pamlico Sound. Sampling was conducted across four dates between the summer and fall of 2022 to encompass greater variations in species compositions in the Pamlico Sound. I initially hypothesized that seagrass habitats would have higher species abundance, richness, and overall diversity compared to unvegetated habitat. I also hypothesized that abundance and richness would decline with seasonality, and that seagrass areas spatially closer to ocean inlets would have a higher richness and abundance than areas further into the sound. In this study, I analyze a novel dataset collected in the northeast part of Pamlico Sound, North Carolina in the area between Oregon Inlet, Roanoke Sound, and Croatan Sound. The dataset is from a beach seine sampling survey of fish and invertebrate assemblages within seagrass habitats, and adjacent unvegetated habitats, that I collected over the course of 2022. YSI data collected from each sampling location, including temperature, salinity, and dissolved oxygen (DO), was used to characterize species compositions across sampling sites. A paired t-test found statistically significant differences between vegetated seagrass habitats and

unvegetated seagrass habitats in both species richness and species abundance, while an analysis of variance (ANOVA) found significant differences in richness and abundance over time in seagrass habitats. Indeed, in paired beach seine hauls, the abundance of fish and invertebrates was an order of magnitude higher over seagrass compared to the adjacent unvegetated bottom. In the months between summer and fall, there was a general decline in nekton abundance. Abundance and richness also declined as the distance from Oregon Inlet increased. Species composition consisted of five dominant species, pinfish, pigfish, silver perch, bay anchovy, and Atlantic silversides. This study will inform fisheries and management groups local to North Carolina’s coastline about changes in species assemblages and their whereabouts within the sound. This study will also serve as a baseline for further research of species assemblages within Pamlico Sound.

Acknowledgments

I would sincerely like to thank the people and organizations that made this study possible: Dr. James W. Morley for his boundless contributions to this project, I genuinely do not think I could have reached out to a better person to help me conduct this study. I would also like to mention the contributions made by the other wonderful people at the Coastal Studies Institute: Andrew McMains, Caid Menzel, and Dr. Verena Wang. Secondly, I would like to thank the Honors College of East Carolina University, had it not been for the Honors College, I doubt I ever would have pushed myself to undertake such a project, and I am grateful for the lessons it taught me along the way. Lastly, I would like to thank Dr. Susan B. McRae, through her seemingly limitless patience I was able to complete this study with all the resources I needed to graduate a proud student of ECU's Honors College.

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Introduction

Habitat is one of the most defining factors in determining species assemblages and distributions in nature. When examining estuarine communities, the importance of habitat becomes exemplified. Research has shown that organisms in juvenile life stages utilize estuarine habitat types as nurseries, including salt marsh, mangrove forest, and seagrass beds (Baillie et al., 2014; Whitfield, 2016). Nursery habitats are defined as habitats that contribute a greater average number of juveniles that recruit to adult populations, in comparison to other habitats that juveniles use (Beck et al., 2001).

Seagrasses are a group of marine flowering plants that that occur in meadows/beds or large groupings of one or more seagrass species (i.e., *Halodule wrightii, and R. maritima*). Waycott et al. (2009) concluded that seagrass area has declined across the globe indiscriminately and that species assemblages have globally suffered due to habitat loss. This study illustrates the importance of gathering data on organisms using seagrass habitats, to demonstrate the importance of seagrass coastal ecosystems (Waycott et al., 2009).

The Albemarle-Pamlico sound presents itself as a specific area of needed investigation and study because of its significance economically, ecologically, and recreationally. This study looks to accomplish the following goals: (1) sample the populations that are using seagrass bed habitats and understand how these populations are distributed, (2) show how seasonality and change in time affects species distributions and assemblages in seagrass, (3) discern how important proximity to ocean inlets is in species distribution, and (4) to show the importance of seagrass habitats by comparing data found in seagrass beds to that of the surrounding unvegetated habitats. In general, seagrass habitats are being threatened by anthropogenic activities within the Pamlico sound, these areas are important to protect, but we must also understand why they are threatened – and what those threats might impact. I hypothesized that data analysis would show significant differences in species abundances and richness with seasonality progressed into later months of the year, and I also expected that abundance, richness, and diversity would be much greater in vegetated habitats compared to unvegetated habitats. The null hypothesis was that there would be no significant difference in habitat usage and species distributions between vegetated and unvegetated habitats.

In this study, I analyzed nekton communities using seagrass bed and unvegetated habitats across different locations in Pamlico Sound over the course of 2022, to test and quantify the significance of habitat type, seasonality, and spatiality, in species assemblages, specifically as nurseries. To reflect the importance of habitat and how it determines species assemblage, seagrass beds were sampled in conjunction with nearby unvegetated habitats.

I also explored how season could influence species assemblages. It is crucial to understand where species occur at specific life stages, what habitats they are using, and when they are present in those habitats. Species distributions change concurrently to seasonality, or the change in seasons (Jackson et al., 2001). With changes in seasonality come changes in factors that may affect species distributions such as water temperature, salinity, DO content, and other factors such as seagrass bed quality (Jackson et al., 2001). Research has shown that species will use different habitats at different points of seasonality, and that proximity or interconnectivity of habitats is crucial to understanding species assemblages (Ballie et al., 2014).

Research has also shown the importance of habitat location within a large ecosystem like Pamlico Sound (Mills & Berkenbusch, 2009). Researcher have found that seagrass habitats within close proximity to larger ocean inlets have higher species abundances depending on their position with regards to the inlets they surround (Mills & Berkenbusch, 2009). With the location of seagrass habitat being an important factor of influence in determining assemblages, I also evaluated how location of habitat within the sound and proximity to Oregon Inlet related to species richness and abundance (Mills & Berkenbusch, 2009).

Along the Atlantic coast of the U.S., Pamlico Sound in North Carolina represents one of the largest estuarine ecosystems in the country. Species observed within this study have commercial, recreational, and ecological value such as blue crabs, mullet, spot, croaker, drum, and sheepshead (Rouleau et al., 2021). The value of the Pamlico Sound also comes from its ecosystem services, aside from the habitat it provides for important species, the estuary system serves to improve water quality through the reduction of erosion and wave activity, the filtering of water, and acting as a carbon sink (Keith & Rashleigh, 2011; Greiner et al., 2013). The recreational fisheries industry in North Carolina had a $1 billion impact on the economy here, and commercial fisheries having a $180 million impact, although these values have been declining in correspondence to habitat loss, overfishing, and pollution (Rouleau et al., 2021). Tourism and recreation are also a large part of North Carolina's economy, with Pamlico Sound drawing over $1.37 billion in impact (Rouleau et al., 2021). Species like silversides (both Atlantic and rough) have both an economic and ecological significance. Silversides are commonly used as bait and for forage stocking in fisheries and recreational connotations – while also filling an important ecological role within the sound, silversides represent just a single portion of the many species found in this study (Noble, 1981).

Methods

Study Site

The Albemarle-Pamlico Sound is the second largest estuary in the United States, and its economic and ecological value cannot be understated (Rouleau et al., 2021). Pamlico Sound's vastness stretches 80 miles long and 20-30 miles wide, with a total area of 3,000 square miles (Rouleau et al., 2021). `All sampling locations were centralized within a 7000-meter radius of Oregon Inlet. The importance of inlets from an ecological perspective is centered around the idea that species will emigrate through the inlet as recently matured adults on their way to the open ocean or immigrate as spawning adults returning to the sound to procreate or to feed (Joyeux, 1999). By centralizing sampling in different locations relative to Oregon Inlet, this study can quantify the effects of spatiality and proximity to tidally important areas (Oregon Inlet) on species assemblage. Each vegetated and unvegetated habitat location was collected using GPS coordinates from the navigation system onboard the research vessel. The collection of raw data on the precise locations of all species found in our survey could be valuable to marine fisheries organizations such as the North Carolina Division of Marine Fisheries (NCDMF).

Part One: Data Collection

To accurately quantify the seasonal changes in species composition using seagrass beds and adjacent unvegetated habitats, I collected data over the course of the summer and fall of 2022, through four field outings from the Coastal Studies Institute in Wanchese, NC. Data collection and field sampling occurred in areas north of Oregon Inlet and locations between the Pamlico Sound just south of Roanoke Sound (Figure 1). At each field site, we first collected latitude and longitude coordinates, and then measured water quality data including water temperature, depth, salinity, and dissolved oxygen using a YSI. Turbidity data was also collected at each site using a Secchi disc. Information regarding the species of seagrass that dominated at each site was also collected. Four separate field outings occurred in this study. Surveying over the course of multiple months and seasons (summer and fall), this study was able to quantify and analyze species' distribution changes with respect to time. During 2022, a total of 27 seine hauls were taken in vegetated and unvegetated bottoms across June 6th, July 28th, September 2nd, and October 12th. I limited sampling to four distinct locations throughout the area, with different proximities to Oregon Inlet, one of the only inlets connecting the sound to the Atlantic Ocean (Figure 1).

Figure 1. Seine haul locations mapped out on google maps based on GPS coordinates taken at each location, the four generalized haul areas can be seen – with Oregon Inlet highlighted by a yellow circle.

To quantify the abundance of fish and invertebrates in the area, two 60 ft beach seine hauls were pulled across paired vegetated and unvegetated habitats (Figure 1). To deploy the seine, two individuals waded the seine out (folded and contained) to 25 meters from the research vessel, while a third individual used a rangefinder to accurately determine the seine's distance from the boat indicating that the haul/pull should begin. The sampling area was avoided when positioning the seine away from the research vessel, to avoid disturbing the habitats. Once the seine was in position the two designated pullers stretched the net out to its complete length parallel to the boat. While pulling the seine, the individuals hauling the seine would take note to keep the lead end poles on the bottom at all times to minimize escapement of organisms. Upon arriving at the side of the vessel, the two lead ends were brought together in a way that forms a U-shape. As we dragged the seine towards the boat, fish and other organisms were forced into the seine bag, and by bringing the ends of the seine together (the lead ends) specimens were collected by pulling on the lead line at the bottom of the seine net bringing the sample/bag closer while minimizing escape (Figure 2, 3, & 4).

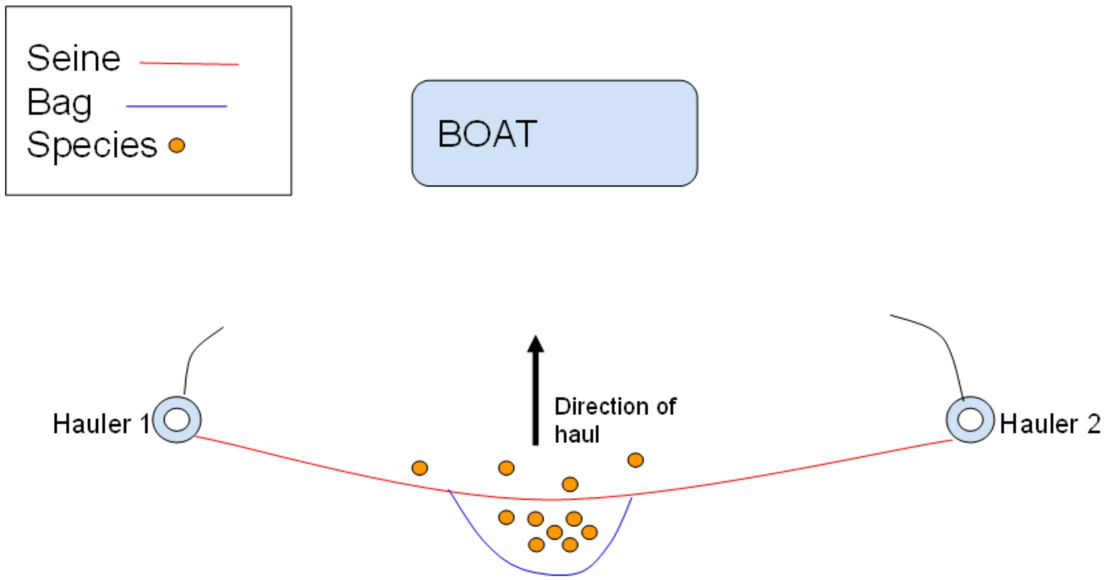

Figure 2. Diagram representing the beginning of a seine haul, with the net fully spread open and being pulled towards the research vessel.

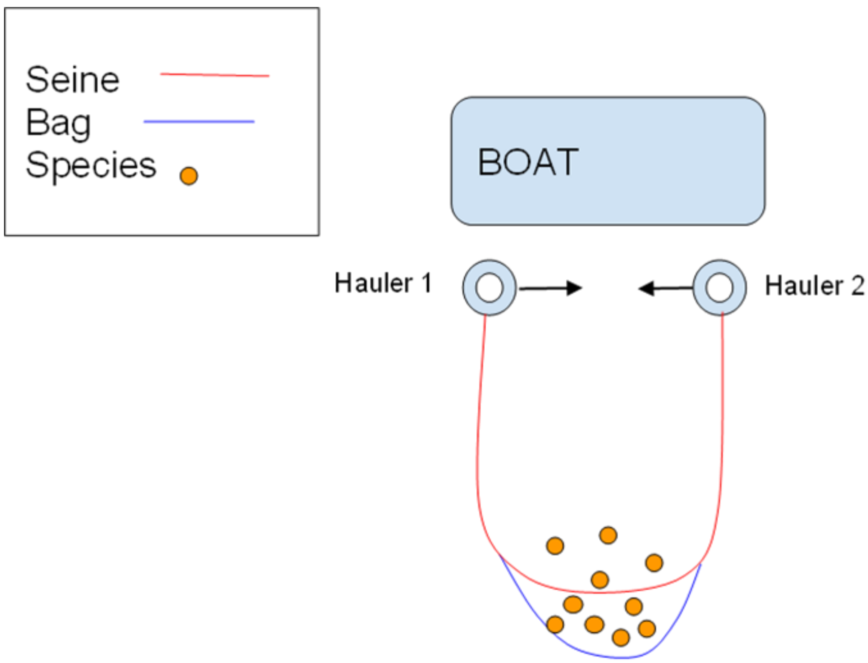

Figure 3. Diagram representing the end of a seine haul demonstrating the “U-shape”, with the net being enclosed around the sample and the bag being pulled towards the researchers for processing and data collection.

Figure 4. View of the research vessel used in collecting the raw dataset, with the seine being pulled in by Caid Menzel (right) and Dylan Whitt (left).

Once the two haulers collected the bag and ensured that all specimens were contained inside, they kept the bag partially submerged to maximize survivability, while the third researcher filled an appropriate number of buckets with water, then subsequently extracted the specimens from the seine’s bag. With catch equally distributed among buckets, multiple researchers individually identified species and released each counted organism. Another researcher took a count/tally of the number of individuals per species per haul while the others sorted. After the entire catch was identified, counted, and released, the seine was taken back out in the water to an area nearby the seagrass bed that consisted of bare sand/mud or that had minimal vegetation (at times avoiding all drifting-uprooted seagrass was impossible). Once the unvegetated adjacent habitat had been sampled and processed in the same way as the vegetated habitat, I moved on to the next sampling site. The beach seine sampling occurred at vegetated and unvegetated habitats at 4 sites over the course of each field outing. Distance from Oregon Inlet was calculated using Google maps, by drawing a straight line from the middle of four the coordinate clusters seen in figure 1 to Oregon inlet.

Species diversity within seagrass and bare habitats was quantified using the Shannon-Weiner diversity index. Using this species diversity index, the number of separate species present can be examined alongside the abundance of each – this index shows the diversity at each of the four sites, at each of the four dates – and in the context of habitat type (vegetation presence). The Shannon-Wiener formula is as follows:

$$H=\sum[(pi)\times\ln(pi)]$$

Shannon-Wiener diversity was calculated for every paired haul (locations that had a seagrass haul and a consequent sandy/unvegetated haul) throughout the four sampling dates – and a vegetated and unvegetated average diversity was produced for each date. Where pi is the abundance of species i in a given seine haul, and all species are summed.

Part Two: Data Analysis

After compiling and digitizing the beach seine dataset, I employed several methods of analysis to examine the data. Firstly, I took the raw dataset and found the species richness, and total abundance of all collected nekton for each of the 27 hauls. Richness was calculated by adding the total number of species found in a single haul, and abundance was calculated by adding together the total number of individuals found in a haul. The first statistical analysis I used was a paired t-test to determine if the average richness and average abundances were significantly different between vegetated and unvegetated habitats. The paired t-test was calculated using the Excel Analysis ToolPak. The paired t-test was used to understand how richness and abundance might be enhanced in seagrass habitat. The paired t-test function generated a table containing the degrees of freedom, t-stat, Pearson Correlation coefficient, the one-tailed p-value, the two-tailed p-value, and the hypothesized mean difference. The P-value represents the probability of the null hypothesis being false, a value of 0.05 gives 95% confidence that the null hypothesis is false.

Another method of data analysis that I employed was an analysis of variance (ANOVA), which was used to compare abundance and richness across sampling dates, to test for seasonal changes. The Excel Analysis ToolPak was also used for all ANOVA calculations. For the seasonal changes analysis (i.e., ANOVAs), only seagrass habitat sampling was considered. The ANOVA table for abundance was log transformed to ensure that residual error distribution was normal (Table 2). The ANOVA function generated a table which contained degrees of freedom, sum of squares, mean of squares, R-squared values, F-ratio, and p-values. Mean of squares values are estimates of variance across groups calculated as the sum of squares value divided by its corresponding number of degrees of freedom. The F ratio is the ratio of two mean square values, a false null hypothesis will result in an F value of 0. Large F ratios indicate the variation among group means is more than can be expected to occur by chance alone. The P value represents the estimated probability of rejecting the null hypothesis where a value of 0.05 gives 95% confidence that the null hypothesis is false.

Results

Throughout the course of this study, 6919 individual organisms were captured and released in total. Out of 6919 individuals, 5995 of them were caught in seagrass (86.65%), with 924 caught in unvegetated habitat. Species were largely found in seagrass habitats, with some species being exclusively found in seagrass (Table 1). Out of 41 different species present in this study, four species were most abundant outside of seagrass beds: menhaden (*Brevoortia tyrannus*), lizardfish (*Synodus saurus*), spot (*Leiostomus xanthurus*) and croaker (*Micropogonias undulatus*). Croaker was the only species exclusively found in unvegetated habitats; all other species were also found in seagrass beds (Table 1). In comparison, 18 out of the 41 species represented in this study were exclusively caught in vegetated habitats. Some of the notable species only found in seagrass bed habitats were rainwater killifish (*Lucania parva*), naked goby (*Gobiosoma bosc*), lyre goby (*Evorthodus lyricus*), northern sennet (*Sphyraena borealis*), and striped blenny (*Meiacanthus grammistes*). All other species not listed were primarily represented in vegetated habitats over unvegetated habitats.

Table 1. Complete species abundances specifying whether individuals were caught in vegetated or unvegetated habitats.

Species	Seagrass	Unvegetated	Species	Seagrass	Unvegetated
Pinfish	3315	461	White Mullet	4	0
Silverside spp.	1339	297	Atlantic Needlefish	2	1
Silver Perch	274	8	Black sea bass	2	0
Bay Anchovy	236	57	Bluespotted Cornetfish	2	0
Brown Shrimp	234	15	Red Drum	2	1
Pigfish	157	6	Sheepshead	2	0
Pipefish spp.	128	11	Striped Burfish	2	0
Blue Crab	70	9	Tautog	2	0
Halfbeak	34	3	Bay Whiff	1	0
Mojarra sp.	34	6	Hermit Crab	1	0
Striped Anchovy	30	1	Lizardfish	1	5
Rainwater Killifish	24	0	Lookdown	1	0
Grey Snapper	21	1	Menhaden	1	3
Black cheek tonguefish	16	4	Norther Pufferfish	1	0
Naked Goby	15	0	Pompano	1	1
Lyre Goby	9	0	Summer Flounder	1	1
Speckled Trout	8	1	White Shrimp	1	0
Northern Sennet	6	0	Slippery Dick Wrasse	1	0
Striped Blenny	6	0	Spot tail Pinfish	1	0
Gulf Flounder	5	1	Croaker	0	4
Spot	5	27			

Habitat Type

Average species richness decreases from June to October, and in vegetated habitats, average species richness is higher than that of unvegetated habits during all months sampled (Figure 5, Table 2). The differences in average species richness between vegetated and unvegetated habitats varied greatly, most notably, in July the average richness in vegetated habitats was over twice as high than that of unvegetated habitats. Vegetated habitats had a statistically significant higher species richness than those habitats without vegetation (T_9 = 3.130, p = 0.0121, the mean richness was 15.4 in seagrass and 6.3 in unvegetated habitats).

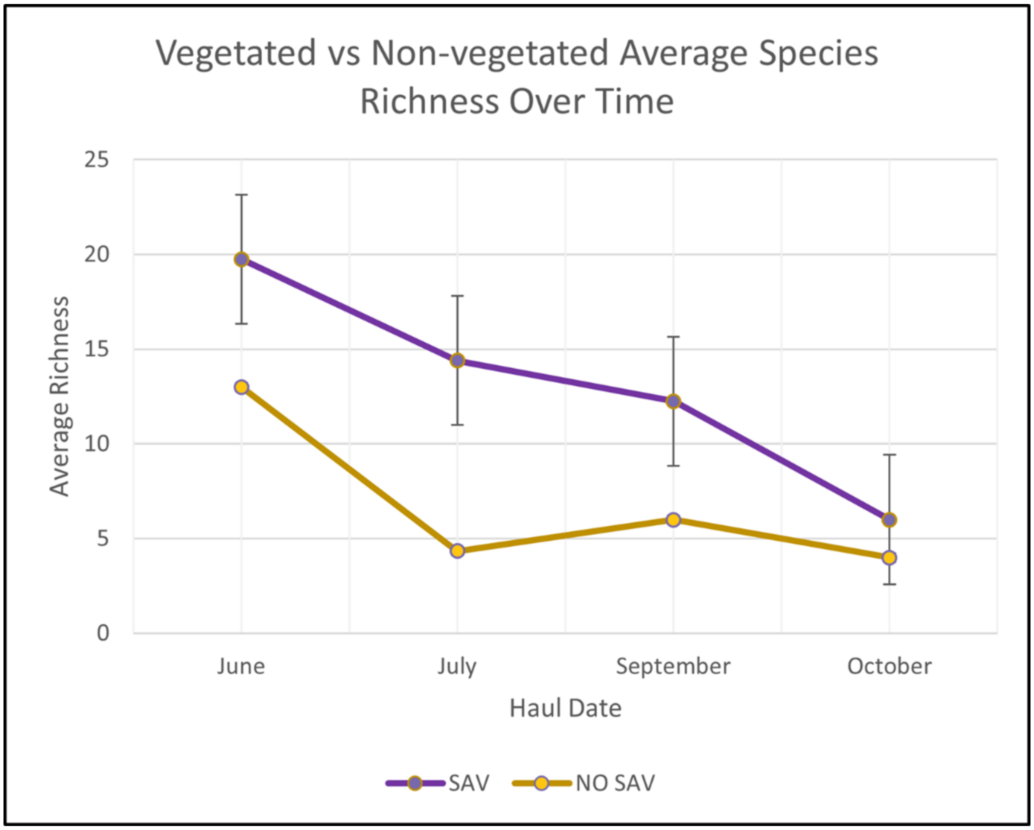

Figure 5. Average species richness during all four sampling months for vegetated and unvegetated habitats. SAV indicates seagrass and NO SAV indicates unvegetated bottom. Note: the standard error for the "NO SAV" bar was smaller than the data points themselves.

Table 2. The paired t-test comparing richness in seagrass and richness without seagrass.

t-Test: Paired Two Sample for Means	*Richness in Seagrass*	*Richness without Seagrass*
Mean	15.4	6.3
Variance	127.3777778	13.34444444
Observations	10	10
Pearson Correlation	0.68129857	
Hypothesized Mean Difference	0	
df	9	
t Stat	3.12967222	
P(T<=t) one-tail	0.006064052	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.012128104	
t Critical two-tail	2.262157163	

Average abundance showed similar trends to average richness, with seagrass beds generally having a higher abundance than the unvegetated counterpart we sampled (Figure 6). The differences in average abundance between vegetated and unvegetated habitats varied greatly, the largest difference being in June, with average species abundance in vegetated habitats over three times higher than average species abundance in unvegetated habitats. September and October species abundances were similar in vegetated vs non-vegetated habitats (Figure 6, Table 3). Vegetated habitats had a statistically significant higher abundance than those habitats without vegetation ($T_9 = 2.347$, $p = 0.0435$, the mean abundances were 458.7 in seagrass and 92.4 in unvegetated habitats).

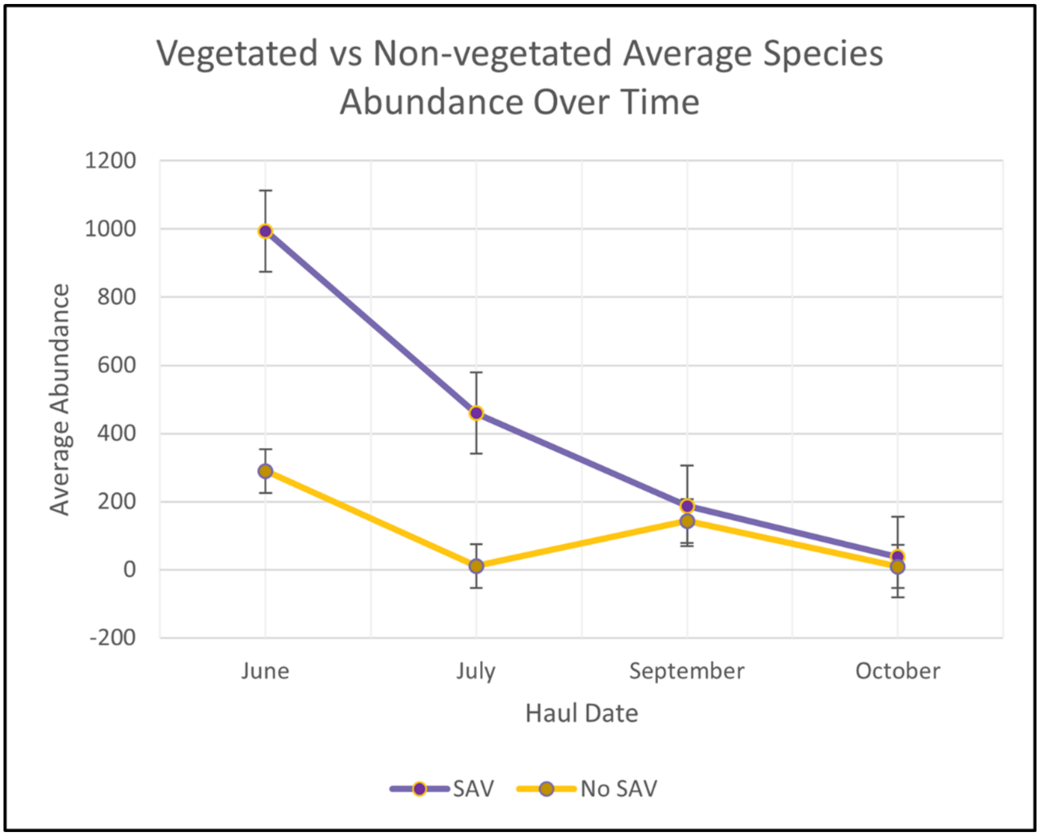

Figure 6. Average species abundance found in vegetated, seagrass habitats plotted over time versus average species abundance found in unvegetated, sandy bottom habitats. SAV indicates seagrass and NO SAV indicates unvegetated bottom.

Table 3. The paired t-test comparing abundance in seagrass and abundance without seagrass.

t-Test: Paired Two Sample for Means	*Abundance in Seagrass*	*Abundance without Seagrass*
Mean	458.7	92.4
Variance	276314.9	20795.6
Observations	10	10
Pearson Correlation	0.353359476	
Hypothesized Mean Difference	0	
df	9	
t Stat	2.347214334	
P(T<=t) one-tail	0.021751576	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.043503152	
t Critical two-tail	2.262157163	

Seasonality

In vegetated habitats, richness declined from June to October, with each month having a lower species richness than the last (Figure 5, Table 4). In vegetated habitats, abundance declined from June to October, with each month having a lower abundance than the last (Figure 6, Table 5).

Table 4. The analysis of variance (ANOVA) conducted for richness in vegetated habitats through June, July, September, and October.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	0.531823277	3	0.177274426	3.88537974	0.037510114
Within Groups	0.547512277	12	0.045626023		
Total	1.079335555	15			

The results of the ANOVA calculation for abundance in vegetated habitats over time are provided in Table 5. There was a significant difference in abundance in vegetated habitats over the course of the survey period. In vegetated habitats, abundance declined from June to October, with each month having a lower abundance than the last.

Table 5. The analysis of variance (ANOVA) conducted for abundance in vegetated habitats through June, July, September, and October.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.261332304	3	1.753777435	18.72427661	8.03758E-05	3.490294819
Within Groups	1.123959534	12	0.093663295			
Total	6.385291838	15				

<u>*Diversity*</u>

The results of the diversity index show I found similar Shannon diversity in vegetated and unvegetated habitats, although, diversity in vegetated habitats was higher in September than in unvegetated habitats (Table 6). Seagrass diversity calculations were often heavily skewed by the presence of one or two dominant species, typically pinfish or silversides. For example, in a haul with 12 different species represented, one or two species would dominate the abundance, while the other ten species were caught in a much lower quantity. However, seagrass habitats did have higher diversities than unvegetated habitats in the later months of the year, with the largest difference in diversity being in September (Table 6).

Table 6. The average Shannon diversity for each field date, for habitats with seagrass and habitats without seagrass.

Date	Diversity in Seagrass	Diversity without Seagrass
June	1.21	1.28
July	1.22	1.28
September	1.56	0.89
October	1.19	1.06

Spatiality

Abundance is highest at the area closest to the inlet in every month, with the other three sampling locations varying in abundance (Figure 7). Richness is generally highest at the area closest to Oregon Inlet (2500m), although in July, the area furthest from the inlet had the highest richness (6950m), and in September the second closest area to the inlet had the highest abundance (4950m) (Figure 8). Figures 7 and 8 only represent data collected from seagrass beds.

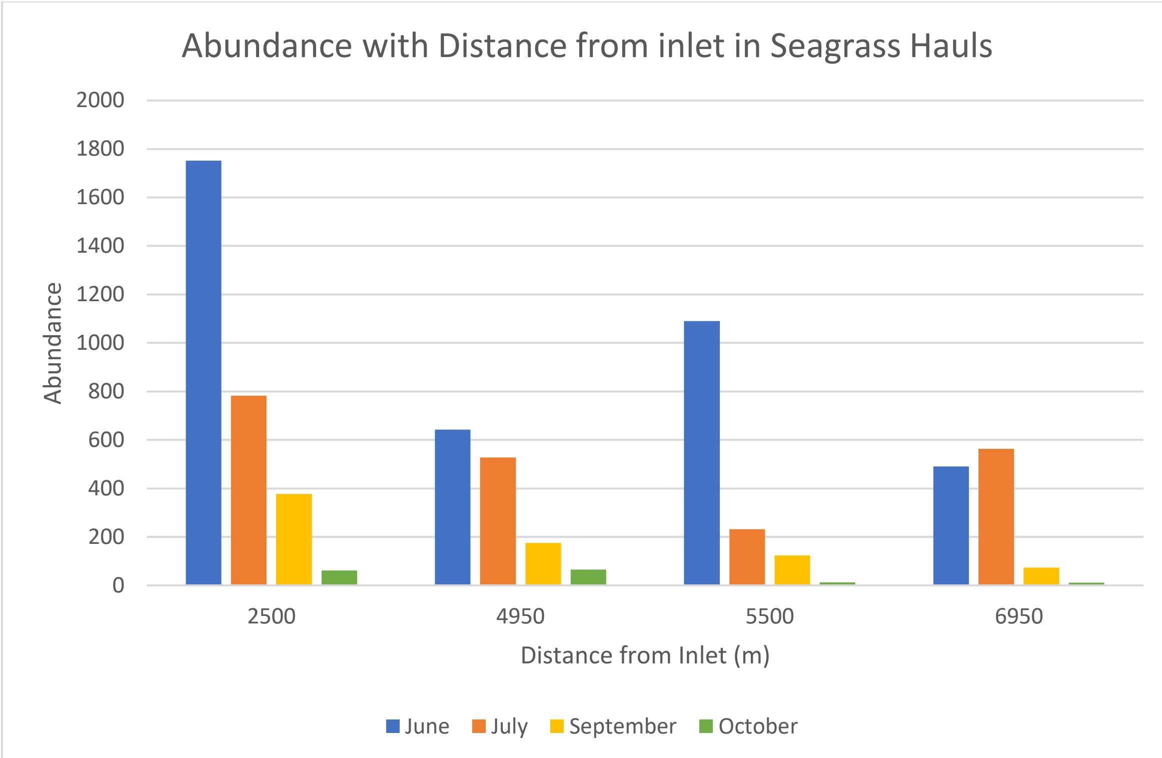

Figure 7. A graph of species abundance with respect to distance from Oregon Inlet, specifically for seagrass habitats.

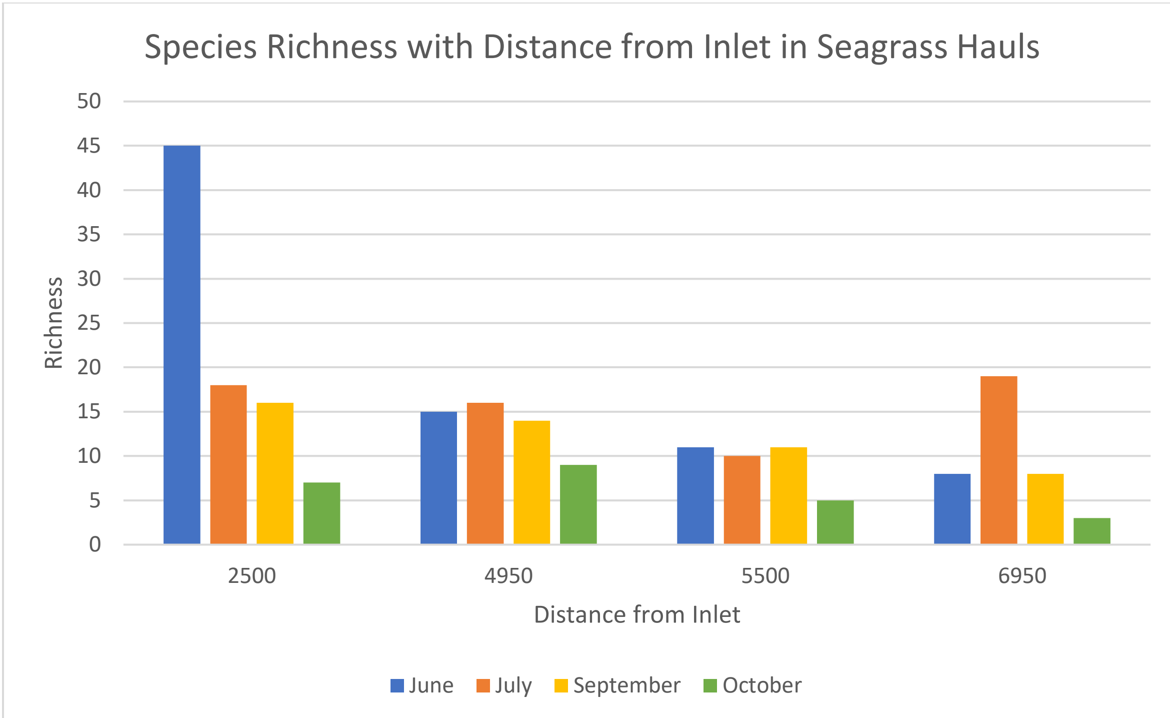

Figure 8. A graph of species richness with respect to distance from Oregon Inlet, specifically for seagrass habitats.

Other Factors

Salinity was almost always highest at the area closest to the inlet – decreasing as distance from the inlet increased (Figure 9). However, the area 4950m away from Oregon Inlet had a slightly higher salinity than the area 2500m away in July. In contrast to other months, in September, salinities were the highest in all sampling locations. After September, salinities in all sites began to decrease in October. The average salinities of each sampling location across all months were 21.99 at 2500m, 20.12 at 4950m, 19.55 at 5500m, and 18.55 at 6950m. Temperature predictably decreased as the study progressed, with the highest recorded water temperature at 29.6 ℃ in July, and the lowest at 18.8 ℃ in October.

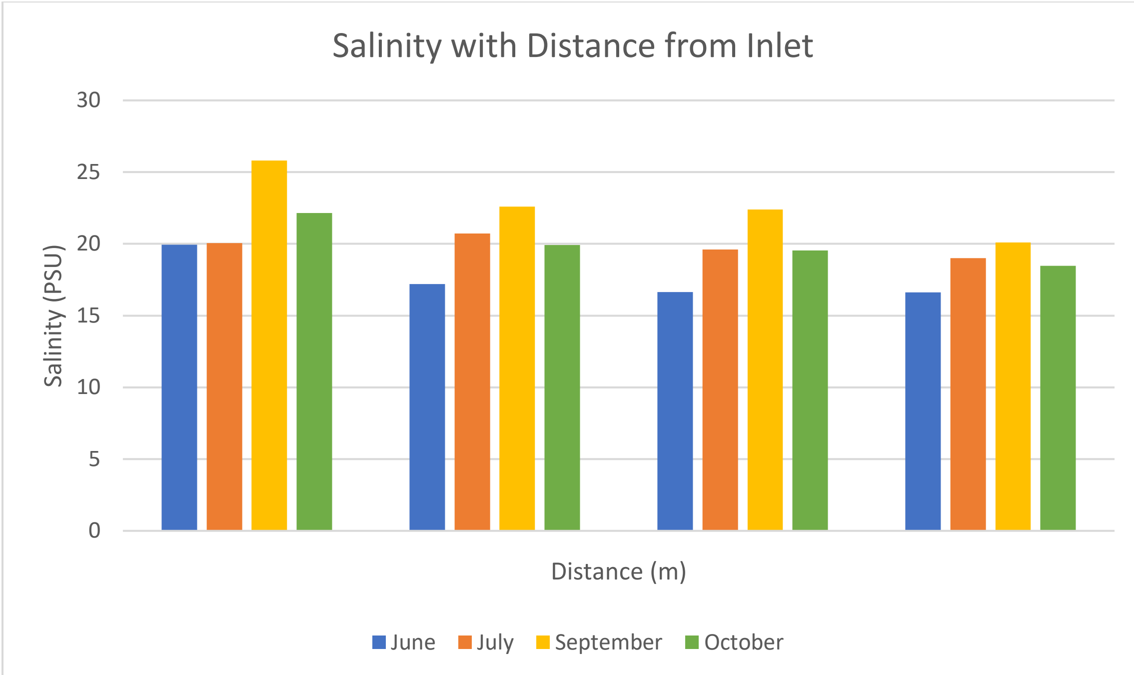

Figure 9. Salinity levels at each sampling site for all four sampling dates.

Discussion

This study found that seagrass beds contained a higher species richness and abundance than habitats with no vegetation at all. This highlights the importance of seagrass habitat, as seagrass beds promote faunal biodiversity. Losses in seagrass habitats that would give rise to unvegetated habitats would have impacts on nekton community structure resulting in a negative cascade of impacts from an ecological and economic perspective (Githaiga et al., 2019). Species abundance within both habitats is almost the same (within error) during the months of September and October. I think that this similarity in abundance, juxtaposed with the higher diversity of seagrass beds in later months, reflects the seasonal changes in habitat use by juvenile species. Thus, an assessment of seagrass habitat should be performed across multiple seasons, to better understand the value of this habitat throughout the year.

Connolly (1994) is a well-structured analysis of seagrass and seasonal dynamics in Australian estuarine fish assemblages, which utilized many of the same methods that were employed in this study. Ultimately, Connolly (1994) concluded that fish assemblages were higher in vegetated (seagrass) habitats during all seasons and demonstrated the impact seagrass losses would have on fish assemblages, in both ecologically and economically significant species. Although there is overlap between research approaches, it is important to note that my study was conducted in the context of North Carolina's estuaries during the present-day time. Globally, the climate has changed significantly since the release of Connolly (1994), and that may change how species assemblages are defined, and this paper strives to provide a more complete analysis given a more modernized range of data and techniques.

Other studies such as Ballie et al. (2014) highlight the importance of interconnectivity and proximity of seagrass habitats as different species move from one location to another during their developmental life stages. Ballie et al. (2014) also hypothesizes that species may use multiple habitats over different seasonal cycles, and that habitat heterogeneity promotes a more diverse assemblage of species. Similarly, Ballie et al. (2014) compared vegetated and unvegetated habitats using analyses such as the Shannon-Weiner diversity index, however, the authors did not focus on seasonality as a driving factor in distributions. Ballie et al. (2014) is also one of the only studies in this literature review that takes place within a similar ecological region (Back and Core Sounds, NC). Estuarine systems can have very complex and diverse species assemblages, and the Pamlico Sound is a unique system with lower average salinities alongside a limited lunar tidal influence.

Species became much less prevalent in the later months of the year. My original hypothesis was that species assemblages would have lower richness and abundance in later months because nekton would seek out habitat other than seagrass. There are many reasons why species abundance declines in the later seasons of the year. Temperature declines drive species into new areas, such as the coastal ocean, and possible degradations in the quality of seagrass habitats could be driving assemblages towards different habitat types such as oyster reefs during later months of the year. Specifically, seagrass has a seasonal cycle, and as the year progresses there is an accumulation of detrital seagrass that changes the nature of this habitat.

Spatiality had a noticeable effect on species assemblages, and although the distance from inlet data was not statistically analyzed, it still shows a trend of larger species abundances more concentrated around the seagrass beds closest to Oregon Inlet. Species assemblages are likely higher closer to the inlet due to the higher diversity of seagrass species found at those sites. *Ruppia maritima* was the dominant seagrass throughout this study with the 4950m, 5500m, and 6950m seagrass beds entirely consisting of *R. maritima*, however, the 2500m sites had a consistent mix of *Halodule wrightii, R. maritima,* and *Zostera marina*, indicating that diversity in the species of seagrass in a bed may correlate to the assemblages of nekton using it as habitat (Valdez et al., 2020).

Seagrass beds contain vital resource species such as red drum (*Sciaenops ocellatus*), summer flounder (*Paralichthys dentatus*), sheepshead (*Archosargus probatocephalus*), and blue crab (*Callinectes sapidus*). Seagrass beds also contain other important organisms, not just resource species. All organisms are important as they each contribute to the ecosystem. For example, many species that largely use seagrass as habitat are economically important as bait fish or prey for higher trophic levels, such as brown shrimp (*Farfantepenaeus aztecus*), and silversides (*Menidia spp.*).

Gear selectivity is an important consideration for the results of any scientific project and although the beach seine was a practical choice, it has its limitations. As with any sampling method, the results of this study were influenced by the choice to use the seine net in many ways. Firstly, the mesh size that makes up the net (aside from the bag), likely contributed to the underrepresentation of the smallest individuals, primarily the recently settled juvenile and post-larval fish, as some slipped through the relatively large openings of the mesh. Secondly, pulling the seine is a rather slow process relative to some fish swimming speeds, and more mobile nekton would likely have been startled and swam outside of the range of the seine, leading to an underrepresentation of larger, more developed, and faster individuals. Lastly, the seine net functionally drags across the bottom, however, contact with the bottom cannot always be consistently established when sampling due to a variety of factors such as changes in benthic elevations, seagrass thickness, and sediment type. Due to the possible inconsistencies in maintaining contact with the bottom of the sound, benthic species such as flounder were likely underrepresented.

Although studies like this one have been conducted across the world, in the context of North Carolina's estuaries, there is a lack of literature surrounding fish abundance and prevalence in vegetated versus non-vegetated habitats on a seasonal time scale. The region being focused on in this study is relatively underrepresented in the literature. Preventing habitat loss is at the forefront of conservation efforts and has been for quite some time. By studying where and when economically and ecologically significant species are located, we may better understand how to support and protect these species in the future.

The Pamlico Sound is not only an area of economic significance, but also a crucial nursery region for a diverse group of species. This study serves to fill a portion of the void in the literature surrounding the importance of these estuarine regions and the changes these nursery habitats are undergoing over time. Oregon Inlet is among the most significant corridors in this portion of North Carolina's coastline for connectivity with the ocean. Studying this area and how juveniles may move through it as they transition to more oceanic life stages is significant from a fisheries perspective. Measurable effect data can be used by North Carolina's Division of Marine Fisheries and other organizations, with a lack of data on what fish species are utilizing seagrasses, this research could provide a highly useful database for many organizations.

As anthropogenic activity continues to drive climate change, it is crucial to characterize species assemblages in ecologically and economically important nursery regions, especially as climate change is shifting the geographic distributions of species. The value of seagrass as habitat is only increasing over time, as climate change contributes to habitat loss and species' ranges change. Climate change is affecting species assemblages and there is a growing need to collect data that represents and contributes to the understanding of what may happen in the future. We are already seeing tropical species in the seagrasses of North Carolina such as grey snapper (*Lutjanus griseus*).

Future goals

Ideally, there are several factors in a more elaborate study that would add a valuable dimension to this project. Firstly, aging fish species using otolith techniques would allow for comparisons to be drawn about what species are using certain habitats, at specific locations, throughout distinct points in their life cycle. Weighing and measuring fish could also lead to a more comprehensive comparison of the last point, I observed fish getting larger as the study approached the late months (September and October), as biomass might have a different relationship with seasonality. Another factor that could be added to this study is the measuring of characteristics of seagrass habitats more comprehensively, including overall area of seagrass cover and patchiness. Nevertheless, the study described here represents an important first step to filling a significant gap in our knowledge of North Carolina species that depend on seagrass habitats.

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